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Winetech
Wine Industry Network of Expertise and Technology
Netwerk van Kundigheid en Tegnologie vir die Wynbedryf

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INSTITUTE FOR GRAPE AND WINE SCIENCES

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INSTITUTE FOR GRAPE AND WINE SCIENCES

The Institute for Grape and Wine Sciences (IGWS) is a joint venture between the wine and table grape industries and Stellenbosch University.

The Institute's vision is to promote world-class teaching, research, and technology transfer in the South African wine industry and to ensure relevance and excellence in wine sciences and technology.

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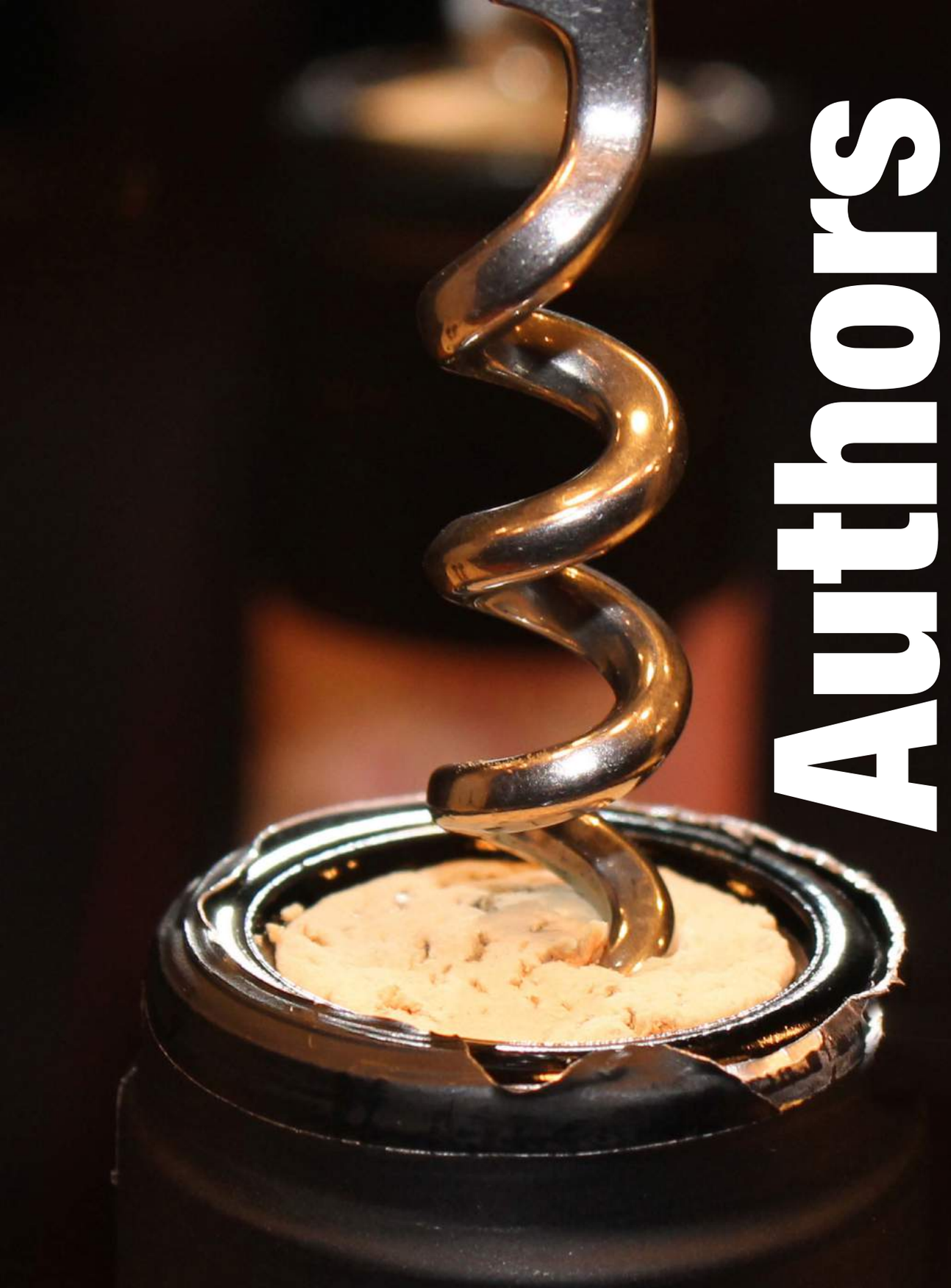

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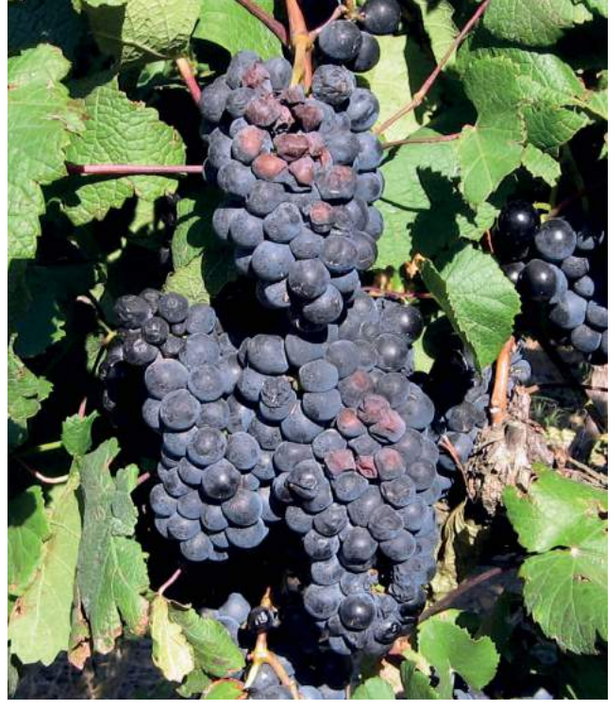
Foreword

Another year has come and gone and with it came many changes to how Winetech operates within the South African wine industry. The most important change we implemented is the overall strategy from being just a coordinator of research funds to becoming a strategic partner in research, development, innovation and knowledge transfer. This strategic change forms part of the wider objectives of the WISE process initiated by industry partners VinPro, SALBA, SAWIS, Winetech and WOSA; namely to work towards a more profitable, competitive and sustainable South African wine industry.

Research, innovation and the transfer of research results form an integral part of this WISE masterplan of technological and economic advancement. The 2016 yearbook, like the previous yearbooks, showcase the results from Winetech funded research. Like its predecessor in 2015 it is an interactive pdf, which allows readers to directly proceed to the sections of the book which interest them. The electronic version also makes the book easier to store. It is Winetech's aim to narrow the gap between researchers and the practitioners of research findings. We hope that this book will partially fulfil that aim.

Karien O'Kennedy
Technology Transfer Coordinator

1 Viticulture



Lesser known red wine cultivars (Part 1)

JANUARY 2016

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KEYWORDS: Vitis, wine grape cultivars, Alvarelhão, Cabernet Cubin, Rondo.

World-wide the search is ongoing for alternative wine grape cultivars to supplement countries' wine spectrum. Furthermore there are numerous cultivation programmes aimed at creating new cultivars with specific characteristics, such as resistance to cold and disease and good wine potential. It is increasingly difficult, however, to import material of such cultivars to South Africa. The cost of importation and material is prohibitive, especially if the material is imported from America. Overseas plant improvement bodies sometimes issue licences for the propagation of newly released cultivars to a limited number of preferred nurseries. Others are not prepared to make the new cultivars' material available to foreign countries, for fear of losing their competitive edge with regard to such cultivars. Nurserymen have rights on some of the cultivars being imported and royalties apply to such wine grape cultivars. To South African wine producers, this state of affairs is unusual.

ARC Infruitec-Nietvoorbij, an institute of the Agricultural Research Council (ARC) in Stellenbosch, has a national grapevine gene pool featuring, inter alia, more than 500 different wine grape cultivars that have been collected from across the country or imported (Van Schalkwyk, 2010 & Van Schalkwyk, 2013). New, promising cultivars are still being imported sporadically and added to this collection. The total wine grape plantings in South Africa have decreased by approximately 6 000 ha from 2001 to 2013. In 2013 93.4% of the total wine grape plantings consisted of mainly 16 cultivars, while 6.6% consisted of other lesser known cultivars (Sawis, 2014). Consequently South Africa does not boast a large variety of cultivars for use in vinification and the industry is continuously looking at the potential of other cultivars to determine whether they might perhaps be able to make a significant contribution to enhancing the South African wine spectrum.

Climate change has also prompted increased interest in alternative cultivars, in warm areas especially. In order to make full use of the grapevine gene pool, production data of cultivars that could potentially make a contribution to the South African wine industry are collected on an annual basis. Wines are made on a small scale in the experiment cellar and evaluated for overall wine quality and

aroma after six months. This evaluation takes place over five consecutive years, whereafter it moves to other cultivars.

The objective of this article is to introduce information about cultivars that could be promising and is a supplement to an article in *Wynboer* (Van Schalkwyk & Schmidt, 2004). The cultivars in this report are not registered for either cultivation or vinification in South Africa in the official varietal list compiled by the Department of Agriculture, Forestry and Fisheries (2014). The cultivars are grafted on Richter 99 rootstocks and trellised on a five strand extended Perold system with adjustable foliage wires and the plant width is 1.5 m x 3.0 m. Standard pruning and canopy management practices were applied. An effort was made to harvest the red wine cultivars at 24.5°B to 25.0°B. At the time of the crush 50 mg/kg SO_2 was added to the grapes, which were then pressed. A must sample was taken for pH, titratable acid and sugar analyses. After one hour's skin contact the crushed grapes were inoculated with pure dehydrated VIN13 at a concentration of 30 g/hℓ. An adjustment of 50 g/hℓ diammoniumphosphate (DAP) was also made. Fermentation on the skins took place at 25°C and the must cap was punched down three times a day. The wine was not aged in wood. The wine was fermented dry, whereafter it was bottled and evaluated for aroma and overall wine quality by a trained tasting panel six months after bottling.

ALVARELHÃO

Alvarelhão is a high quality red wine cultivar originating from the north of Portugal and cultivated intensively for high quality wine in the Monção subregion (www.wine-searcher.com), but it is generally not suitable as a cultivar wine. In Portugal it is mostly used in a blend with Touriga Naçional as a dry red wine and in port and in the Dão province as a blend in red wines. The cultivar has several synonyms, namely Alvarelhao, Alvarelho, Alvarello, Alvarello Gallego, Alvarelo, Alvarelyo, Avarilhao, Brancelho, Brancellao, Brancello, Brencellao, Broncellao, Locaia, Pilongo, Pirruivo, Serradelo, Serradillo, Uva, Gallega, Uva Negra, Varancelha and Verancelha. Alvarelhão has medium large, loose to well-filled bunches. Yields obtained were similar to those of Pinotage under the given conditions. Alvarelhão does not have an intense skin colour and good colour extraction was not obtained during vinification, the result being light ruby red wines. This concurs with overseas findings. The experimental wines had a mildly intense berry/tree fruit aroma.

CABERNET CUBIN

Cabernet Cubin (We 70-281-35) is a red wine cultivar that was cultivated at the *Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau* in Weinsberg in Germany in 1970. It is a cross between Blauer Limberger (Blaufrankisch) and Cabernet Sauvignon (Levadoux clone). According to the literature it is a cultivar that ripens late, produces high yields and is drought resistant (www.wein-plus.eu). Cabernet Cubin also has good resistance to most fungal diseases and is moreover cold resistant, which makes it suitable for cultivation in cold regions where snow is a factor.

Cabernet Cubin was imported by ARC Infruitec-Nietvoorbij in 2007 and after its release from quarantine, it was grafted onto Richter 99 from the grapevine gene pool at Nietvoorbij. An evaluation site was established under contract at Ernita once the plant material had undergone virus cleansing. The desired ripeness was achieved at Nietvoorbij from mid-February to mid-March, earlier than Cabernet Sauvignon and with higher yields (no production data was collected at Ernita). Results show that the cultivar adapts well to warm areas. The bunches are medium-sized and bunch compactness ranges from loose to well-filled. A degree of millerandage occurs at times. Experimental wines were made separately from the two different sites by the respective bodies and evaluated organoleptically at Nietvoorbij by an experimental wine judging panel. Experimental wines had an intense deep red colour, with reasonable tannins and a prominent berry character. The wine made from Cabernet Cubin is very similar to Cabernet Sauvignon, for which it is a possible replacement, because it ripens slightly earlier and also produces higher yields. It should be borne in mind, nevertheless, that cultivation rights on the cultivar have been registered and that royalties will have to be paid if grapevines are planted.

RONDO

Rondo (Geisenheim 6494-5) is a German cross (Zarya Servera x Saint Laurent) bred by Prof V Kraus from the then Czechoslovakia in 1964 (www.winegrowers.info). The cultivar was further developed by Dr Helmut Becker of the Geisenheim Grape Breeding Institute. Rondo is an early cultivar and resistant to winter frost and as a result of one parent being *Vitis amurensis*, it is also resistant to downy mildew. Due to the cultivar's early ripening, it is subject to feeding damage by birds. In wet seasons the cultivar tends to get botrytis rot and it must also be sprayed against oidium. In the Stellenbosch area yields are slightly higher than

Average viticultural and oenological performance of Alvarelhão over five seasons at Nietvoorbij, Stellenbosch.

Viticultural data		Oenological data	
Harvest date	7-31 March	Alcohol volume	14.1%
Dry rot	0.2%	Extract	25.0 g/ℓ
Sour rot	0.2%	Sugar	1.5 g/ℓ
Sunburn	0.6%	Titratable acid	5.8 g/ℓ
Run-off	3.4%	pH	3.64
Crop mass/grapevine	6.8 kg	Wine colour 420 nm	0.721
Crop mass/ha	15.7 t	Wine colour 520 nm	0.827
Bunch mass	242.0 g		
Berry mass	2.4 g		
Berry volume	2.4 mℓ		
Sugar	23.6°B		
Titratable acid	6.9 g/ℓ		
pH	3.34		
Skin colour 420 nm	0.205		
Skin colour 520 nm	0.630		

Average viticultural and oenological performance of Cabernet Cubin over five seasons at Nietvoorbij, Stellenbosch.

Viticultural data		Oenological data	
Harvest date	12 February-16 March	Alcohol volume	13.0%
Dry rot	0%	Extract	32.1
Sour rot	0%	Sugar	2.2 g/ℓ
Sunburn	0%	Titratable acid	7.2 g/ℓ
Run-off	7.5%	pH	3.62
Crop mass/grapevine	4.5 kg	Wine colour 420 nm	5.973
Crop mass/ha	10.1 t	Wine colour 520 nm	9.210
Bunch mass	170.7 g		
Berry mass	1.4 g		
Berry volume	1.4 mℓ		
Sugar	24.0°B		
Titratable acid	6.6 g/ℓ		
pH	3.51		
Skin colour 420 nm	0.495		
Skin colour 520 nm	2.390		

Average viticultural and oenological performance of Rondo over five seasons at Nietvoorbij, Stellenbosch.

Viticultural data		Oenological data	
Harvest date	24 January-27 February	Alcohol volume	13.7%
Dry rot	2.6%	Extract	35.6 g/ℓ
Sour rot	1.7%	Sugar	2.4 g/ℓ
Sunburn	0.4%	Titratable acid	5.7 g/ℓ
Run-off	1.3%	pH	4.08
Crop mass/grapevine	4.8 kg	Wine colour 420 nm	9.836
Crop mass/ha	10.7 t	Wine colour 520 nm	11.788
Bunch mass	165.9 g		
Berry mass	1.8 g		
Berry volume	1.7 mℓ		
Sugar	24.1°B		
Titratable acid	7.10 g/ℓ		
pH	3.61		
Skin colour 420 nm	0.488		
Skin colour 520 nm	2.588		

that of Cabernet Sauvignon. The grapes are very dark in colour and also have red juice. Good colour extraction is achieved during vinification and therefore Rondo is blended with other red wine cultivars that struggle to obtain colour. Rondo produces full-bodied, balanced wines with an intense dark red colour and a prominent blackcurrant/spicy character with tree fruit/vegetative and nutty nuances. The cultivar performs especially well in cooler areas and is suitable for the vinification of cultivar wines.

ACKNOWLEDGEMENTS

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SUMMARY

Alvarelhão, Cabernet Cubin and Rondo could make a contribution to the existing South African red wine cultivation, to a lesser or greater extent. However, in order to evaluate them properly, these cultivars should be planted on a semi-commercial basis in different climatic regions. It should be noted, nevertheless, that cultivation rights have been registered on Cabernet Cubin and Rondo and any planting may only take place under contract with the applicable licence holder/owner.

In Part 2 we look at other lesser known red cultivars that could possibly make a contribution to the enhancement of the South African wine spectrum.

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Lesser known red wine cultivars (Part 2)

FEBRUARY 2016

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KEYWORDS: Vitis, wine grape cultivars, Chambourcin, Regent, Yama Sémillon.

In this sequel about lesser known red cultivars that could possibly make a contribution to the South African wine industry (Van Schalkwyk & Schmidt, 2016), we look at Chambourcin, Regent and Yama Sémillon.

Chambourcin, Regent and Yama Sémillon are three lesser known red cultivars which offer good vinification and disease resistance qualities, the latter being conducive to a reduction of spraying cost against fungal diseases. These cultivars are currently being used at Nietvoorbij as cultivation parents with a view to cultivating disease resistant wine grape hybrids.

CHAMBOURCIN

Chambourcin (Johannes Seyve 26.205) is a French-American interspecific hybrid (Seyve Villard 12-417 x Seibel 7053) (Galet, 1979). The cultivar offers good resistance to downy mildew, oidium and botrytis and was used to cultivate other disease resistant cultivars, such as for example Regent. For this very reason it is being planted throughout the world in regions with a high humidity especially. At Nietvoorbij in Stellenbosch this cultivar ripens midseason. Chambourcin is fertile and has reasonable vigour. Even though the bunches are fairly loose, they are well-filled. Coulure, and a degree of millerandage, with hard green berries, occur at times. Chambourcin yields (10-17 t/ha) are considerably higher than that of Cabernet Sauvignon in the Stellenbosch area. The yields were also higher than that of Shiraz and compared well to Pinotage yields.

The grapes have a good sugar/acid balance and pH. Although the skin colour appears intense, good colour extraction was not obtained during vinification in all years, consequently the wine colour was sometimes slightly too light for a good red wine. Experimental wines were well-balanced and had a moderately prominent spice and berry character. The wine displayed none of the wild hybrid tastes. Although this cultivar was intended for areas with high humidity, the results show that it also fares well in the Stellenbosch region. Overseas the cultivar is

Average viticultural and oenological performance of Chambourcin over five seasons at Nietvoorbij, Stellenbosch.

Viticultural data		Oenological data	
Harvest date	1-18 March	Alcohol volume	13.9%
Dry rot	0%	Extract	26.7 g/ℓ
Sour rot	0%	Sugar	1.6 g/ℓ
Sunburn	0%	Titratable acid	6.6 g/ℓ
Run-off	4.0%	pH	3.64
Crop mass/grapevine	5.9 kg	Wine colour 420 nm	2.133
Crop mass/ha	13.8 t	Wine colour 520 nm	2.582
Bunch mass	254.2 g		
Berry mass	2.0 g		
Berry volume	2.0 mℓ		
Sugar	23.4°B		
Titratable acid	7.3 g/ℓ		
pH	3.34		
Skin colour 420 nm	0.613		
Skin colour 520 nm	3.505		

mostly used to make rosé, and as a blending cultivar, but in some instances also as a cultivar wine. Some overseas cellars recommend that Chambourcin be enjoyed as a young wine, while Chambourcin has also been reported to require a lengthy period in maturation barrels (7-15 years) to achieve its full aroma potential. Chambourcin is also suitable for cultivation in summer rainfall regions and has already been registered on the South African Varietal List for planting in South Africa (2014).

REGENT

Regent (GF. 67-198-3) is a German red wine cultivar that was created by Prof Gerhardt Alleweldt from the Institute for Grape Cultivation in Geilweilerhof (www.winegrowers.info). It is a hybrid between Diana (Silvaner x Müller Thurgau) and Chambourcin. Regent should not be confused with Regal Seedless, which was also originally registered as Regent at Upov and is a table grape. There was some confusion when the registration was submitted, because it had not been ascertained that the name Regent had already been registered for a wine grape cultivar. This was later rectified by registering the table grape "Regent" as Regal Seedless. The cultivar was mainly intended for cool climatic conditions and is planted in cool areas. It has moderate-strong vigour and an upright bearing. Regent is resistant to frost and also known for its resistance to most fungal diseases, such as downy mildew and oidium.

Regent ripens fairly early, but not before Pinot Noir, as some overseas winemakers have claimed. The grapes achieved full ripeness mostly in the midseason. Although a fairly high degree of sunburn occurred during a heatwave in 2005, the other seasons saw hardly any sunburn damage. Regent has smallish, well-filled bunches and produces good yields. The berries have a thick skin with a dark red colour and reddish pulp. Although Regent wines had relatively low total acids and tended to be high in pH, the wines were well-balanced with a lovely dark purple-tinged colour and a prominent spice/berry aroma. Vegetative aromas such as green beans, cloves and also nutmeg were perceived on the palate. World-wide and in South Africa there is currently much interest in this cultivar, but there are breeding rights and it may only be grown under contract with the breeder or the institution where the cultivar was bred. Although Regent is mainly recommended for cool areas, a small planting has been established under contract in the Swartland to determine whether the cultivar will fare well in warm regions. These grapevines have yet to come into production.

Average viticultural and oenological performance of Regent over five seasons at Nietvoorbij, Stellenbosch.

Viticultural data		Oenological data	
Harvest date	2 February-17 March	Alcohol volume	14.3%
Dry rot	0.8%	Extract	31.4 g/ℓ
Sour rot	0.3%	Sugar	2.0 g/ℓ
Sunburn	10.7%	Titratable acid	6.5 g/ℓ
Run-off	1.0%	pH	3.91
Crop mass/grapevine	4.9 kg	Wine colour 420 nm	1.926
Crop mass/ha	11.6 t	Wine colour 520 nm	3.428
Bunch mass	149.0 g		
Berry mass	1.9 g		
Berry volume	1.6 mℓ		
Sugar	24.2°B		
Titratable acid	5.1 g/ℓ		
pH	3.78		
Skin colour 420 nm	0.801		
Skin colour 520 nm	3.627		

YAMA SÉMILLON

Yama Sémillon was created at the Yamanashi University in Japan. It is a hybrid between Sémillon and the wild Japanese grape, Yama Bodou (*Vitis coignetiae*) (Yamakawa, Y. & Tanaka, H., 1991). The cultivar was specially cultivated for the

Japanese climate with its high humidity. Yama Sémillon is quite resistant to downy mildew, oidium, botrytis rot and berry burst. Its growth is vigorous and upright with huge leaves that are prone to damage by strong winds, which subsequently results in a fair amount of sunburn damage. A fair amount of coulure occurs at times. The bunches are loose, long and skittle shaped, with average weight and fairly small berries. Yama Sémillon is quite fertile and produces higher yields than most of the red cultivars in the Stellenbosch area. Yields of 7 to 9 kg per grapevine have been obtained in some seasons.

Yama Sémillon tends to have high titratable acid. In one particular season the must analysis during the harvest showed a sugar of 28°B and the wine had a total acid of 7.2 g/ℓ with a pH of 3.45. Although the skins are not very dark in colour and the analytical wine colour was not intense either, the tasting panel that evaluated the wines indicated that the colour was fairly intense. Yama Sémillon produces complex wines and has a prominent tree fruit/berry and spice character. Sometimes it has a distinct vegetative character (herbal, green beans, eucalyptus and asparagus). Yama Sémillon with its good resistance to diseases is eminently suitable for cultivation in cool, moist climatic regions.

Average viticultural and oenological performance of Yama Sémillon over five seasons at Nietvoorbij, Stellenbosch.

Viticultural data		Oenological data	
Harvest date	14 February-18 March	Alcohol volume	15.4%
Dry rot	0.1%	Extract	35.2 g/ℓ
Sour rot	0%	Sugar	2.4 g/ℓ
Sunburn	11.4%	Titratable acid	7.6 g/ℓ
Run-off	7.5%	pH	3.55
Crop mass/grapevine	5.09 kg	Wine colour 420 nm	0.854
Crop mass/ha	12.2 t	Wine colour 520 nm	1.409
Bunch mass	170.0 g		
Berry mass	1.2 g		
Berry volume	1.1 mℓ		
Sugar	25.5°B		
Titratable acid	7.0 g/ℓ		
pH	3.25		
Skin colour 420 nm	0.463		
Skin colour 520 nm	1.976		

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SUMMARY

Chambourcin, Regent and Yama Sémillon may make a lesser or greater contribution to current South African red wine cultivation. All three these cultivars have good disease resistant characteristics that should reduce spraying costs. Chambourcin is already being grown in certain areas and there are no breeding rights on the cultivar. Regent and Yama Sémillon should be planted on a semi-commercial basis in different climatic regions so that their potential can be properly evaluated. While there are no breeding rights on Yama Sémillon, they do apply to Regent, as mentioned earlier. Breeding rights on Regent only expire in 2024; it will therefore be necessary to negotiate with the licence holder/owner to establish bigger plantings under contract.

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Tissue cultures and viticultural applications

JANUARY 2016

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KEYWORDS: Grapevine, tissue cultures, embryogenic, somatic, berry cultures.

INTRODUCTION

Plant cells are considered totipotent, meaning that all plant cells have the capacity to regenerate into a complete new individual. Totipotency, in combination with the ability to differentiate (cells/tissues/organs develop to acquire a specialised function) and dedifferentiate (cells/tissues/organs undergo changes to revert to cells that are without specialised function and meristematic in nature) underlies the ability to manipulate plants in tissue culture. More than a century ago these findings sparked the field known today as plant tissue culture. Plant tissue culture research and knowledge has progressed rapidly in the last 40 years and is now recognised as a major discipline playing an important role in fundamental plant biological studies and product orientated research.

GRAPEVINE TISSUE CULTURE USES

The first grapevine tissue cultures were reported in 1944, with the objective of establishing sterile in vitro tissue cultures. Since then several advances have been made in manipulating grapevine explants in culture (the term for the starting material used in establishing a tissue culture). Several motivating factors propelled the research in grapevine tissue cultures and include:

(i) The development of micro propagation protocols for grapevine materials (Harris & Stevenson, 1982; Torregrosa *et al.*, 2001);

(ii) The elimination of viruses from plant material using the meristem cultures and somatic embryogenesis (Harris & Stevenson, 1979; Goussard *et al.*, 1991);

(iii) Development of methods to perform embryo-rescue in breeding programs that target seedless grapevine cultivars (Perl *et al.*, 2000; Burger *et al.*, 2003; Tian & Wang, 2008; Ji *et al.*, 2013);

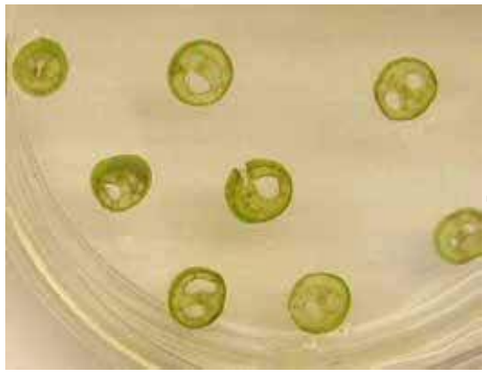
(iv) Development of culturing systems to support genetic manipulation of grapevine cultivars (Martinelli & Mandolino, 1994; Kikkert *et al.*, 1996; Perl *et al.*, 1996; Vidal *et al.*, 2003);

(v) The use of cell-based cultures to study-specific aspects of grapevine development and/or berry ripening (Symons *et al.*, 2006; Ramaschandra *et al.*, 2011), as well as regulation of metabolic pathways (Cakir *et al.*, 2003; Ferri *et al.*, 2011); and

(vi) The production of valuable metabolites for grapevine suspension cultures (Calderón *et al.*, 1993; Gagné *et al.*, 2011). Lately, grapevine organ-specific cultures, such as berry cultures, are being evaluated as model systems to overcome specific problems with the study of grapevine organs, specifically grapevine berries in field conditions.

Berries at different developmental stages

Sterilisation



Berry slices

1 month

Cells start to dedifferentiate

1-2 months

Non-embryogenic callus cells forming

Selection

3-6 months

Homogenous callus plate culture

1-2 months

Synchronised suspension culture

Uses:
Grape berry ripening studies
Model system for regulation studies
Secondary metabolite production
Fungal elicitor testing

GRAPEVINE IS DIFFICULT TO MANIPULATE IN CULTURE

Grapevine is considered recalcitrant to tissue cultures, mostly because significant optimisations are needed to implement efficient protocols for different grapevine explants and cultivars. Although a range of tissue culture methods and techniques have been published (Martinelli & Gribaudo, 2009), considerable optimisations per experiment, explants, variety, cultivar and/or clone are often needed, making the work time-consuming, technically demanding and not generally available to all labs working with grapevines. The initial drive to develop grapevine tissue culture and transformation technologies also suffered significant setbacks with consumer resistance against genetically modified foods. Notwithstanding, the technologies developed and are available in a few laboratories around the world and some countries and industries invested resources and efforts in implementing genetic improvement. Research groups typically use the technologies to provide proof-of-principle results (Terrier *et al.*, 2009, Lashbrooke *et al.*, 2013). In vitro grapevine plants can also be used for transient transformation experiments using *Agrobacterium* infiltration (Du Preez *et al.*, 2010), a technique widely applicable in functional studies for crop improvement.

SOMATIC VS EMBRYOGENIC GRAPEVINE TISSUE CULTURES – WHAT'S THE DIFFERENCE?

Callus is defined as unorganised, undifferentiated plant cells forming as a result of a wound response. Grapevine cultures can be established to form either embryogenic or non-embryogenic callus types (Stander *et al.*, 2009). Embryogenic

callus consists of small, tightly packed meristematic cells which have excellent regeneration potential. In other words, somatic embryogenic callus develop from ordinary plant body (somatic) tissues, but can develop into reproductive embryo structures, ultimately germinating in culture to form plantlets. Non-embryogenic callus, on the other hand, are not maintained for the purpose of regeneration into plants. These cells consist of highly prolific, thin walled cells containing mostly vacuoles and are typically used to study cellular and metabolic processes in culture. Here the use of non-embryogenic berry cultures will be considered further.

NON-EMBRYOGENIC SOMATIC BERRY CALLUS CULTURES AS RESEARCH TOOLS

Callus cells theoretically will grow and proliferate if provided with appropriate nutrients and a supportive environment. It is well-known that these callus cells can maintain metabolic functions characteristic from the organ they originate, but the extent of this "memory" needs to be experimentally proven and not assumed.

The process involved to develop such cultures from grapevine berries are outlined in Figure 1 and starts with berries being collected and surface sterilised after which the berries are cut into slices and pips are removed. The slices are placed on media optimised for callus formation. The callus cultures are rigorously selected and transferred to fresh medium until homogenous cultures are obtained after four to six selection and transfer cycles.

Sharathchandra *et al.* (2011) reported an analysis from these somatic grape berry cell cultures, comparing them to evaluate if the callus forming process would eliminate the characteristic features of the berry development stages (green to ripe). The first interesting and significant result was that the cultures from green, véraison and ripe berry explants formed different proteins, providing evidence that the process to initiate callus formation does not abolish "signatures" linked to the different developmental stages of the explants. Moreover, the results showed that soluble proteins produced by the cell lines were comparable to those of berries from the field in corresponding developmental stages. In the study reported by Sharathchandra *et al.* (2011) the different cell cultures were incubated in the dark, eliminating light as a modulating factor.

Interestingly, in a subsequent study the analyses of the green, véraison and ripe cultures were extended to evaluate how the cultures will respond to exposure to light (Noqobo, 2015). The results of this study confirmed that the berry cultures originating from explants in different developmental stages maintained characteristic differences in metabolite production (pigments and aroma compounds) when exposed to different light regimes. These berry cultures therefore retained the capacity to respond differentially to the changing light environment, providing support for their use as potential test systems when studying berry metabolism (Noqobo, 2015).

These berry suspension cultures were also used to study pathogen responses in grapevine cells (Mutawila *et al*., 2012; Mutawila, 2014). The suspensions were treated with elicitors (extracts from the fungi that will trigger a defence response in the plant cells) originating from *Eutypa lata*, a destructive wood-infecting pathogen, as well as with elicitors originating from a *Trichoderma* biocontrol agent that is used as a protective treatment on pruning wounds. The suspension cultures treated with these elicitors were used to study the early responses of the grapevine cells to the elicitors and to study the expression of defence-related genes and the development of protective metabolites such as polyphenols (Mutawila, 2014). These cultures showed some of the mechanisms the biocontrol agent employs to protect grapevines against this destructive pathogen, as well as the response that is triggered by the pathogen.

MAINTAINING INTACT BERRIES IN CULTURE TO STUDY DEVELOPMENTAL AND RIPENING PROCESSES

Berries comprise skin, pulp and seed tissues and several processes and metabolic changes are uniquely different in these tissue types. One of the problems with generating callus cultures of grapevine organs such as berries, is the fact that tissue-specificity is lost when initiating callus formation. Exciting techniques have however recently been developed to adapt intact grape berries to tissue cultures and maintain their development/ripening under controlled conditions in culture (Dai *et al.*, 2014, 2015). These cultures allow the berries to be studied and sampled as intact organs, but with the possibility to control the media and environmental conditions.

These examples and literature illustrate that grapevine tissue culture techniques are important as study tools, but also important to support plant improvement strategies for cultivars and clones.

SUMMARY

Tissue culture is a relatively old plant science, originated in the early 1900s and still plays a crucial role in many modern technologies. It has contributed significantly to grapevine biology and biotechnology and is facilitating our understanding of how grapevine functions in terms of gene-expression, metabolism and development. Grapevine tissue culture techniques are continuously updated, establishing systems and protocols that allow these cultures to be used for exciting new applications.

ACKNOWLEDGEMENTS

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The effect of cover crops on ring nematodes and their management in a drip irrigated vineyard

JANUARY 2016

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KEYWORDS: Biofumigation, plant-parasitic nematodes, cover crops, grapevines.

INTRODUCTION

Plant-parasitic nematodes can reduce crop production by as much as 15% in grapevines under stress (inter alia water stress). An important plant-parasitic nematode associated with grapevines is ring nematode (*Criconemoides xenoplax*). Ring nematodes damage the roots of all grapevine rootstocks.

Nematode control is currently based on the use of registered chemicals. Grapevine rootstocks resistant to root-knot nematode (*Meloidogyne javanica*) are planted as well. However, none of the available rootstocks are resistant against ring nematode.

PHOTO: A MALAN.

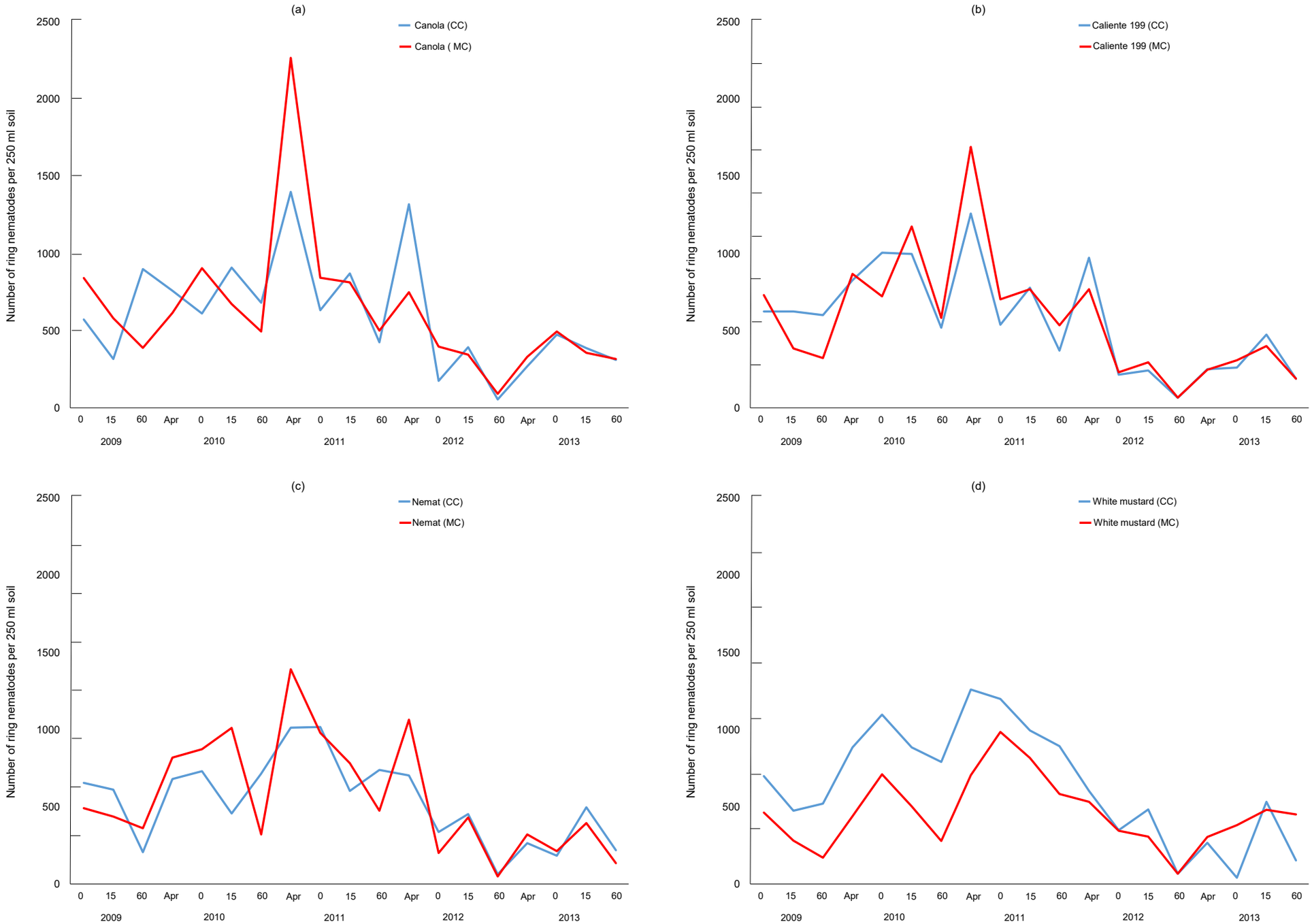
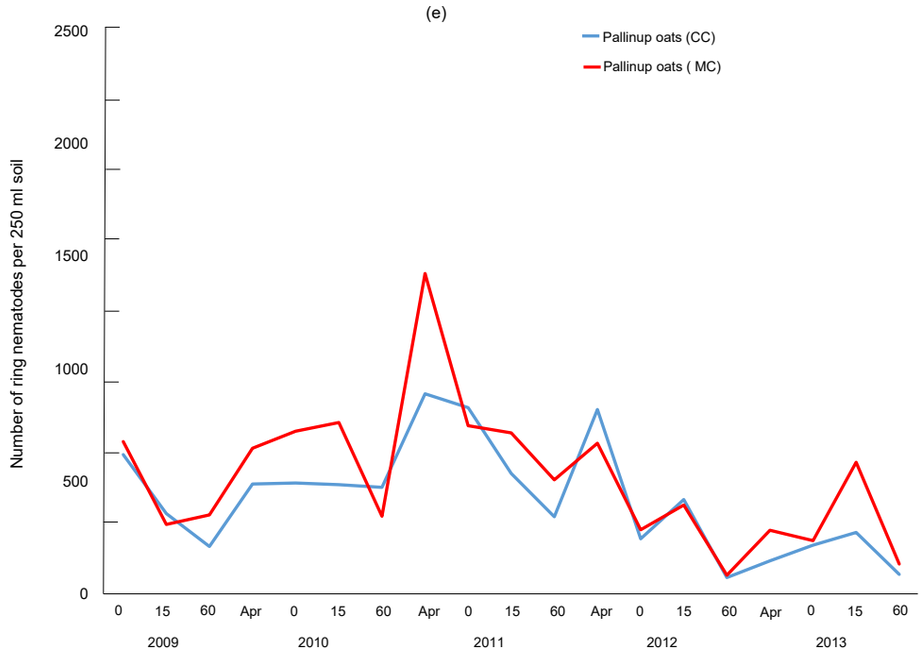


FIGURE 1. The effect of two management practices, namely CC and MC (see Table 2), applied to different cover crops and weeds in the grapevine inter-row on the ring nematode (*Criconemoides xenoplax*) population in a sandy to sandy loam soil near Stellenbosch from day 0 in 2009 to day 60 in 2013 (April = Apr, day 0 = 0, day 15 = 15, day 60 = 60). LSD ($p \leq 0.05$) = 252. Canola (*Brassica napus* cv. AV Jade).

a) Canola (*Brassica napus* cv. AV Jade). b) Caliente 199 (*Brassica juncea* cv. Caliente 199). c) Nemat (*Eruca sativa* cv. Nemat). d) White mustard (*Sinapis alba* cv. Braco).



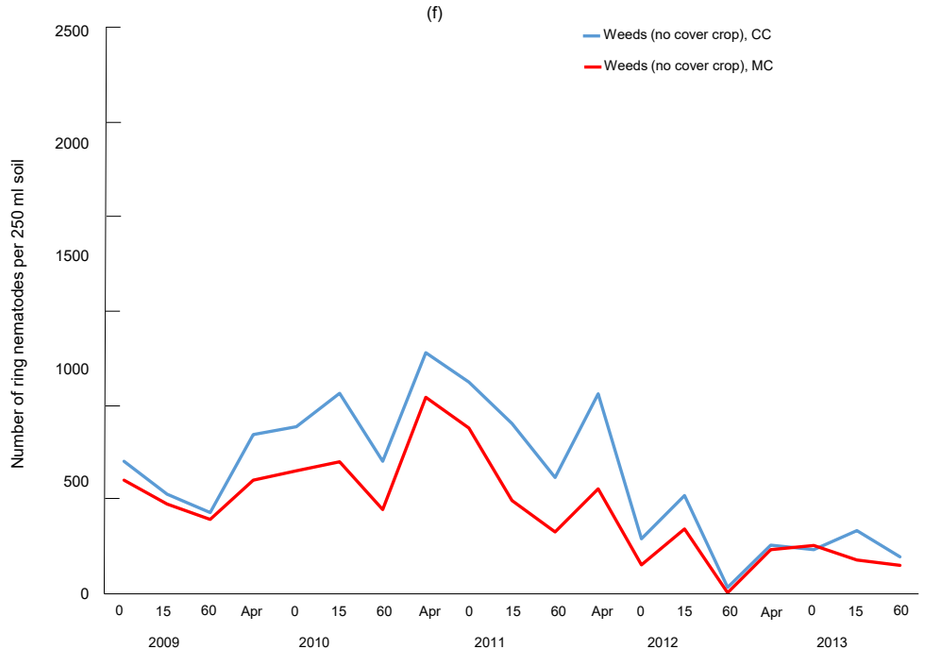


FIGURE 1 Cont. The effect of two management practices, namely CC and MC (see Table 2), applied to different cover crops and weeds in the grapevine inter-row on the ring nematode (*Criconemoides xenoplax*) population in a sandy to sandy loam soil near Stellenbosch from day 0 in 2009 to day 60 in 2013 (April = Apr, day 0 = 0, day 15 = 15, day 60 = 60). LSD ($p \leq 0.05$) = 252. Canola (*Brassica napus* cv. AV Jade).

e) Pallinup oats (*Avena sativa* L. cv. Pallinup). f) Weeds (winter growing weeds).
g) Weedsnem (winter growing weeds combined with the application of a nematicide in the vine row during grapevine bud break).

TABLE 1. The rainfall during the cover crop growing season (May to August), 0-60 days after the management practices were applied (September and October) and late summer/early autumn (November to April).

Treatment phase	Seasonal rainfall (mm)				
	2009/10	2010/11	2011/12	2012/13	2013/14
May to August September to October November to April	435 171 187	391 77 152	360 80 228	535 231 181	578 151 NA
Total	793	620	668	947	NA

TABLE 2. The two soil management practices applied during bud break.

Management practice applied	Code
The cover crops and winter growing weeds were controlled chemically full surface by glyphosate (1.8 kg/ha) during grapevine bud break, whereafter the cover crops/weeds were left standing to be flattened by the tractor traffic during the grapevine growing season.	CC
The cover crops were controlled between late bloom and early seed/pod formation, which coincided with grapevine bud break. The cover crops and winter growing weeds in the work row were macerated by slashing the above-ground growth and incorporating the fibre to a depth of 200 mm immediately thereafter with a disc harrow. This was done before the soil started drying out, as water plays an important role (hydrolysis) during biofumigation. Chemical control in the vine row during grapevine bud break was achieved with glyphosate at a rate of 0.6 kg/ha.	MC

Certain crops, inter alia brassica species, produce glucosinolates (GSL), which are hydrolysed by the enzyme myrosinase to form isothiocyanate (ITC) when cellular disruption occurs during the maceration and mechanical incorporation of these growing crops into the soil (biofumigation). To maximise the presence of ITC in the soil during biofumigation, brassica species with a high GSL concentration should be selected. However, the minimum tillage system in which cover crops are controlled chemically during grapevine bud break to leave a summer mulch, are currently employed by the grapevine industries. This system reduces water runoff and erosion, restricts evaporation from the soil surface, reduces soil temperature fluctuations, suppresses winter and summer growing weeds effectively, as well as helps maximise the production of quality grapes.

The objectives of this study were:

- To determine the ability of selected cover crops, some known for their biofumigation properties, to reduce the ring nematode population in a commercial vineyard.
- To compare the effect of minimum tillage and mechanical cultivation (green manuring) applied to these selected cover crops during grapevine bud break on the control of ring nematodes.

MATERIALS AND METHODS

A five year study was conducted in a seven year old Shiraz/101-14 drip irrigated (from December to March) vineyard, established on a sandy to sandy clay loam soil near Stellenbosch. The rainfall during the study is shown in Table 1. The fourteen treatments were replicated five times and consisted of two soil management practices, namely CC and MC (Table 2), applied to canola, Caliente 199, Nemat, white mustard, Pallinup oats (cover crop frequently used in vineyards), a treatment in which no cover crop was sown (Weeds) and a weeds treatment in which a nematicide was applied in the vine row during grapevine bud break (Weedsnem) (Figure 1). The nematode status of the soil was determined just before the management practices were applied (day 0), 15 days and 60 days thereafter, as well as at the beginning of April (before the cover crops were established).

RESULTS AND DISCUSSION

The ring nematode populations in the work row were in all cases very low (significantly lower than those in the vine row), which did not allow for specific conclusions or trends regarding the impact of the cover crops on these nematodes to be made (data not shown). The ring nematode populations in the vine row are shown in Figure 1.

Three periods were considered, namely:

1) April to day 0 in a specific year, which indicates the impact that the actively growing cover crops and winter weeds had on the ring nematode numbers;
2) Day 0 to day 60 for a specific year, indicating the impact of the cover crops after the management practices were applied; and
3) Day 60 to April the following year, indicating the ability of the ring nematodes to recover during the 'inter-treatment period'.

April to day 0

During 2010, the ring nematode population declined in canola (CC) and Caliente 199 (MC) (Figure 1a & b). This trend was observed for all treatments during 2011 and 2012, with the exception of Nemat (CC), white mustard (MC) and Weedsnem (CC) during 2011 (Figure 1a-g). During the relatively wet winter of 2013 (Table 1) this trend was observed in the two Nemat treatments, white mustard (CC), Pallinup oats (CC), Weedsnem (MC) and Weeds (CC) (Figure1c-g). The ring nematode population increases during the growing season of the cover crops and were on average 61% for canola, 35% for white mustard, 26% for the weeds, 20% for Pallinup oats, 17% for Caliente 199 and 7% for Nemat.

Day 0 to day 60

A continuous decline in ring nematode numbers from day 0 to day 60 was detected throughout the study for canola (MC) (Figure 1a). This was also observed for white mustard (MC) and Weedsnem (CC), with the exception of 2013 and 2009, respectively (Figure 1d & f). Pallinup oats (CC) resulted in the ring nematode numbers declining continuously from day 0 to day 60 during 2009, 2010 and 2011 (Figure 1e). As far as the other treatments are concerned, the ring nematode numbers were suppressed irregularly.

Day 60 to April

With the exception of canola (CC) in 2009/10, as well as the two white mustard treatments, Nemat (CC) and Weedsnem (CC) in 2011/12, the ring nematode populations repeatedly increased from day 60 to April the following year (Figure 1a-g). The rainfall during this period varied between 152 mm and 228 mm, which seemed sufficient to allow the ring nematode population to recover at least partially. The average percentage increase during this period was much higher than during the winter (April to day 0), namely 247%. This is an indication that a follow-up treatment with a nematicide is necessary in an attempt to maintain the downward trend achieved during the sixty day period after grapevine bud break. Biofumigation should, therefore, not be applied in isolation, but as part of an integrated nematode control program.

Day 0 2009 to day 60 2013

A significant decline in the ring nematode population was observed from day 0 in 2009 to day 60 in 2013, irrespective of the treatments applied (Figure 1a-g), with the exception of white mustard (MC), although the rainfall was higher during the 2012/13 and 2013/14 seasons than during the 2010/11 and 2011/12 seasons. It is possible that the roots of the permanent sward, which was maintained in the grapevine inter row before the treatments were applied by regular slashing, supplied an additional food source to the ring nematodes throughout the year. In contrast, the soil management practices applied during the trial removed this food source during spring and early summer (also in the Weeds treatment in which no biofumigation crop or nematicide were applied), which reduced the availability of roots during the grapevine growing season.

SUMMARY

During winter, Nemat, Caliente 199 and Pallinup oats did not facilitate a ring nematode population increase to the same extent as the weeds growing in this region. In some years, the ring nematode population even showed a decline during winter, whether a cover crop was established or not. During the early grapevine growing season, the most effective ring nematode suppression was achieved when canola and white mustard were mechanically incorporated into the soil, as well as where a nematicide was applied or oats was sown and combined with full surface chemical control. However, the drastic increase in the ring nematode population during summer and early autumn indicates that a follow-up treatment with a nematicide is necessary in an attempt to maintain the downward trend achieved during the sixty day period after grapevine bud break. Biofumigation should, therefore, not be applied in isolation, but as part of an integrated nematode control program. Controlling the weeds/cover crops during the grapevine growing season may help reduce the ring nematode population over the medium term.

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Esca in SA vineyards

FEBRUARY 2016

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KEYWORDS: Esca, *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., Basidiomycete, vineyard.

Esca is a fairly unknown grapevine disease in South Africa, although the disease has probably existed locally for decades. This article discusses the causative organisms, the symptoms by which the disease may be recognised and basic control strategies.

In Europe esca is currently one of the biggest causes of grapevines dying, which lead to loss of income. In certain regions it is known as the 'second phylloxera'.

What exactly is esca? The definition of esca has changed regularly over the past few years. Certain researchers have suggested that the disease is caused by a succession of different fungi, while others consider it a complex of various diseases, inter alia a vascular and a wood rot disease. The vascular disease is linked to *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. which cause Petri disease, and wood rot with Basidiomycete fungi.

WHAT DO THE SYMPTOMS LOOK LIKE?

A cross section of a trunk or arm of a grapevine with esca displays sections with soft yellow rot (Photo 1). The soft yellowing may be accompanied by a black or dark brown margin, while V-shaped browning and black or brown spots are also regularly observed. Leaves sometimes display characteristic *tiger stripe* symptoms (Photo 2). These symptoms start out as yellow spots between the veins (Photo 3). When the spots become larger, they coalesce and later appear as necrotic

PHOTO 1. Cross section of a grapevine trunk with esca. Note the soft yellow rot, brown discolouration, black lines and black spots. The various fungi involved in the disease are responsible for the different symptom types.

PHOTO 2. Typical *tiger stripe* leaf symptom on a white cultivar (2a) and on a red cultivar (2b).

PHOTO 3. The first symptoms to be observed on a leaf are pale green to yellow, round or irregular spots that develop between the veins.

PHOTO 4. Purplish brown spots on berries, known as *black measles.*

PHOTO 5. Apoplexy usually occurs during the warmer summer months when the water requirements of the plant are not being met.

sections between the veins, hence the *tiger stripe* appearance. Leaf symptoms do not necessarily appear each year and the reasons for this are unknown. These characteristic symptoms are not very common in South African vineyards and may partially explain why the disease is not well known. In South Africa the disease usually goes hand in hand with a general decline of the plant. Berries may display characteristic purplish brown spots, although these symptoms have only been observed in isolated instances in South Africa (Photo 4). However, these symptoms are very common in California where it is known as *black measles.* Apoplexy, or the sudden wilting and dying of grapevines, is known as the acute form of esca (Photo 5). Symptoms are more common in older grapevines, although symptoms have been observed on seven to 10-year-old grapevines in South Africa.

ECONOMIC IMPORTANCE OF THE DISEASE

Esca considerably shortens the productive lifetime of a grapevine. Must and wine quality are also compromised. Spots on berries may render it impossible to market table grapes. The loss of income as a result of smaller crops and inferior quality grapes, together with the cost of new plantings, undoubtedly contribute to the economic impact of the disease. Large-scale monitoring and surveys in French vineyards have found esca to be currently responsible for an annual loss of 1 to 1.5 billion euros. (The total income of the French wine industry is 7 billion euros.) This figure only reflects the loss due to unproductivity and does not include the costs associated with re-establishment. Recent surveys in South Africa have shown that the disease occurs in all the wine-growing regions of the Western and Northern Cape, while it has also been observed in grapevines in Limpopo and Mpumalanga. Producers are mostly unaware of the disease and are under the impression that the deterioration in the plants' performance is due to the age of the grapevines.

CONTROL MEASURES

The best advice is to prevent the disease. There is no remedy to heal the rotten and dead tissue. Producers are encouraged to buy certified nursery plants. Such grapevines have the best chance during establishment to develop into healthy, balanced grapevines with the ability to handle stress. *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. are able to infect plants at a very early stage. Wounds, in particular pruning wounds, are the most important infection portals in established grapevines and therefore pruning wound protection is of cardinal importance. A registered biological control agent, based on *Trichoderma*, is highly recommended. The *Trichoderma* fungus has the ability to colonise pruning wounds and to form a long lasting biological protective layer inside the pruning wound to fight pathogen infections. Basidiomycete fungi most probably infect plants through wounds, although this occurs at a later stage than pathogens of Petri disease. If symptoms of esca are observed, the affected section or arm can be sawn off and an extension made. This treatment will only be successful, however, if the symptomatic tissue is removed completely. It is good practice also to remove 10 to 15 cm of the 'visually healthy' wood, because the pathogens usually colonise the healthy wood before symptoms become visible. This wound then has to be treated with a wound sealer or biological product, because it is usually large and there is a reasonable chance that it may be re-infected.

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PHOTO: F VILJOEN.

Performance of cover crops with biofumigation properties in a drip irrigated vineyard

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KEYWORDS: Biofumigation, cover crop, grapevine, soil, weed control.

INTRODUCTION

Alternatives to the chemical control of nematodes, such as biofumigation (maceration and mechanical incorporation of a crop with a high glucosinolate content into the soil), are being considered. Brassica species have shown promising results. An increasing number of herbicide-resistant weeds, makes the use of cover crops essential as a non-specific biological method of pre-emergence weed control in vineyards. In addition, a cover crop mulch during the grapevine growing season reduces water runoff, erosion and evaporation from the soil surface, conserves soil water and reduces temperature fluctuations in the soil when compared to bare soils. These advantages may be forfeited if the cover crops are mechanically incorporated into the soil during grapevine bud break to facilitate biofumigation.

The aim of this study was to determine the performance of cover crop species with biofumigation properties when controlled chemically or incorporated mechanically into the top soil during grapevine bud break.

MATERIALS AND METHODS

The trial was conducted in a full-bearing seven year old Shiraz/101-14 vineyard established on a sandy to sandy clay loam soil near Stellenbosch. Two management practices (CC and MC in Table 1) were applied to five cover crops (Table 2) and a treatment in which no cover crop was established (Weeds), totalling twelve treatments. These treatments were replicated three times in a fully randomised block design. Seedbed preparation in the work row was done to a depth of approximately 150 mm with a disc harrow (two passes in opposite directions). The seeds were broadcast by hand at the seeding densities indicated in Table 2, and were covered by means of shallow cultivation (approximately 30 mm deep) with a rotary harrow. The cover crops were sown annually during the end of April/early May, after the first winter rain of more than 16 mm. The late onset of winter rain in 2013 resulted in the cover crops not being established until 23 May. To help ensure a sufficient supply of P, K and N to the cover crops in this sandy to sandy loam soil, while not exceeding the norms for grapevines, these macro-nutrients were broadcast in the amounts specified in Table 3.

RESULTS AND DISCUSSION

Cover crop performance

All the cover crops produced more dry matter in 2011 than in 2010 (Table 4), due to the additional P and N applied one week before sowing, as well as the K applied at the two to six leaf stages. Although it did not rain during the second and third

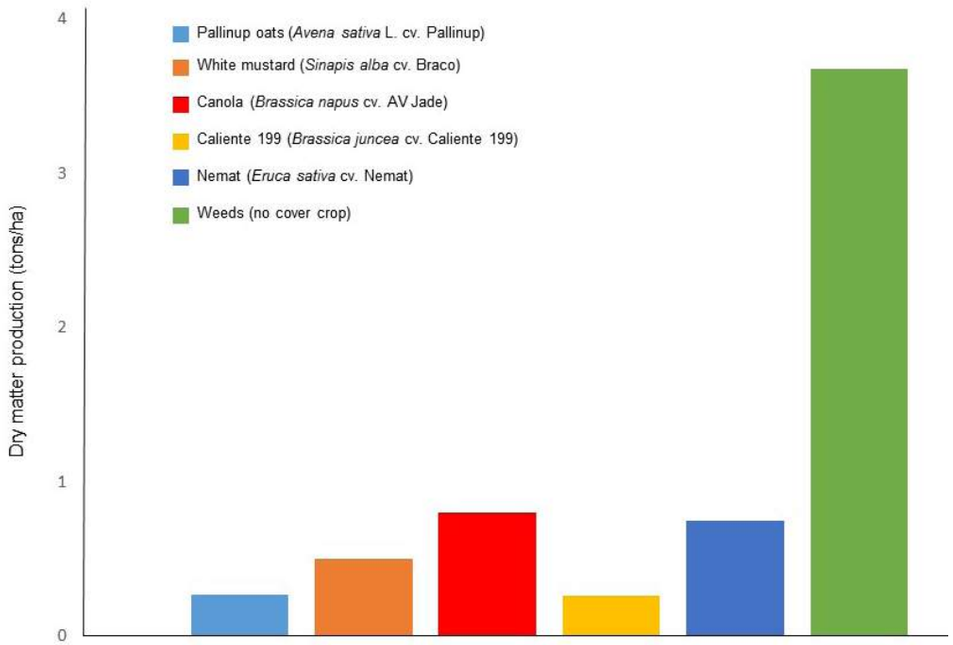

FIGURE 1. Effect of cover crops on the average winter weed stand in a Shiraz/101-14 vineyard established on a sandy to sandy clay loam soil near Stellenbosch.

week after the cover crops were sown, the 71 mm rain that fell between 24 April and 7 May 2011 (Table 5), seemed sufficient to ensure the successful germination of the cover crop seeds and sustain growth during the first three weeks.

The four broadleaf cover crops selected for their biofumigation properties did not perform as well as Pallinup oats during 2012 and produced less fibre in 2012 than in 2010 and 2011 (Table 4). This was attributed to the average temperature from May 2012 to August 2012 being approximately 1.4°C lower than that of the previous two seasons (Table 6). This indicated that the four broadleaf species were more sensitive to lower temperatures than Pallinup oats. Although the cover crops could only be sown as late as 23 May 2013, the dry matter production of Pallinup oats still improved slightly, compared to 2012 (Table 4). This was attributed to the relatively high rainfall during the first five weeks following the sowing of the cover crops (Table 5) and the slightly cooler temperatures during June, July and August compared to 2010 and 2011 which seems to favour the oats (Table 6). The four broadleaf cover crops, however, still performed poorer than during the 2011 season, indicating that these species need to be sown earlier.

Suppression of winter growing weeds

The average weed stand was significantly reduced by all the cover crops (Figure 1). Effective suppression (less than 10% of the weed stand measured in the Weeds treatment) was achieved with Pallinup oats and Caliente 199.

TABLE 1. The two management practices applied to each of the cover crops from grapevine bud break to harvest.

Management practice applied	Code
The cover crops and winter growing weeds were controlled chemically full surface by glyphosate (1.8 kg/ha) during grapevine bud break, after which the cover crops/ weeds were left standing to be flattened by the tractor traffic during the grapevine growing season. Full surface, post-emergence weed control during the first week of December was achieved with glyphosate at a rate of 1.8 kg/ha.	CC
The cover crops were controlled between late bloom and early seed/pod formation, which coincided with grapevine bud break. The cover crops and winter growing weeds in the work row were macerated by slashing the above-ground growth and incorporating the fibre to a depth of 200 mm immediately thereafter with a disc harrow. This was done before the soil started drying out, as water plays an important role (hydrolysis) during biofumigation. Chemical control in the vine row during grapevine bud break was achieved with glyphosate at a rate of 0.6 kg/ha. Full surface, post-emergence weed control during the first week of December was achieved with glyphosate at a rate of 1.8 kg/ha.	MC

TABLE 2. The cover crop species and the seeding densities at which they were established.

Cover crops	Seeding density (kg/ha)
Pallinup oats (*Avena sativa* L. cv. Pallinup)	100
White mustard (*Sinapis alba* cv. Braco)	8
Canola (*Brassica napus* cv. AVJade)	8
Caliente 199 (*Brassica juncea* cv. Caliente 199)	10
Nemat (*Eruca sativa* cv. Nemat)	5

TABLE 3. Nutrients applied in the Shiraz/101-14 vineyard to promote cover crop growth and to supply the needs of the grapevines.

Year	Date	Stage	Amount (kg/ha)		
			N	P	K
2010	12/06/2010	Two to six leaf stages of Pallinup oats	28	0	0
	11/11/2010	Grapevine flowering stage	28	0	30
2011	28/04/2011	One week before sowing of cover crops	28	30	0
	09/06/2011	Two to six leaf stages of Pallinup oats	28	0	30
	30/11/2011	Grapevine full bloom	28	0	0
2012	09/05/2012	Just after sowing cover crops	28	15	30
	21/06/2012	Two to six leaf stages of Pallinup oats	28	0	0
	06/12/2012	Grapevine full bloom	0	0	30
2013	23/05/2013	Just after sowing of cover crops	28	0	30
	20/06/2013	Two to six leaf stages of Pallinup oats	28	0	0

TABLE 4. Cover crop performance as measured at the end of August.

Treatment	Dry matter production (t/ha)*			
	2010	2011	2012	2013
Pallinup oats (*Avena sativa* L. cv. Pallinup)	2.48b	4.09a	5.34a	5.86a
White mustard (*Sinapis alba* cv. Braco)	4.09a	4.80a	3.25b	3.12bc
Canola (*Brassica napus* cv. AVJade)	2.29b	4.19a	1.29d	3.43b
Caliente 199 (*Brassica juncea* cv. Caliente 199)	3.08ab	5.43a	2.83bc	2.19bc
Nemat (*Eruca sativa* cv. Nemat)	2.48b	3.46b	1.91cd	1.74c

* Values in columns followed by different letters differ significantly at the 5% level.

Suppression of summer growing weeds

The average weed stand in the CC treatments was significantly lower than that of Weeds (MC), with the exception of Caliente 199 (CC) (Table 7). The average weed stand in the CC treatment of a cover crop species also tended to be lower than the corresponding MC treatment. The observed trends were attributed to the presence of a surface mulch in the CC treatments, as well as to the mechanical cultivation applied during September in the MC treatments which may have promoted the germination of weeds during early summer.

SUMMARY

On sandy to sandy clay loam soils, cover crops should receive N, K and P from just before planting up until the six leaf stage to maximise their performance. However, the application of P should be done judiciously, as it may accumulate and reach very high concentrations in the top 75 mm of the soil within a very short period of time. Relatively low winter temperatures and the late establishment of the cover crops due to a dependency on the winter rain (drip irrigation), had a greater negative effect on the performance of the small seeded white mustard, canola, Caliente 199 and Nemat, than on the larger seeded Pallinup oats. All the cover crop species can compete successfully with the winter growing weeds of the region, especially Pallinup oats and Caliente 199. Chemical control of the cover crops or weeds during grapevine bud break seemed to improve the control of summer growing weeds.

Caliente 199, canola, white mustard and Nemat can therefore be included in a cover crop rotation system for grapevines without compromising weed control efficacy.

TABLE 5. Winter rainfall for the period 24 April to 20 August at the nearest weather station.

Period	Rainfall (mm)			
	2010	2011	2012	2013
24 April-7 May	37	71	60	9
8 May-21 May	75	0	23	7
22 May-4 June	29	85	33	123
5 June-18 June	92	54	75	44
19 June-2 July	18	79	40	36
3 July-16 July	63	2	75	9
17 July-30 July	6	15	61	108
31 July-13 August	25	48	88	87
14 August-20 August	6	10	47	52
Total winter rainfall	351	364	502	475

TABLE 6. Average monthly winter air temperatures from May to August measured at the nearest weather station.

Month	Temperature (°C)			
	2010	2011	2012	2013
May	16.0	16.7	15.5	17.5
June	14.8	14.2	14.1	13.8
July	14.5	15.3	13.2	14.2
August	14.8	14.5	12.3	13.1

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TABLE 7. Effect of the cover crops managed according to two management practices during grapevine bud break on the stand of summer growing weeds (average over four seasons) measured at the end of November.

Treatment	Dry matter production (t/ha)
Pallinup oats (*Avena sativa* L. cv. Pallinup), CC[1]	0.34bc[2]
Pallinup oats, MC[1]	0.69ab
White mustard (*Sinapis alba* cv. Braco), CC	0.38bc
White mustard, MC	0.49abc
Canola (*Brassica napus* cv. AV Jade), CC	0.26c
Canola, MC	0.50abc
Caliente 199 (*Brassica juncea* cv. Caliente 199), CC (Caliente), CC	0.44abc
Caliente 199, MC	0.47abc
Nemat (*Eruca sativa* cv. Nemat), CC	0.37bc
Nemat, MC	0.38bc
Weeds, CC	0.33bc
Weeds, MC	0.79a

[1] For explanation see Table 1.
[2] Values followed by different letters differ significantly at the 5% level.

Investigating grapevine cell walls

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KEYWORDS: Cell walls, polymers, grapevine pathogens, grape ripening, winemaking.

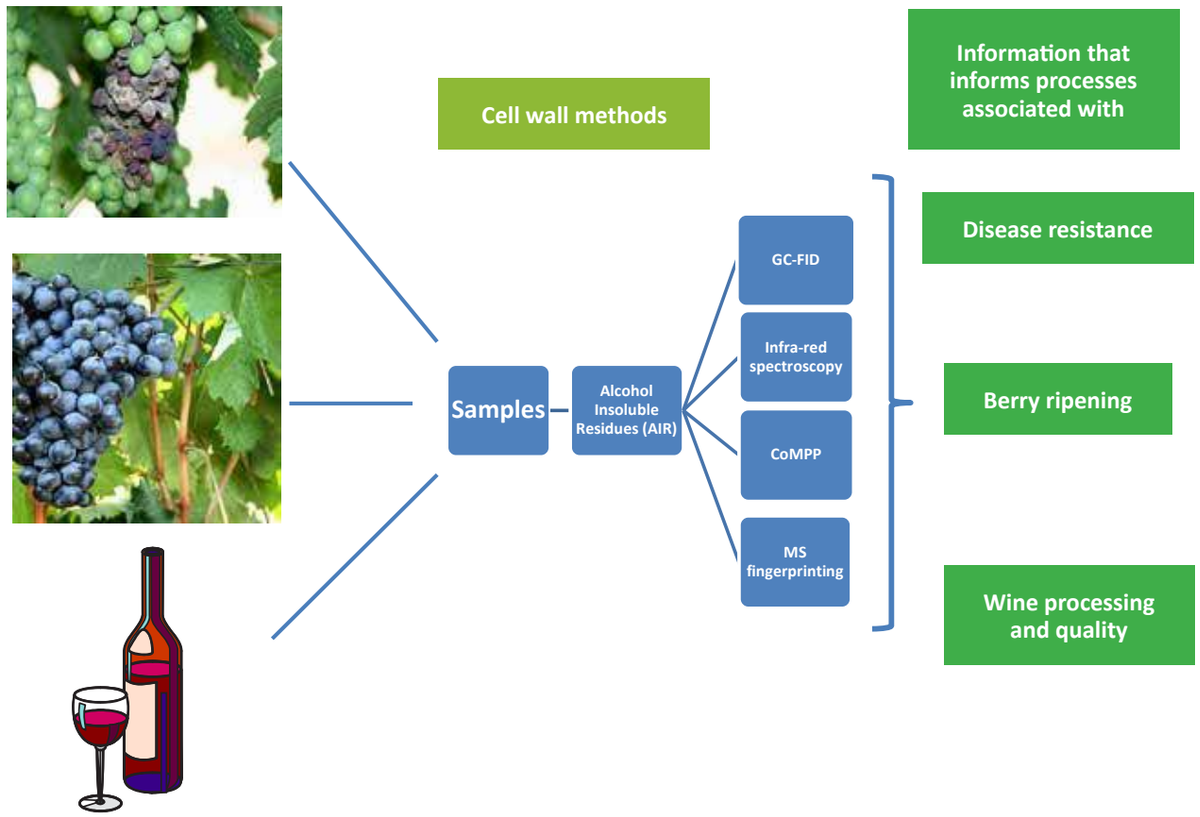

FIGURE 1. Grapevine (berry and leaf) cell walls are important role players in disease resistance, determining optimal ripeness and in wine processing and quality. These can be studied by using 'high-throughput' cell wall profiling methods (see Table 1 for advantages and disadvantages – photographs courtesy of Prof. Piet Goussard).

INTRODUCTION

Wine quality is strongly connected in various ways to the cell wall properties of the grapevine tissues. We have implemented methods to analytically profile cell wall components in tobacco (used for plant-pathogen studies) and grapevine (leaves and berries). These methods have been very useful in various industry-relevant research objectives. This article briefly describes the following:

- How these methods work.
- How they have been applied in fundamental studies in understanding grapevine disease resistance to fungal pathogens.
- How it is useful in developing ripening biomarkers and assessing progression of berry maturation in wine and table grapes.
- How these approaches provide useful information on the effectiveness of enzyme maceration approaches and the impact on final wine composition.

WHY IS STUDYING GRAPEVINE CELL WALLS IMPORTANT IN GRAPE AND WINE RESEARCH?

Plant cell walls are complex, interconnected structures of polysaccharides (pectin, cellulose and hemicellulose), cell wall proteins and polyphenols, and play an important role in many agricultural and industrial processes such as fruit ripening, plant-pathogen interactions and as substrates in bio-refineries (Albersheim, P. *et al*., 2010). In the grapevine, the cell walls of the leaves are the first line of defence against pathogenic attack (Gomès, E. & Coutos-Thévenot, P., 2009). Fungal diseases such as grey rot (*Botrytis cinerea*) and mildew (e.g. *Plasmopara viticola*) usually first infect the young leaves and then spread to the berries. These pathogens secrete enzymes that degrade the cell walls of these tissues in order to gain access to the vine nutrients. To understand the details of the pathogen attack and the disease resistance mechanisms of the plant which will enable us to develop effective disease preventative methods, we need knowledge on the composition and three-dimensional architecture of the grape berry and leaf cell walls.

On another level a portion of the proteins, polysaccharides and polyphenols from the cell walls of the grape berry end up in the wine (Ayestarán, B. *et al*., 2004). Arabinogalactan-proteins (AGPs) and rhamnogalacturonan-II (RG-II) polymers are the main grape-derived polysaccharides in wine (Doco, T. *et al*., 2003), but arabinans and homogalacturonans (HG) can also be present (Guadalupe, Z. *et al*., 2012). These polysaccharides together with the polyphenols such as hydroxycinnamic acids, anthocyanins and tannins contribute towards the mouthfeel, astringency, bitterness, aroma, colour and colour stabilisation, tannin aggregation and precipitation, protein haze formation and aroma retention of must and wine (Garrido, J. & Borges, F., 2013) and are therefore dominant role players in determining wine quality. At present the exact location and specific cell wall polymer interaction of every phenolic compound is unknown, but we do know that the proanthocyanidins are predominantly bound to the pectin polymers and to a lesser degree to the xyloglucan and cellulose (Ruiz-Garcia, Y. *et al*., 2014). Other polyphenols are found in the vacuole of the grape skin cells and thus require a disrupted cell wall to be released during the fermentation. Due to this relationship between the cell wall and polyphenols, it follows that the extractability of phenolic compounds during the wine fermentation depends on the recalcitrance of the cell wall, but also on the grape cultivar (low extraction from thick skinned cultivars, e.g. Mourvèdre), the degree of ripeness (higher extraction from riper grapes) and the winemaking practices used (skin contact and use of maceration enzymes) (González-Neves, G. *et al*., 2008; Hernández-Hierro, J.M. *et al*., 2014; Sacchi, K.L. *et al*., 2005). Given the crucial importance of grapevine-derived polysaccharides in

TABLE 1. Highlighting the major advantages and disadvantages of the 'high-throughput' cell wall profiling methods developed and applied in grape disease, ripening and winemaking studies.

Method	Benefit	Drawback
GC-based compositional tools (Nguema-Ona, E. *et al*., 2012)	Highly accurate and quantitative (requires 1-3 mg of AIR).	Requires degradation, time consuming and infers polymer presence.
CoMPP (Nguema-Ona, E. *et al*., 2013)	mAbs and CBMs are highly specific, direct confirmation of polysaccharides.	Semi-quantitative, sample intensive (30-40 mg of AIR).
FT-MIR spectroscopy Moore, J.P. *et al*., 2014; Zietsman, A.J.J. *et al*., 2015)	Rapid (less than 1 minute per scanned samples), many variables probed, non-destructive and non-invasive, 5-10 mg of AIR, identification of chemical functional groups.	Quantitative analysis is not ideal, identification of specific polymers present is not possible due to resonance overlaps.
Enzymatic oligosaccharide fingerprinting using mass spectrometry (Moore, J.P. *et al*., 2014; Zietsman, A.J.J. *et al*., 2015)	Excellent structural information due to high mass accuracy. Sample amount *ca.* 5 mg of AIR.	Enzyme digestion time consuming (12-16 hours), quantitation is not easily possible.

disease resistance and in the quality of the wine produced, it is therefore of critical value to develop methods to trace and assess these carbohydrate polymers in the vine and in the grape berry through maceration and fermentation to the final wine.

HOW DO WE STUDY GRAPEVINE (BERRY AND LEAF) CELL WALLS?

To analyse grape-derived cell wall polysaccharides we have developed and applied a combination of high-throughput techniques including monosaccharide compositional analysis, comprehensive microarray polymer profiling (CoMPP) analysis, FT-IR spectroscopy and oligosaccharide mass fingerprinting, as well as more in-depth approaches using chemical and enzymatic fractionation methods (Figure 1). Before cell walls can be analysed, it has to be extracted from the plant tissue which involves mechanical grinding and treatment with a series of organic solvents and the final alcohol insoluble residue (AIR) obtained, represents the cell walls. This material can now be used in various ways:

a) We can break it down with acids to see the different sugars (e.g. glucose etc.) that make up the polymers;

b) We can probe with monoclonal antibodies (mAbs) and carbohydrate binding modules (CBMs) to identify specific types of polysaccharides (e.g. pectins, the gummy 'sticky' stuff that clogs filters and presses during winemaking);

a) We can use infra-red (IR) spectroscopic tools with chemometric methods for a quick overall picture of the polymers; and

b) We can use more detailed analytical and fractionation methods to understand the more intricate complexity of the cell walls (e.g. using enzymes with liquid chromatography such as HPAEC or mass spectrometry).

A useful schematic of how we proceed with our studies is provided (Figure 1), as well as a table showing the benefits and drawbacks of the different methods (Table 1). Studying grape cell walls is like a scientific detective story; we get pieces of information from the different methods which we add together to form a more complete picture. This picture provides a hypothesis on certain cell wall characteristics which we are able to test under different conditions (e.g. disease studies; berry ripening progression and wine processing with enzymes). The results of our testing allow us to make more accurate predictions and develop a new understanding which can be used by the industry to implement better practises in the future. In the last section we describe how these methods have been put to good use in helping to understand industry-relevant grape and wine problems and questions.

DECIPHERING THE GRAPEVINE CELL WALL

The methods described have been optimised and used extensively over the last five years at the IWBT. In the first study[11] we confirmed the accuracy of these profiling methods for determining the polysaccharide composition of tobacco leave cell walls (tobacco is the model plant on which to perform plant-pathogen studies) and that it can be used as a high-throughput screening method. Enzymatic oligosaccharide fingerprinting methods were used to determine the specific structure and diversity of the arabinoxyloglucan species found in tobacco. A follow-up study (Nguema-Ona, E. *et al*., 2013) investigated how the wall-associated grapevine gene VvPGIP1 (Polygalacturonase-Inhibiting Protein) which can protect plants against fungal infection, changed the cellulose-xyloglucan network when expressed in tobacco. This gave a new understanding on disease resistance mechanisms. Another study (Moore, J.P. *et al*., 2014) established the baseline cell wall profiles for grapevine leaves which is necessary for further investigations on grapevine disease resistance. We did extensive studies on the changes happening in the grape berry cell wall (Crimson Seedless and Cabernet Sauvignon) during ripening (Moore, J.P. *et al*., 2014) and specific cell wall polymers were identified

that can serve as ripening biomarkers. Cell wall ripening changes were also part of the topic of another study on Pinotage grapes (Zietsman, A.J.J. *et al*., 2015), but the focus was on the Pinotage berry skin and the cell wall dynamics due to maceration in the presence and absence of maceration enzymes. De-pectination and cell wall unravelling due to enzyme treatment were observed and the impact of the grape ripeness was also demonstrated. Our most recent study investigated the polysaccharides released into the wine, as well as those left over in the pressed Cabernet Sauvignon grape cell walls (Gao, Y. *et al*., 2015) and suggested the existence of an enzyme-resistant pectin layer that shields the xyloglucan network in the skin cells. This has important implications for understanding the interaction between the polysaccharide degrading enzymes and the grape tissues.

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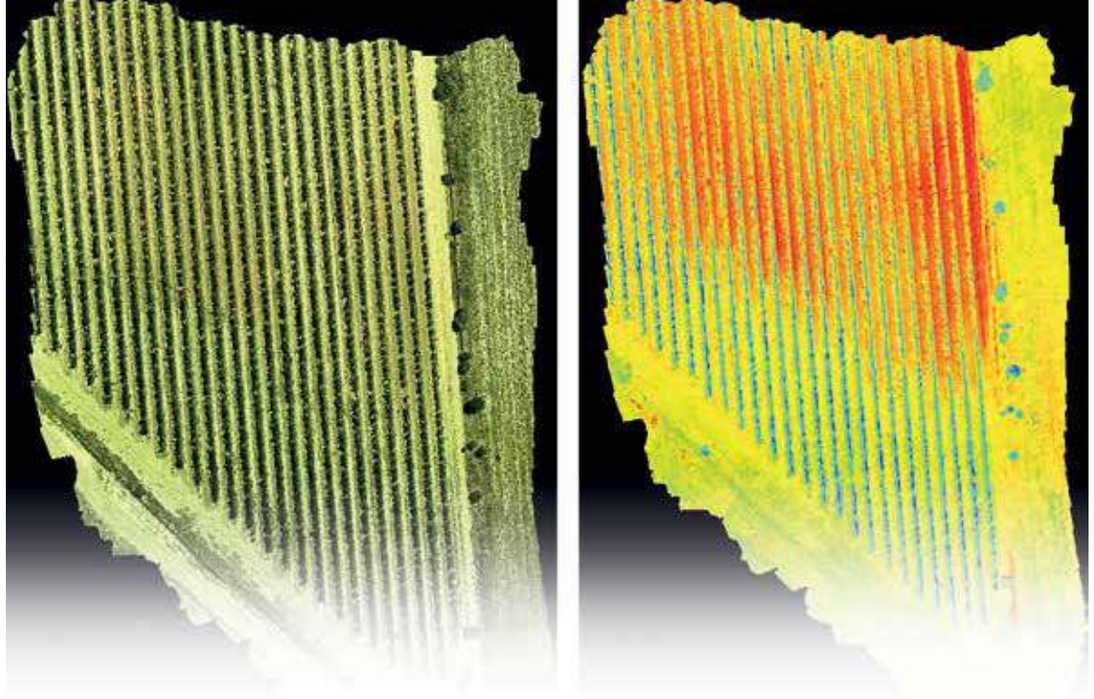

Multispectral image calibration

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KEYWORDS: Remote sensing, multispectral, grapevine.

Multispectral remote sensing has been in use in the South African wine industry for more than 10 years, with applications ranging from segmented or sequential harvesting, to adapting future block layout, amongst other applications. Its utilisation as a research tool has also been explored in the form of finalised Winetech/THRIP funded projects, as well as a currently running project. In this article it is discussed how the difficult challenge of calibrating imaging to the often diverse environment of the grapevine can be approached, using imaging along with single-vine measurements of vigour.

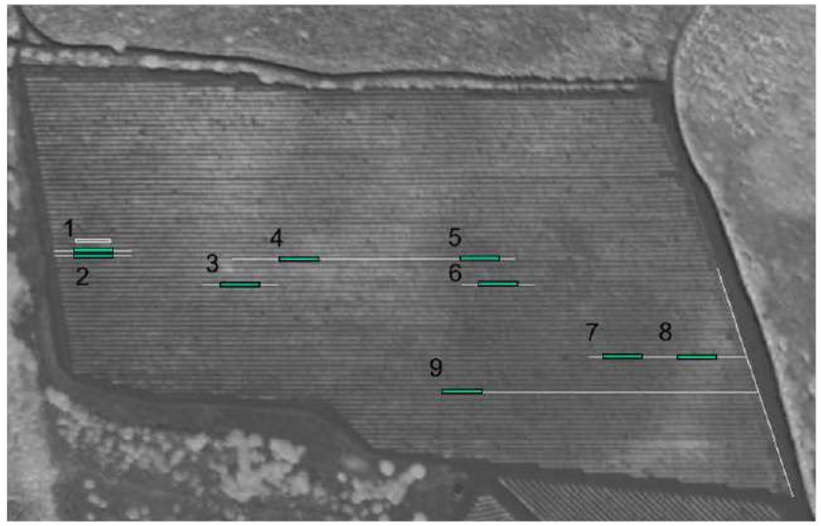
One key difficulty in working with non-calibrated images, or images from different service providers/imaging platforms is to be able to compare images over differing seasons. One approach is to use relatively simple measurable grapevine parameters, such as dormant cane mass.

Results from three studies will be presented here, one aimed at investigating the correlation of within-vineyard field measurements conducted in different areas at different times with Normalised Difference Vegetation Index (NDVI) image pixel values extracted from measured vines. Pruning mass is evaluated temporally and spatially against NDVI image pixel values.

Results from three studies in the Stellenbosch wine growing region are presented where high resolution multispectral images were collected over different growing seasons from 2005 to 2007, along with field measurements at selected mini-plots consisting of 10 to 12 vines each. After creation of NDVI images, pixel values were extracted for these plots, coinciding with differing vigour levels in the vineyard, and compared to pruning and yield data, as well as other selected parameters for the same plots. A simple mask-scan method of image pixel value extraction was used from open source software (Image J, NIH, Bethesda, Maryland, USA) (Figure 1). Note that the NDVI pixel values correspond to ratios of a maximum pixel value of 255 in the 8-bit greyscale NDVI image.

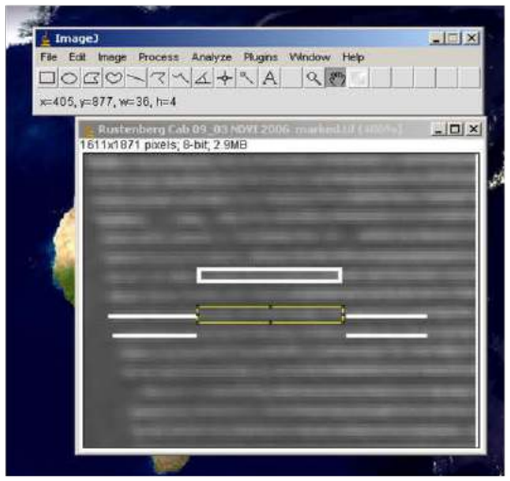
The correlation between the mean plot pruning mass and mean NDVI pixel values over two consecutive seasons showed a higher R^2 value (strength of correlation) in the first season (Figure 2), which shows that a season (or vineyard) where a smaller range of pruning mass values occur, would lead to more difficulty for the NDVI index to differentiate pruning mass differences. Another challenge is shown in the data, namely that for these two years the vineyard's main vigour classes' pruning mass was relatively similar, but the NDVI values were different over years

1



Greyscale (8bit) NDVI image of a vineyard indicating plot layout

2



Extraction of pixel values on measurement plot consisting of 12 vines aided by Image J open source software.

3

Average NDVI pixel value = 93.0

- Steps 1 to 3 were repeated for each plot

FIGURE 1. Steps to extract plot mean pixel values for field data comparison.

for the same plots (Figure 3). This highlights the need to calibrate NDVI values over years with vineyard reaction.

The offset in NDVI values between the two years were corrected by normalising the NDVI values to the 2005 season (correcting offset) and this was used to predict the 2006 mean pruning mass from the normalised NDVI values from 2005 using the linear equation, as an example showing that with these corrections predictions can even be performed over seasons (Figure 4). Creating predictions of pruning mass from NDVI values can yield very well classified images "tuned to the vineyard" and this approach is recommended over "artificial" classification systems which yields arbitrary classes. A viticulturist can respond much better to statements like: "The low vigour area of vineyard block A has a pruning mass of approximately 0.6 to 0.8 kg per grapevine" than: "The low vigour area has a mean NDVI value of 0.6 to 0.8". Pruning mass values can be related to indices, such as the Ravaz index (yield to pruning mass ratio), which can then be used to make spatial bud load adaptations in these zones.

In another example from a more variable vineyard, with less suspected cover crop/weed influence on the pixel classification, the correlation between plot pruning mass and NDVI was even better ($R^2 = 0.99$) (Figure 6). This yielded an excellent improvement over the raw NDVI classification of the image, with the result shown in Figure 7.

CONCLUSION

Updated and automated image calibration software can be developed to improve image classification and to ensure stability of image observations between different seasons and between different vineyards. It was clear in this investigation that a better approach is to calibrate images using field data, but in order for this to happen, producers need to establish plots where pruning mass and/or other parameters can be measured consistently over different growing seasons. Furthermore remote sensing service providers (independent of the platform) need to ensure that their software has the capability to incorporate grapevine measurements into a calibration algorithm for the data to be useful and "tangible" to the producer. Further work was also done to evaluate the possibility to extract single-vine pixel information in a similar approach to the mini-plot extraction, and to automate the method using software algorithms (Smit *et al.*, 2010).

ACKNOWLEDGEMENTS

Conrad Schutte, Zelmari Coetzee, Dr Victoria Carey, Dr Julian Smit, THRIP and Winetech for funding of the projects.

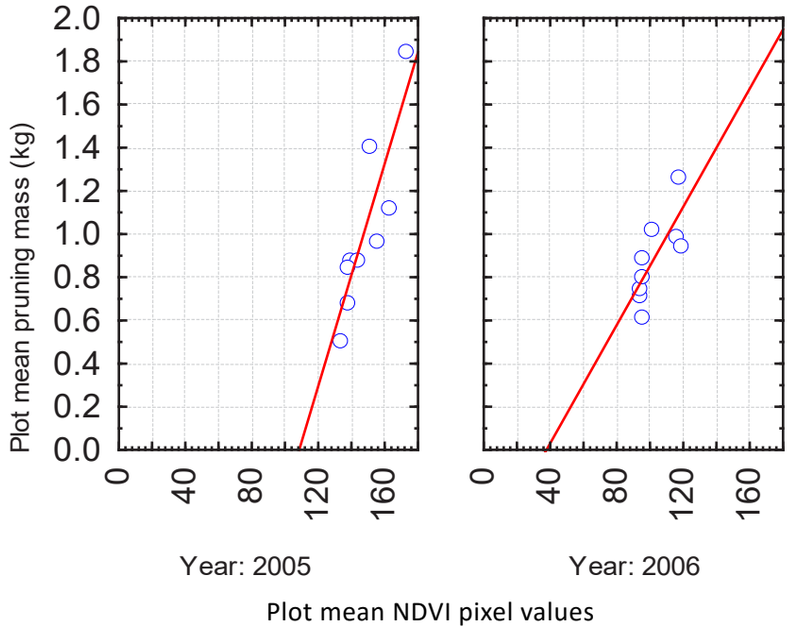


FIGURE 2. Correlation between plot average pruning mass (kg) and plot average NDVI over two consecutive years, 2005 R^2 = 0.74 and 2006 R^2 = 0.58.

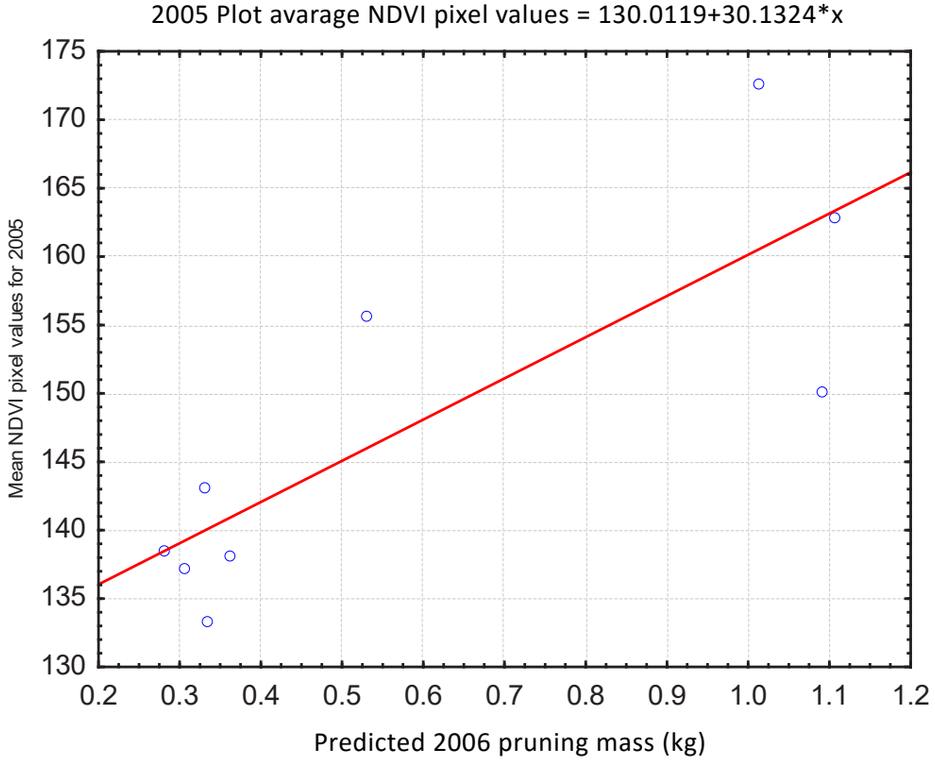


FIGURE 4. Correlation between 2005 plot average NDVI pixel values and predicted 2006 pruning mass (kg) (R^2 = 0.67).

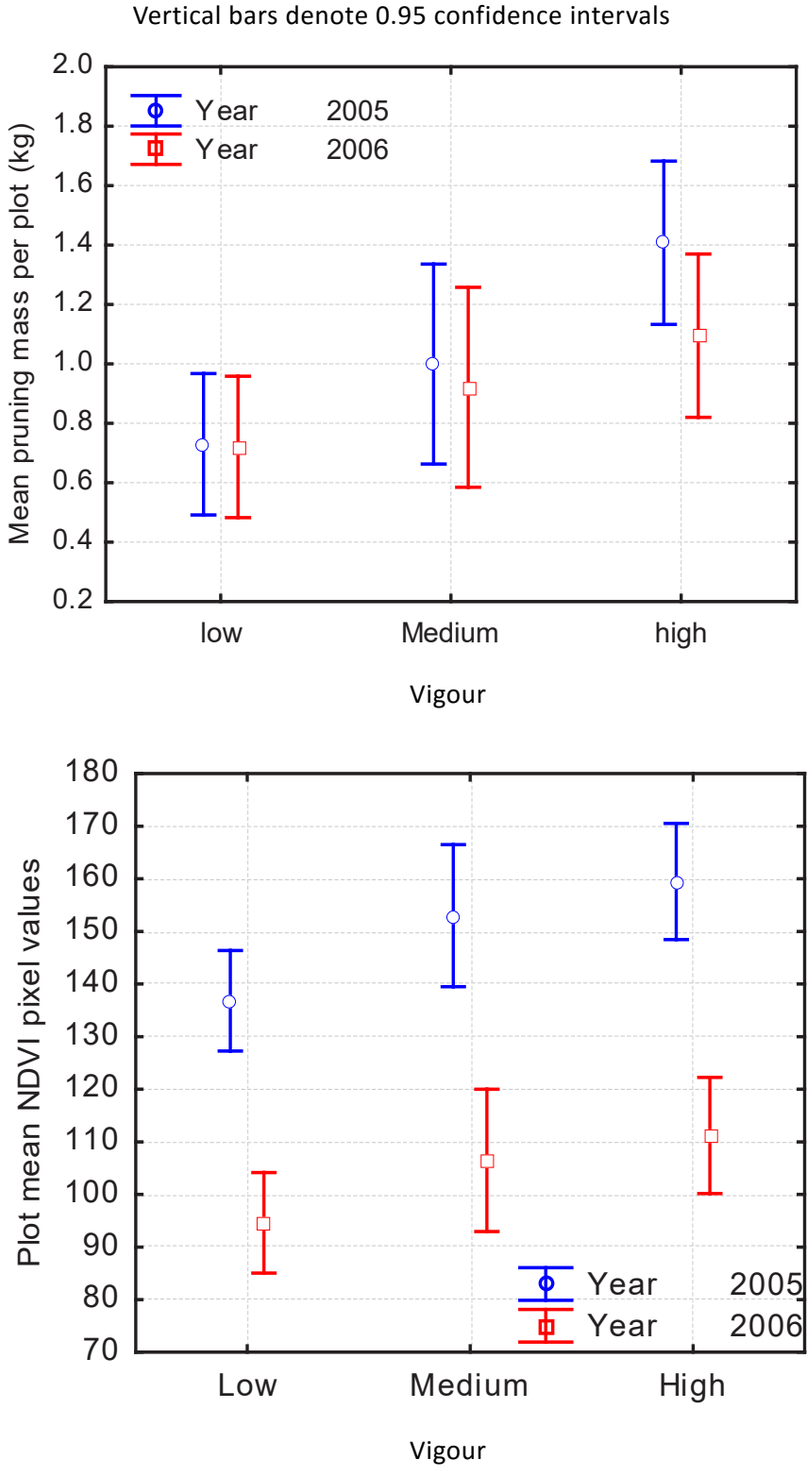


FIGURE 3. Mean pruning mass per plot (left), as well as plot mean NDVI values (right), for different vigour level plots over two consecutive seasons.

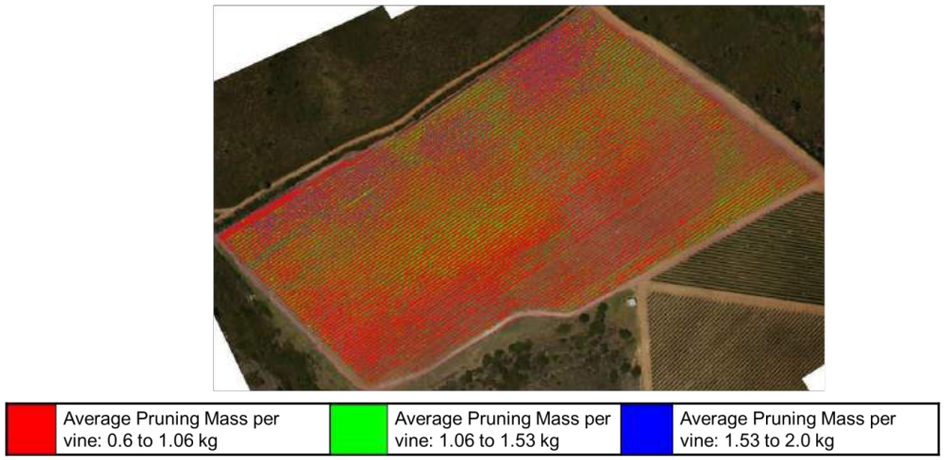


FIGURE 5. Multispectral NDVI image classified to three pruning mass levels determined from pruning mass values predicted from NDVI:pruning mass linear regression equations.

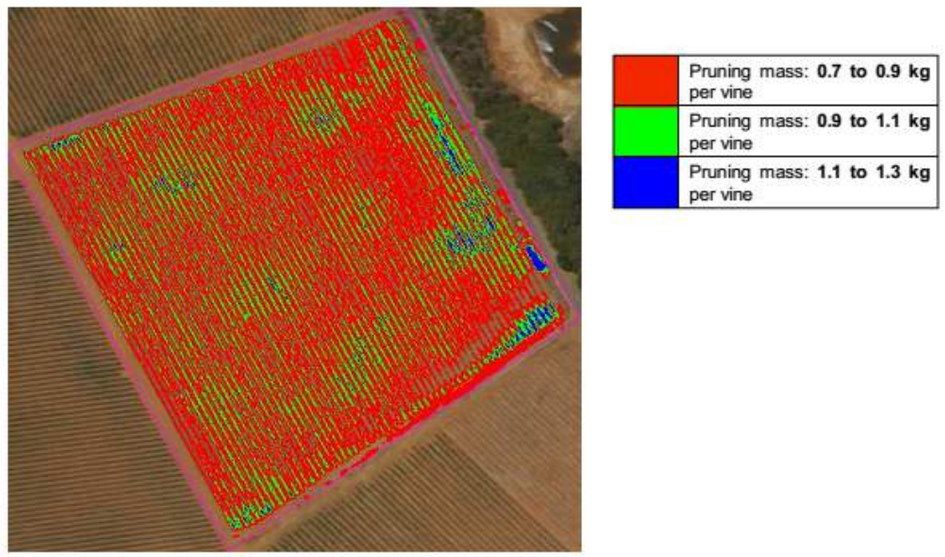


FIGURE 7. Multispectral NDVI image classified to three pruning mass levels determined from pruning mass values predicted from NDVI:pruning mass linear regression equations.

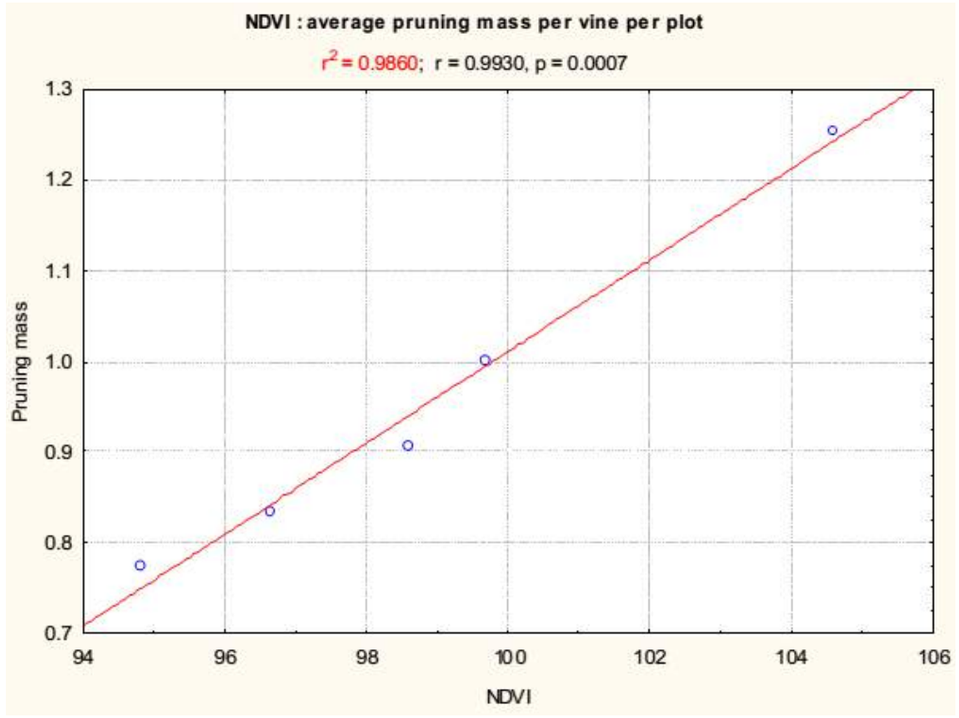


FIGURE 6. Relationship between mean pruning mass per grapevine in the mini-plots (kg) and the mean NDVI values for the mini-plots in the 2006 growing season for a Stellenbosch vineyard.

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Functions of volatile terpenes in grapevines

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KEYWORDS: Volatile terpene, grapevine.

At some point between the 7th and 4th millennia BC in the Caucasus region (spanning modern day Iran, Turkey and Georgia), our ancestors unknowingly began the process of grapevine selection that would lead to the eventual domestication of this crop species (Terral *et al.*, 2010). During the domestication process; selection was based on observable phenotypes within the naturally occurring ("wild") populations, and potentially included: size/yield, survival/resistance to stresses (biotic and/or abiotic), flavour/aroma (taste), colour, and any combination of a number of quality-associated attributes. The >6 000 years of selection have culminated in the diversity of germplasm we currently have for grapevines, estimated at between 5 000 and 8 000 accessions (Moreno-Sanz *et al.*, 2008). If only grape berry colour is considered as a trait; the degree of diversity present within cultivated grapevines is visually evident with a spectrum of shades ranging from white and greys, through pinks and reds to black. In the current genomic era, these germplasm collections, and specifically the genetic basis (mutations) of these varietal differences, provides valuable tools for plant breeders to improve grapevines for a variety of prospective targets.

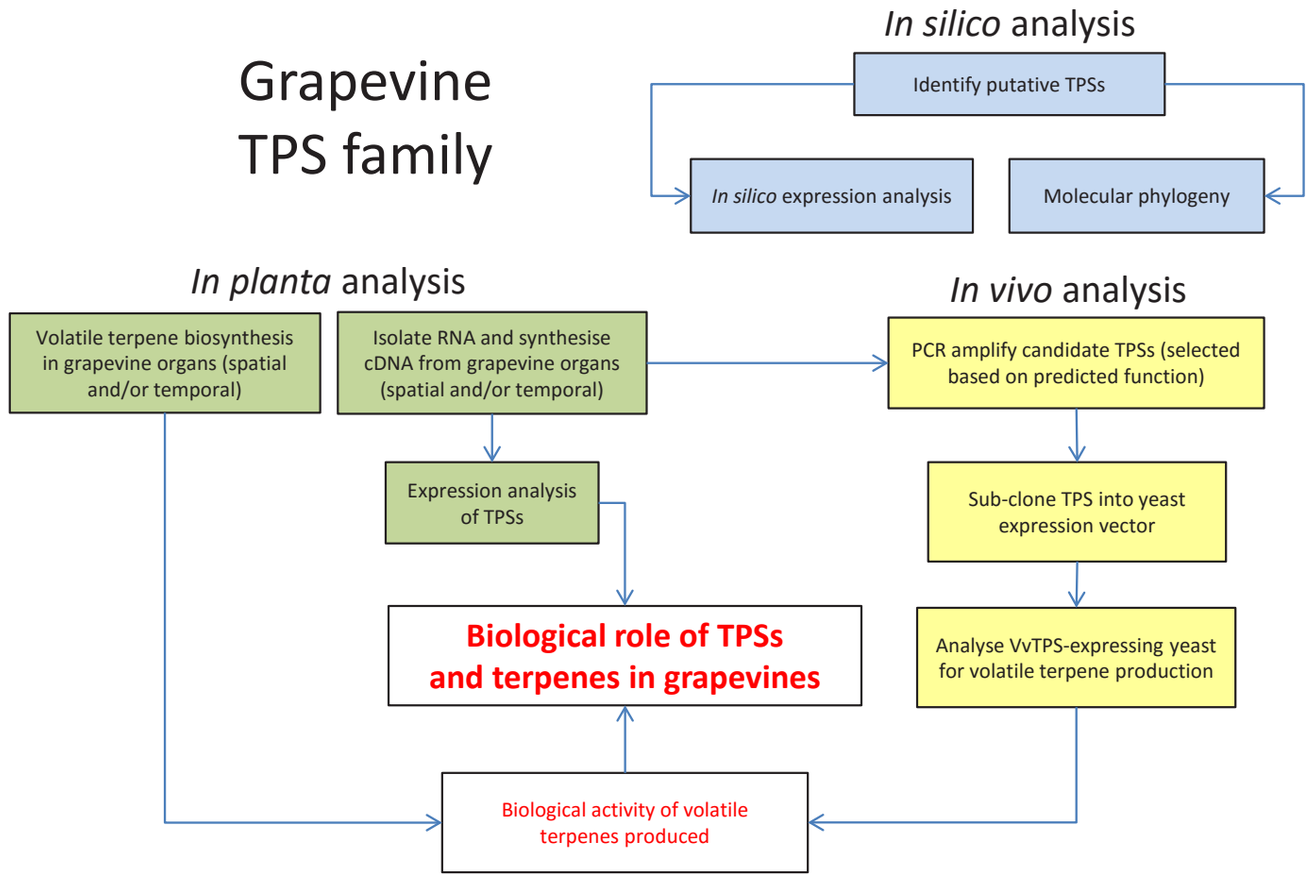

FIGURE 1. Discovery and analytical pipeline for the study of terpene synthases and the volatile terpenes in grapevine.

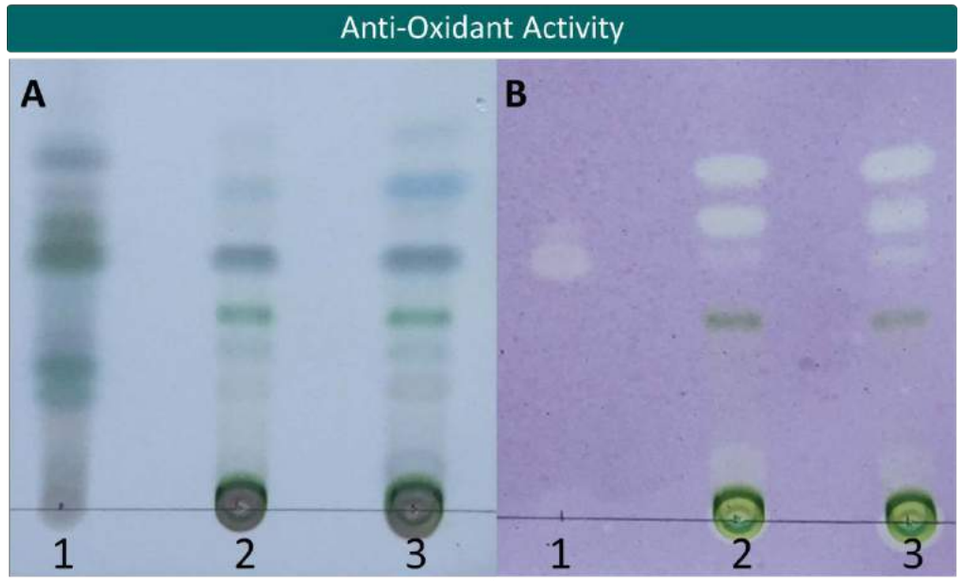

FIGURE 2. Presence and potential biological activity of terpenes from grape flowers. (A) Vanillin-stained terpenes in grapevine flowers (stained coloured bands indicative of terpenic compounds); (B) DPPH assay for antioxidant activity (pale yellow zones indicative of anti-oxidant activity). (1) Authentic standard of the sesquiterpenoid valencene; (2) Terpene extracts from Shiraz; and (3) Sauvignon Blanc flowers.

Terpenes are the collective term for a large group of chemical compounds that all share a common C_5-precursor, isopentenyl diphosphate, yet perform diverse biological functions that range from light-harvesting for photosynthesis to herbivory defence. Some terpenes are essential for plants (e.g. the carotenoids in light-harvesting and photoprotection, and the phytohormones in growth and development); whereas others are referred to as secondary metabolites and are involved in "specialised metabolism" and are not essential for the plant, but typically provide some or other survival and/or fitness benefit.

Due to their characteristic properties, volatile terpenes have a long association with humans as flavours, fragrances, dyes, cosmetics, insecticides and numerous therapeutic applications. The presence of volatile C_{10}-monoterpenes, C_{13}-norisoprenoids and C_{15}-sesquiterpenes are responsible for the characteristic flavours, aromas and scents associated with a number of plant species. These compounds are dominant in many plants, e.g. lemons (limonene), eucalyptus (eucalyptol), pine (pinene), mint (menthol), geranium (geraniol) or roses (ß-damascenone). Specifically in grapes and wine, these volatile monoterpenes contribute predominantly to the floral aromas (e.g. geranium, rose, lily, lavender and citrus blossom). Terpenes can be present in the free (volatile) form or stored as non-volatile, water-soluble glycosidically (sugar) bound conjugates. Monoterpenes are prevalent in the varietal character of the Muscats (e.g. Muscat d'Alexandrie and Morio Muscat), but can also contribute to that of Weisser Riesling, Bukettraube and Gewürztraminer. Sesquiterpenes are less common with rotundone being a well-known example with a very low odour threshold (16 ng/ℓ in wine), and is responsible for the peppery aroma found in, e.g. Shiraz grapes and wine (and pepper). Wine is, however, a complex blend of thousands of metabolites that can originate from the grape (e.g. sugars, organic acids and phenols) or as products of the winemaking process (via the fermentation and/or bioconversions during fermentation and/or ageing). The ultimate wine flavour and aroma are therefore determined by the complex assortment of literally hundreds of metabolites with both the absolute concentration and/or ratios of specific metabolites playing a role in the ultimate perceived flavour and aroma.

The most abundant terpenes in Muscat and other aromatic wines are linalool, geraniol, nerol, α-terpineol and hotrienol (Marais, 1983). The aroma thresholds of these compounds are low (ng/ℓ to µg/ℓ range), and their abundance can vary with several orders of magnitude. From a wine quality perspective the volatile terpenes are important contributors to the overall bouquet of wines, but the actual biological function of these compounds in grapevines is poorly understood. Numerous studies in other plant species have demonstrated that volatile organic compounds (e.g. terpenes and green leaf volatiles (i.e. C_6-hexanols) play an important role in plant defence. Volatiles can perform various functions in defence, e.g. they can directly act as repellents to insects and other herbivores, or indirectly as attractants to predators of herbivores, or act as volatile signals where they prime defence responses against pathogens in adjacent leaves and neighbouring plants.

The completion of a grapevine (cv. Pinot noir) genome sequence provided researchers with a molecular snapshot of the full genetic potential of grapevine. The grapevine genome represented the fourth plant-, and the first woody fruit-bearing plant genome sequenced, highlighting the importance of the grapevine to many of the world's economies. Numerous interesting findings have been reported in the literature that fall outside of the scope of this article, but with specific reference to terpenes it was found that grapevines possess one of the largest terpene synthase (TPS) families of all the sequenced plant genomes (second only to that of Eucalyptus): ~89 genomic loci were identified that encoded for putative TPSs (where a TPS is the enzyme that catalyses the conversion of a prenyl phosphate precursor (i.e. geranyl diphosphate and farnesyl diphosphate) to a mono- or sesquiterpene volatile product, respectively. Functional characterisation of a number of the identified TPSs and their volatile products highlighted the complexity: a single TPS enzyme could potentially form multiple volatile terpenes, and the volatile terpenes produced are subsequently susceptible to further rearrangements (both enzymatic and non-enzymatic) to produce numerous potential terpenic products (from a single precursor).

With its large over-represented TPS family; it is surprising that relatively little is still known on the role of terpenes in grapevines. A number of grapevine studies have, however, contributed to our current understanding. A study by Martin *et al.* (2009) showed that sesquiterpene synthesis was predominantly localised to the anthers and pollen in Cabernet Sauvignon flowers and emission peaked before bloom. This production of volatiles in the male flower parts, and the timing (before bloom) is biologically unique for floral scent production: most plant species form volatile terpenes during bloom and then typically not in the male flower parts. Volatiles can act as attractants for pollinators and complex mixtures have co-evolved to attract specific agents. The domesticated grapevine, unlike its cross-pollinating wild relatives, is self-pollinating and therefore does not require pollinators for fertilisation. Tasin *et al.* (2006) showed that the female grapevine moth (*Lobesia botrana*) was attracted by a ratio-specific blend of three terpenoids: β-caryophyllene, β-farnesene and dimethyl-nonatriene (DMNT) for oviposition (egg-laying). The authors speculated that the ratio was required for discrimination between host and non-host plants, which is essential for maintenance of associations between insects and suitable plant hosts. It has also been demonstrated that methyl jasmonate elicits the production of monoterpenes and sesquiterpenes in grapevine leaves (Hampel *et al.*, 2005) and in grape cell cultures (D'Onofrio *et al.*, 2009). Jasmonates are well-studied elicitors of the defence response in plants. A study by Lawo *et al.* (2011) on the volatiles emitted

by rootstock Teleki 5C (a Hungarian interspecific cross of *Vitis vinifera* x *V. riparia*) roots following phylloxera (*Daktulospaira vitifaliae* Fitch) infection; showed that the infested roots released significantly more volatiles than the uninfested roots. Interestingly, 25% of the volatile metabolites significantly upregulated in the infested roots were terpenes. Interestingly, ß-caryophyllene (a sesquiterpene) was exclusively formed in the infested roots and was not detected in the control roots. Teleki 5C has moderate resistance to nematode feeding, and good resistance to phylloxera. From the preceding it is clear that terpenes occur in complex, structurally diverse mixtures and fulfil various ecological roles.

With ~89 potential grapevine TPSs, it becomes particularly challenging to correlate a specific TPS-encoding gene (or genomic locus) to the production of a specific volatile terpene.

Our research focusses on a number of fundamental biological questions:

- Where and when are TPSs formed?
- Which TPS(s) produce which volatile terpene(s)?
- How do grapevine cultivars differ in their TPSs and volatile terpene profiles?; and
- Why does a grapevine produce volatile terpenes (biological activity/ significance)?

These questions are addressed by isolating and characterising candidate TPSs in an optimised heterologous yeast system. Yeast do not naturally produce any endogenous terpenes and, therefore, provide a suitable null background for these studies. Heterologous expression of a TPS in yeast results in the *de novo* production of volatile terpenes that can be analysed by gas chromatography (GC) mass spectra (MS). The volatile terpene(s) produced are used to assign a function(s) to the isolated TPS and, by extension, the respective genomic locus. This locus is then a potential functional marker for future breeding/selection. The volatile terpene(s) produced by the respective TPS are further analysed to elucidate their possible activity (e.g. against reactive oxygen species (ROS), pathogen) in order to determine the *in planta* function of the volatile terpenes.

Using the described pipeline (Figure 1), three TPS-encoding genes (*VvTPS3*, *VvTPS12* and *VvTPS15*) have been isolated from grapevine cDNA, and successfully expressed in yeast. The recombinant yeasts were shown to produce predominantly sabinene (TPS3), Germacrene D (TPS12) and eucalyptol (TPS15), respectively. Sabinene is a monoterpene ($C_{10}H_{16}$) and is one of the compounds that contribute to the spiciness of, e.g. black pepper; Germacrene D is a sesquiterpene ($C_{15}H_{24}$) with antimicrobial and anti-insecticidal activity, and eucalyptol (1,8-cineole) is a monoterpene ($C_{10}H_{18}O$) and its scent is described as fresh/camphor-like. These volatile terpenes formed are currently being tested for their biological activity (Figure 2). Furthermore, additional TPSs are being identified and isolated from a number of commercially relevant wine grape cultivars for functional analysis. Sequence data and the functional characterisation provide potential varietal-specific differences that contribute to the typicity of grapevine cultivars. Linking TPS sequences and expression of specific TPS-encoding genes to the production of volatile terpenes in different grapevine organs (spatial and/or temporal) or in response to viticultural manipulations is required to understand, and ultimately exploit, these versatile biological compounds.

SUMMARY

The terpenic composition of grape berries contributes to the varietal character of the grape and subsequent wine. The biosynthesis of terpenes is catalysed by terpene synthase enzymes. The recent grapevine genome sequence revealed a large (>89) terpene synthases family, making the study of this family particularly challenging. Understanding the basis of the varietal aromas of cultivars is still an active field of study, as is the impact of production practices in modulating the aromatic potential of the berries. Although the functioning of the enzymes are understood mechanistically, the relationship between the enzymes (presence and activity) and the origin (and fate) of terpenes in the complex grape matrix is still unknown. The availability of the grapevine genome sequence and the molecular tools developed in grapevine studies (i.e. genomics, transcriptomics and metabolomics) make it possible to identify, isolate and study individual terpene synthase-encoding genes. A specific terpene synthase encoding gene can then be linked to the production of a specific terpenic product and the derived functional information, together with gene expression data, can be used to evaluate the effect of, e.g. a stress and/or viticultural treatment to the metabolism of terpenes in grapes to understand, and ultimately exploit, these versatile biological compounds.

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Merlot responses to irrigation

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KEYWORDS: Growth, low frequency drip, PRD, quality, yield.

INTRODUCTION

Irrigation may be used for manipulating growth and yield to obtain specific wine quality objectives. In most grape growing regions, water resources are limited. Therefore, irrigation must be applied judiciously. This implies that the least amount of water should be applied to obtain a specific wine quality objective. In this regard, various aspects play an important role. Drip irrigation, for example, requires less irrigation water than micro-sprinklers to obtain comparable growth, yield and wine quality. Another approach that could improve irrigation water use efficiency (WUE), expressed as kg grapes per m^3 water, is partial root zone drying (PRD). With this approach, one half of the root system is well-watered, while the other half is allowed to dry out. After approximately 14 days, irrigation is switched to the dry side, while the wet side is allowed to dry out. By repeating this switching throughout the growing season, approximately 50% water may be saved without compromising yield and wine quality. The objective of the study was to compare low frequency drip irrigation (less than 10 irrigations per season) and PRD to dryland (rain fed) cultivation.

EXPERIMENTAL BACKGROUND

The field experiment was carried out with Merlot/99R near Wellington in the Coastal Region during the 2004/05 and 2005/06 seasons. Six irrigation treatments were applied as indicated in Table 1. In the case of the three irrigations per season, irrigations were applied (i) at pea size berries, (ii) *véraison* and (iii) post-harvest.

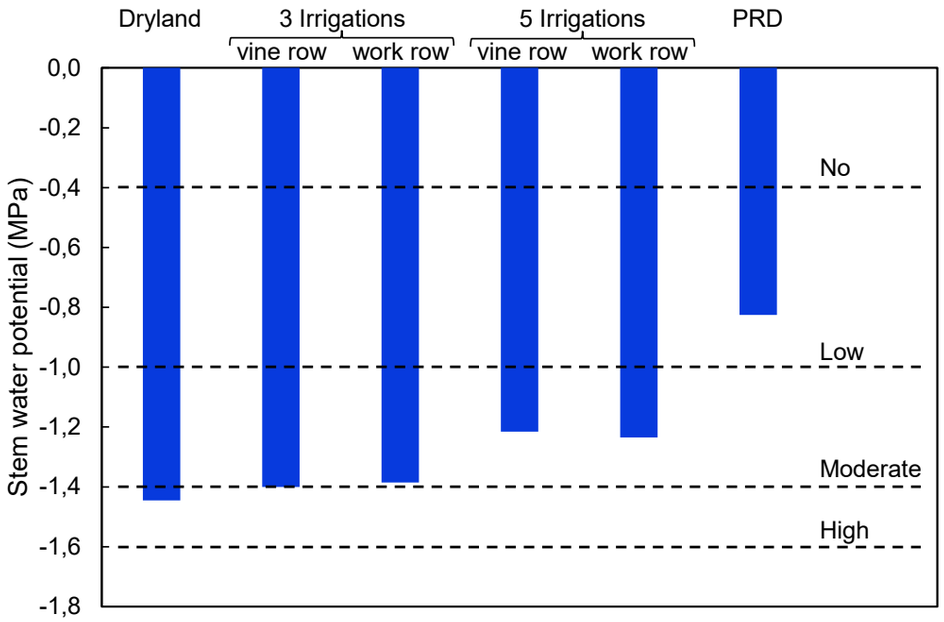

FIGURE 1. Effect of irrigation on midday stem water potential in Merlot/99R during ripening near Wellington. Dashed lines indicate thresholds of water constraint classes for Merlot.

For the five irrigations per season, irrigations were applied (i) at pea size berries, (ii) between pea size berries and *véraison*, (iii) at *véraison*, (iv) during ripening and (v) post-harvest. These two groups of low frequency irrigations were either applied on the grapevine rows or subsurface in the middle of the work rows. In the case of the PRD approach, dripper lines were also installed subsurface in the middle of the work rows. Irrigation was applied in alternating rows. After approximately 14 days, irrigation was switched to the dry work rows. One to two irrigations per week were required to keep the soil moist. The irrigation switching between alternating work rows was applied from pea size berries until harvest. Each irrigation treatment was replicated four times. Irrigation volumes applied and measured grapevine responses are listed in Table 1 and Figure 1. For more details of the experiment layout and measurements, refer to Myburgh (2011a & b).

RESULTS AND DISCUSSION

PLANT WATER CONSTRAINTS

According to stem water potential measured around 13:00 by means of a pressure chamber, the dryland grapevines experienced high water constraints during berry ripening (Fig. 1). Water constraints only tended to be lower where three irrigations were applied compared to dryland cultivation. Where five irrigations were applied, grapevines experienced moderate water constraints. Subsurface irrigation in the middle of the work rows did not affect grapevine water status, irrespective of the number of irrigations. Grapevines irrigated according to the PRD approach experienced only low water constraints (Fig. 1).

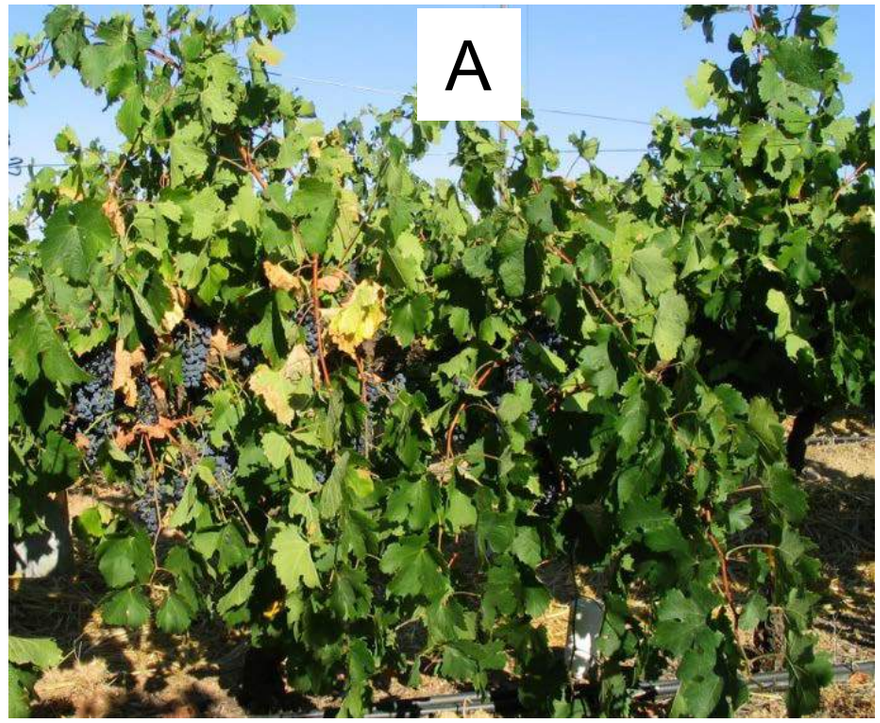

FIGURE 2. Effect of (A) dryland cultivation and (B) PRD irrigation on the foliage of Merlot/99R during berry ripening near Wellington.

TABLE 1. Effect of number of irrigations, position of dripper lines and partial root zone drying (PRD) on the performance of Merlot/99R near Wellington. Water use efficiency (WUE) is grapes produced per unit irrigation water. Results are means of two seasons.

Measured variable	Dryland	Three irrigations		Five irrigations		PRD
		Vine row	Work row	Vine row	Work row	
Irrigation (mm)	0	96	96	132	132	215
Cane mass (t/ha)	1.1	1.2	1.3	1.5	1.4	1.5
Berry mass (g)	1.0	1.1	1.1	1.2	1.2	1.4
Bunch mass (g)	73	93	87	105	103	116
Yield (t/ha)	8.5	10.2	10.6	12.6	11.9	13.7
WUE (kg/m^3)	n/a	10.5	10.9	9.6	9.1	6.7
Juice sugar (°B)	25.2	25.7	25.8	25.4	25.1	24.2
Juice acidity (g/ℓ)	6.3	6.2	6.2	6.0	6.0	5.6
Juice pH	3.29	3.33	3.34	3.33	3.30	3.32
Wine colour (%)	80	76	78	70	74	64
Berry character (%)	54	56	59	51	50	48
Wine quality (%)	58	57	63	50	50	45

SHOOT GROWTH

Cane mass at pruning increased with the number of conventional drip irrigations compared to dryland cultivation (Table 1). During berry ripening, leaves of the dryland grapevines showed yellowing (Fig. 2A). This typical response to water constraints was not observed in the irrigated grapevines. Irrigation in the work rows did not affect growth vigour, irrespective of the number of irrigations. During berry ripening, limited active shoot growth tips occurred on the PRD grapevines (Fig. 2B). Despite this trend, the PRD approach did not increase cane mass compared to five irrigations. In another study, the PRD approach had a similar effect on cane mass of Merlot in the Breede River Valley (Lategan & Howell, 2010a). The results confirmed that PRD can restrict shoot growth in spite of higher irrigation volumes.

YIELD

At harvest, berry mass increased with the number of irrigations compared to dryland cultivation (Table 1). During berry ripening, berries on the dryland grapevines showed crimping and shrivelling. Irrigation in the work rows did not have any effect on berry mass, irrespective of the number of irrigations. In the case of PRD, more irrigation increased berry mass compared to five irrigations. The effect of the irrigations on berry mass clearly reflected in bunch mass and yield (Table 1). Yield of Merlot in the Breede River Valley also declined as water constraints increased (Lategan & Howell, 2010b).

WATER USE EFFICIENCY

The WUE decreased as the irrigation volumes increased (Table 1). The WUE of the PRD grapevines was the lowest. Likewise, PRD did not improve WUE of Merlot in the Breede River Valley (Lategan & Howell, 2010b). These results confirmed that PRD will not necessarily improve WUE compared to conventional, single line drip irrigation. In many of the previous PRD experiments, the control grapevines were also irrigated by means of double dripper lines. Consequently, the control grapevines received double the volume irrigation compared to PRD, where irrigation was only applied via alternating dripper lines. Due to the biased comparison, WUE of the PRD approach was substantially higher than the other irrigated treatments.

SUGAR, ACIDITY AND PH

The objective was to pick the grapes at approximately 25°B (Table 1). In the case of PRD, berry ripening was slower compared to the other treatments. Consequently, the PRD grapes had to be picked at 24°B to reduce the risk of yield losses due to rot. The total titratable acidity decreased with the volume of irrigation water (Table 1). In general, irrigation tended to increase juice pH compared to dryland cultivation. This could possibly be due to more leaves being shaded as irrigation increased grapevine vigour, which resulted in lower total titratable acidity and higher pH (Iland, 1989).

WINE SENSORIAL CHARACTERISTICS

In comparison to dryland cultivation, sensorial wine colour decreased with the number of irrigations, irrespective of where the water was applied (Table 1). The high irrigation volumes, which induced the low water constraints in the PRD grapevines, resulted in the poorest wine colour. More prominent berry character was obtained with dryland cultivation and three irrigations compared to five irrigations and PRD. The PRD approach produced the poorest overall wine quality (Table 1). The combination of better colour and more prominent berry character

caused overall wine quality to be better where grapevines experienced higher water constraints. Previous studies in the Breede River Valley (Lategan & Howell, 2010b) and the Lower Olifants River Region (Myburgh, 2011c) also showed that higher water constraints improved Merlot wine quality. For more detailed results and discussion refer to Myburgh (2011a & b).

CONCLUSIONS AND RECOMMENDATIONS

Under the prevailing conditions, Merlot grape yield responded positively to increasing volumes of irrigation water compared to dryland cultivation. In contrast, less irrigation increased grapevine water constraints, sensorial colour, berry character and overall wine quality. If high wine quality is the objective, and to ensure higher yields compared to dryland cultivation, up to three irrigations can be applied. If yield is the primary objective, Merlot should be irrigated when the midday stem water potential reaches -1 MPa to maintain a balance between yield and wine quality. The low WUE and poor wine quality obtained with the PRD approach clearly did not meet the expectations. Therefore, this practice cannot be recommended for irrigation of wine grape vineyards.

SUMMARY

The effect of low frequency drip irrigation and PRD (partial root zone drying) on grapevine responses were compared to dryland cultivation, i.e. rain fed, in a warm climate. The field trial was carried out with Merlot/99R near Wellington in the Coastal Region. Under the prevailing conditions, grape yield responded positively to the increased volume of irrigation water applied compared to dryland cultivation. In contrast, higher grapevine water constraints under dryland cultivation or only three irrigations improved sensorial wine quality. If high wine quality is the objective, three irrigations, i.e. at pea size, *véraison* and post-harvest should be applied. In addition to high wine quality, this irrigation approach will also sustain higher yields. Five or more irrigations should be applied if high yield is the objective if adequate irrigation water is available. In the latter case, Merlot must be irrigated when midday stem water potential reaches -1 MPa to achieve a balance between yield and quality. The low water use efficiency, as well as low wine quality of the PRD did not meet the expectations. Therefore, PRD cannot be recommended for wine production.

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PHOTO: P MYBURGH.

Interpretation of stem water potential measurements

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KEYWORDS: Berry mass, Cabernet Sauvignon, juice, vegetative growth, wine, yield.

INTRODUCTION

Water potential is useful for making decisions concerning the timing of irrigations to achieve specific yield and wine quality objectives. During the day, water lost by transpiration exceeds water absorbed by roots. Consequently, a water potential gradient develops in grapevines. At night, hardly any transpiration occurs and water deficits, or water constraints, that developed in the preceding day can be supplemented again. Therefore, water potential is highest just before dawn. It is referred to as pre-dawn leaf water potential (Ψ_{PD}). As the soil dries out, the water replenishment during the night becomes less and slower. Consequently, water constraints before dawn and during the day increases in due course. Since water is retained in plants at a suction or negative pressure, water potential values are negative.

Minimum water potential is usually reached between 12:00 and 14:00 on sunny days. Under cool, cloudy conditions, water constraints in grapevines are less. To reduce the effect of changing weather conditions, leaves can be covered with bags at least 30 minutes before measurements are made. Water potential in covered leaves is referred to as stem water potential (Ψ_S). Measuring Ψ_S provides a better indication of the impact of soil water content on grapevine water status than leaf water potential (Ψ_L), which is measured in leaves that are fully exposed to sunlight. Based on research results, different thresholds were proposed for water constraint classes according to Ψ_{PD}, Ψ_S and Ψ_L (Table 1).

EXPERIMENTAL BACKGROUND

Midday Ψ_S was related to growth and yield, as well as juice and sensorial wine quality characteristics of Cabernet Sauvignon. Measurements were carried out in 54 experiment plots in the Stellenbosch, Swartland (Mehmel, 2010), Lower Olifants River (Bruwer, 2010) and Lower Orange River regions. Grapevines were either grown dryland, or irrigated by means of single or double dripper lines. In some plots, grapevines were subjected to water deficits by reducing irrigation frequencies to expand the range of responses. Stem water potential was measured as described previously (Myburgh, 2010). Grapes were harvested when the sugar reached 24°B. All wines were made at ARC Infruitec-Nietvoorbij as described by Myburgh (2011). Measurements were repeated over two seasons, except for the Stellenbosch region where limited resources restricted measurement to one season.

TABLE 1. Water constraint classes and thresholds for pre-dawn (Ψ_{PD}), leaf (Ψ_L) and stem (Ψ_S) water potential in Cabernet Sauvignon.

Water constraints	Class	Water potential thresholds (MPa)		
		Ψ_{PD}	Ψ_L	Ψ_S
No	I	$\Psi_{PD} \geq -0.2$	$\Psi_L \geq -1.15$	$\Psi_S \geq -0.60$
Mild	II	$-0.2 > \Psi_{PD} \geq -0.4$	$-1.15 > \Psi_L \geq -1.30$	$-0.60 > \Psi_S \geq -0.85$
Moderate	III	$-0.4 > \Psi_{PD} \geq -0.6$	$-1.30 > \Psi_L \geq -1.45$	$-0.85 > \Psi_S \geq -1.15$
High	IV	$-0.6 > \Psi_{PD} \geq -0.8$	$-1.45 > \Psi_L \geq -1.60$	$-1.15 > \Psi_S \geq -1.40$
Severe	V	$\Psi_{PD} < -0.8$	$\Psi_L < -1.60$	$\Psi_S < -1.40$

GRAPEVINE RESPONSES ACCORDING TO WATER CONSTRAINTS

Class I – No water constraints

Based on midday Ψ_S, grapevines in only one plot fell in Class I. This was insufficient to identify consistent grapevine response trends. However, vegetative growth is expected to be more vigorous, compared to Class II, i.e. mild water constraints. Usually, shoot growth tips are active throughout the season and internodes are long. Light penetration into the bunch zone is poor and older leaves in the bunch zone may turn yellow. Berry mass could exceed 1.3 g. Due to excessive shading of buds early in the season and competition with vegetative vigour, yield may be lower than Class II. If leaves are shaded, juice potassium (K^+) is usually high, total titratable acidity (TTA) low and pH high (Iland, 1989). If berry temperature is reduced by shading, juice TTA could be high and pH low. Sugar accumulation might be delayed and juice dilution may occur. Wine colour and overall quality is expected to be less than 45%. In fact, where mean Ψ_S of Cabernet Sauvignon was higher than -0.6 MPa over three seasons, juice pH was 3.6 and wine quality 38% (Howell *et al.*, 2014b). Mean berry mass was 1.4 g and yield 14.6 t/ha in the sandy soil (Howell *et al.*, 2014a).

Class II – Mild water constraints

Active growth tips occurred late in the growing season. Light penetration into bunch zone was poor and leaf yellowing occurred inside the canopy. Strongest growth, biggest berries and highest yield were obtained in all soils, compared to the other classes (Fig. 1A-C). Berry mass was between 1.2 and 1.4 g. Juice K^+ ranged from 1 600 to 1 800 mg/ℓ, TTA from 7.3 to 8.0 g/ℓ and pH from 3.4 to 3.6 (Fig. 1D-F). Since vigorous growth caused more shaded leaves, juice TTA was lower and pH higher in heavier than in sandy soils. Sugar accumulation may be retarded and juice dilution may occur. In sandy soils, wine colour was 65% to 70% and overall quality 45% to 50% (Fig. 1G & I). In heavier soils, wine colour was 50% to 55% and overall quality 40% to 45%. Berry character was stronger in sandy soil than in heavier soil (Fig. 1H).

Class III – Moderate water constraints

In the pre-*véraison* period, drooping of the second tendril from the shoot apex may occur. Limited active shoot tips occurred in the post-*véraison* period. Leaves could show para-helionastic movement. Leaves did not show yellowing. Vegetative growth was reduced by c. 25% and 30% in sandy and heavier soils, respectively, compared to Class II (Fig. 1). Berry mass was between 1.1 and 1.3 g. In heavier soils, yield was comparable to Class II, but was reduced by c. 5% in sandy soils. Juice K^+ ranged from 1 200 to 1 400 mg/ℓ, TTA from 7 to 7.5 g/ℓ and pH from 3.4 to 3.5 (Fig. 1D-F). Since the stronger growth reduced berry temperature, juice TTA was higher and pH lower in heavier than in sandy soils. In sandy soils, wine colour was 75% to 80% and overall quality 55% to 65%. Wine colour was 60% to 70% and overall quality 45% to 55% in heavier soils (Fig. 1G & I). Berry character was stronger in sandy soil, compared to heavier soil (Fig. 1H).

Class IV – High water constraints

Shoot tips were inactive in the pre-*véraison* period. Limited drying of tendrils occurred in some plots. Leaf folding, drooping and para-helionastic movement may occur. Basal leaves showed limited yellowing. Bunch exposure was increased. Vegetative growth was reduced by c. 30% and 40%, respectively, in the sandy and heavier soils, compared to Class II (Fig. 1A). Limited berry shrinkage occurred. Berry mass was between 1 and 1.2 g. Yield was reduced by c. 35% and 15% in the sandy and heavier soils, respectively, compared to Class II (Fig. 1C). Juice K^+ ranged from c. 1 200 to 1 300 mg/ℓ, TTA from 6.5 to 7.5 g/ℓ and pH from 3.35 to 3.45 (Fig. 1D-F). Juice TTA was higher and pH lower in heavier soils than in sandy soils, since stronger growth reduced berry temperature (Iland, 1989). Wine colour was 65% to 75%, irrespective of soil texture (Fig. 1G & I). In sandy soil, overall quality was 50% to 55%, compared to 55% to 60% in heavier soil. Berry character was lower in the sandy soil, compared to heavier soil (Fig. 1H).

Class V – Severe water constraints

Shoot elongation stopped when berries reached pea size. Desiccated shoot tips occurred in the pre-*véraison* period, particularly in sandy soils. Basal and bunch zone leaves showed yellowing. General tendril drying and leaf shedding may occur. Bunches were excessively exposed, particularly in sandy soils. Vegetative growth was reduced by c. 50%, compared to Class II in all soils (Fig. 1A). Berries showed shrinkage or shriveling. Berry mass was between 0.9 and 1.1 g. Yield was reduced by c. 40%, compared to Class II in all soils (Fig. 1C). Negative carry over

effects to next season's yield may occur, particularly in sandy soil. Juice K^+ ranged from c. 900 to 1 000 mg/ℓ and TTA from 6.5 to 7.5 g/ℓ (Fig. 1D & E). Juice pH was c. 3.5 and 3.35 in sandy and heavier soils, respectively (Fig. 1F). Since the sparse growth allowed berry temperatures to increase, juice TTA was lower and pH higher in sandy than in heavier soils (Iland, 1989). Sugar accumulation is likely to be limited. In sandy soil, sensorial wine colour was 70% to 75% and overall quality 50% to 55% (Fig. 1G & I). In heavier soil, wine colour was 75% to 85% and overall quality 55% to 65%. Berry character was stronger in heavier soil, compared to sandy soil (Fig. 1H).

CONCLUSIONS AND RECOMMENDATIONS

If Cabernet Sauvignon grapevines in sandy soil experience no water constraints, yield might be slightly higher, but wine quality will be low. Ideally, they should experience moderate water constraints to obtain high quality with c. 5% reduction in yield. Since high to severe water constraints will reduce yield substantially in sandy soil, it must be avoided. Grapevines in heavier soil should experience high water constraints to obtain high quality with c. 15% reduction in yield. If yield is the primary objective, grapevines should experience mild water constraints to obtain a balance between yield and wine quality. In this regard, -0.6 MPa is considered as a critical Ψ_S threshold. Although high wine quality is expected where grapevines experience severe water constraints, it will only be economically viable if high wine prices can compensate for the c. 40% yield loss.

Response of water potential to soil and atmospheric conditions, i.e. the anisohidric behaviour, can vary between cultivars. Consequently, other cultivars might not respond in a similar way to water constraints as Cabernet Sauvignon.

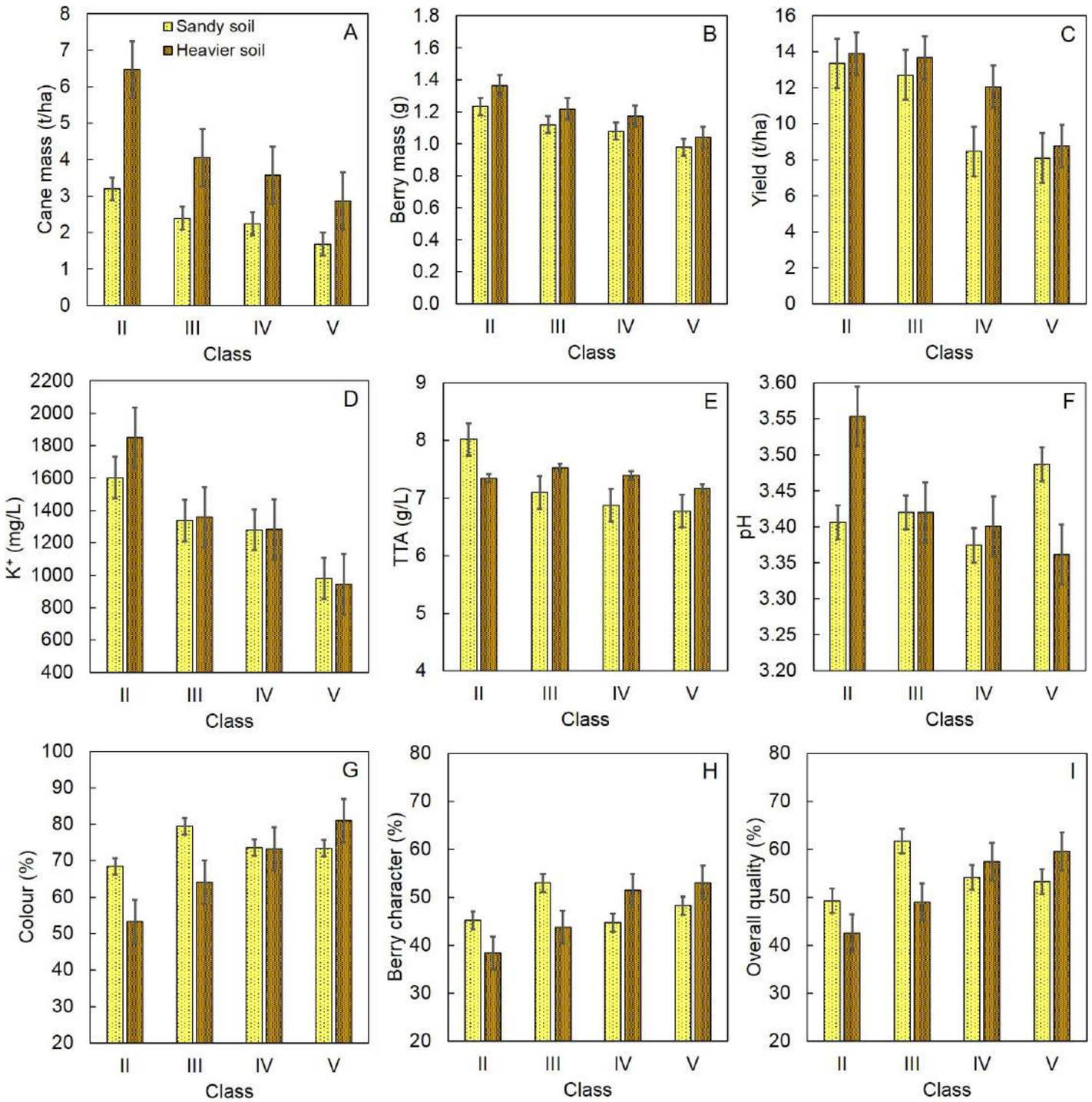

FIGURE 1. Cane mass (A), berry mass (B) and yield (C), as well as juice (D, E & F) and wine (G, H & I) characteristics of Cabernet Sauvignon within different water constraint classes based on midday stem water potential. Vertical bars indicate ± one standard deviation.

For example, there is evidence that water potential in Shiraz is lower, compared to other cultivars under comparable soil and atmospheric conditions. Anisohydric behaviour and related growth, yield and quality responses of the more popular cultivars should be determined by further research. Since Ψ_S is better related to soil water content and more convenient than pre-dawn measurements, it is recommended for commercial application.

SUMMARY

Stem water potential is useful for making decisions concerning the timing of irrigations to achieve specific yield and wine quality objectives. Therefore, midday stem water potential was related to growth and yield, as well as juice and wine quality characteristics of Cabernet Sauvignon in 54 experiment plots in the Stellenbosch, Swartland, Lower Olifants River and Lower Orange River regions. If Cabernet Sauvignon grapevines in sandy soil are over-irrigated, yield might be slightly higher, but wine quality will be low. Ideally, grapevines in sandy soils should experience moderate water constraints to obtain high wine quality with c. 5% yield reduction. Since high to severe water constraints will reduce yield and wine quality in sandy soil, it must be avoided. Grapevines in heavier soil should experience high water constraints to obtain high wine quality with c. 15% yield reduction. If high yield is the objective, grapevines in heavier soils should experience mild water constraints to obtain a balance between yield and wine quality. In contrast, severe water constraints will ensure high wine quality, but this is only recommended if high wine prices compensate for the c. 40% yield loss. Midday stem water potential is better related to soil water content and more convenient than pre-dawn measurements. Therefore, it is recommended for commercial application.

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Aster yellows symptoms on white cultivar. Yellow leaves have a wrinkled appearance, leaves are thicker than usual, and the growth tip dies back.

Aster yellows and leafhoppers

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KEYWORDS: Aster yellows, leafhopper, vector, control.

In view of the fact that Aster yellows infects wine, table and raisin grapes, the disease poses a serious threat to the entire viticultural industry and especially the availability of clean plant material.

Aster yellows is a phytoplasma disease which was first identified in the Vredendal area in 2006. Meanwhile the disease has also been recorded in the Waboomsrivier, Robertson and Montagu areas and classified as a quarantine disease. Serious crop losses and dieback of grapevines occur in all these areas and within two to four years infected blocks may deteriorate to such an extent that they have to be replaced in totality. No direct control measures for this disease exist anywhere in the world.

Various symptoms are associated with Aster yellows disease. Early in the season leaves have a wrinkled appearance and shoots have short nodes and may display delayed growth. Later in the season the leaves of white cultivars become yellow and those of red cultivars show a reddish discolouration. Affected leaves are thicker than

Leafhoppers (*Mgenia*).

Leafhoppers (*Acia*).

normal, brittle and curl downwards. Shoots lignify only partially or not at all, with dieback of shoots from the growth tip and partial dieback of bunches. Infected grapevines deteriorate and eventually die.

Overseas, Aster yellows is transmitted by leafhoppers and this plays a big role in the spread of the disease within blocks and to other blocks in the immediate vicinity. Only one leafhopper species, *Acia lineatifrons*, is a known pest in South African vineyards. It causes *hopper burn*, a condition where leaf edges dry out and the leaves fall prematurely. It is a sporadic pest which only attains pest status in certain years.

Surveys were conducted in vineyards infected with Aster yellows in Vredendal, Waboomsrivier and Robertson to determine the following:

- Which types of leafhoppers occur in the vineyards.
- Which leafhoppers carry the Aster yellows phytoplasma in their digestive tract, making them potential vectors of the disease.
- Whether any of the leafhoppers are definitely a vector of the disease.

To determine whether leafhoppers occur in the vineyards infected with Aster yellows, a motorised backpack-type vacuum sampler was used. The leafhoppers were identified under a stereo microscope and the numbers of each genus or species recorded.

At least five sticky, yellow traps were hung in various locations in each block and after two to three weeks leafhoppers were identified and counted under the microscope. Identifications were confirmed by M. Stiller, an expert in the field of leafhopper taxonomy at the ARC Biosystematics Division in Pretoria.

Altogether 26 leafhopper species in 24 genera and five families were found in the three Aster yellows infected areas. Apart from *Acia lineatifrons* which is a well-known grapevine pest, only a few of these species were previously known to occur in South African vineyards. Although all genera occurred on grapevines and on weeds in the vineyard during the growing season, the numbers of *Acia lineatifrons* and *Mgenia fuscovaria* are high on grapevines and low on weeds.

Molecular methods were used to determine whether Aster yellows phytoplasma occurs in the digestive tract of the various leafhopper species. The University of Pretoria conducted transmission studies on the leafhopper species in which the phytoplasma was found. These studies proved that *Mgenia fuscovaria* can act as a vector of Aster yellows phytoplasma in grapevines.

Field trials in the three viticultural areas where high infestations occurred, showed that the contact insecticides Steward® (active ingredient: indoxacarb) and Dursban® (active ingredient: chlorpyrifos) gave excellent results with regard to control of leafhoppers. Products should be applied when leafhoppers become active in the vines and, if necessary, repeated after 14 days. When applying these products, their withholding periods should be borne in mind. Dursban should not be applied within four weeks after bud burst, because it is phytotoxic to young vine leaves.

Commercial applications with the systemic insecticide Kohinor® (active ingredient: imidacloprid) also offered very good control up to 17 months following application. This product has a particularly long withholding period (112 days) which has to be managed very carefully. Steward®, Dursban® and Kohinor® are currently registered for the control of leafhoppers on wine grapes.

Although no product is known to kill the phytoplasma in grapevines with Aster yellows, the grapevines may be managed to curb losses. Shoots with yellow leaves and the infected cordons must be removed regularly throughout the season. Post-harvest removal of the entire vine with its roots will considerably reduce transmission of the disease.

SUMMARY

Altogether 26 leafhopper species were identified during a survey in vineyards infected with Aster yellows. Further studies showed that the indigenous leafhopper *Mgenia fuscovaria* is able to act as a vector of Aster yellows phytoplasma in grapevines.

The results of field trials led to the registration of Steward®, Dursban® and Kohinor® for the control of leafhoppers on wine grapes. To curb losses in infected vineyards, shoots with yellow leaves and the corresponding cordons should be removed regularly during the course of the season. Post-harvest removal of the entire infected grapevine, with roots, helps to limit the spread of the disease.

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Field application and persistence of EPNs to control *Planococcus ficus* on grapevine roots

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KEYWORDS: EPNs, *Planococcus ficus*, grapevine, roots.

INTRODUCTION

Planococcus ficus (the vine mealybug) is currently regarded as the dominant mealybug species, with it being a major pest insect of the South African table grape and wine industries. The most common method of mealybug control in South Africa is the use of such chemical insecticides as organophosphates. An alternative to using chemicals in controlling *P. ficus* is to use entomopathogenic nematodes (EPNs) within an integrated pest management (IPM) scheme. The current rise of interest in the use of EPNs within an IPM scheme is a progressive and positive move that has been taken towards reducing chemical pesticide use, in the pursuit of sustainable agricultural practice.

The use of EPNs is generally more suited to controlling soil-dwelling insect stages than above ground insect pests. Although the greater part of the mealybug population is found above ground, they also occur on vine roots to a depth of 30 cm. This is encouraging for the use of EPNs, when considering the fact that the vine mealybug has a distinctive vertical seasonal movement on grapevines.

The main objectives of the current study were to establish the efficiency of controlling soil populations of *P. ficus*, using EPNs in vineyards. The persistence of nematodes was established in the laboratory, as well as after application in two vineyards.

IN-FIELD SOIL APPLICATION AND INFECTIVITY OF *STEINERNEMA YIRGALEMENSE*

Nematodes at concentrations of 80, 40, 20, and 0 nematodes (infective juveniles) /cm^2 were applied to an area of 80 x 100 cm measured around each treatment vine in a vineyard in Welgevallen and Nietvoorbij (Fig. 1). The soil temperature, 15 cm below the soil surface during the 48 hours following on nematode treatment, was a mean of 24°C, with a minimum of 17°C and a maximum of 32°C.

The concentration of 80 infective juveniles/cm^2 of *S. yirgalamense* was responsible for the greatest percentage of *P. ficus* mortality, being 50% at Welgevallen and 52% at Nietvoorbij, respectively (Fig. 2).

IN-FIELD SOIL PERSISTENCE OF *S. YIRGALEMENSE*

Testing for *S. yirgalemense* efficacy one, two, four, and 12 weeks post application showed high efficacy and persistence in the Welgevallen vineyard using codling moth larval mortality as the measurement concerned (Fig. 3). After 12 weeks, zero mortality for codling moth larvae was found in the Nietvoorbij vineyard (Fig. 3).

LABORATORY PERSISTENCE OF *STEINERNEMA YIRGALEMENSE* AND *HETERORHABDITIS ZEALANDICA*

No significant difference was found in mortalities over the six dates for *S. yirgalemense.* However, codling moth larval mortalities due to *H. zealandica* were reduced from 100% to 5% (Fig. 4).

CONCLUSION OF THE STUDY

In both vineyards, *S. yirgalemense* performed well, despite the fact that the mealybugs were left in the soil for only 48 hours, whereas in practice, the nematode would have had an indefinite time period for infection. In addition to the above findings, the mealybugs were buried 15 cm beneath the soil, which means that the nematodes had to detect, and to infect, the insects within a short period of time. These are promising results, when considering in South Africa, *P. ficus* spends the winter months in colonies on the lower trunk, under the bark,

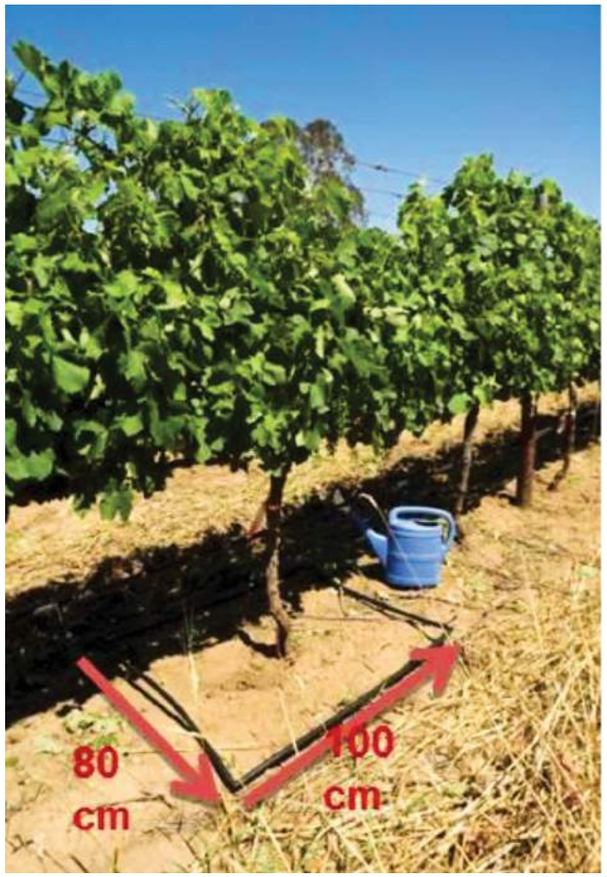

FIGURE 1. A marked treatment vine with a measured area of 80 x 100 cm for EPN application.

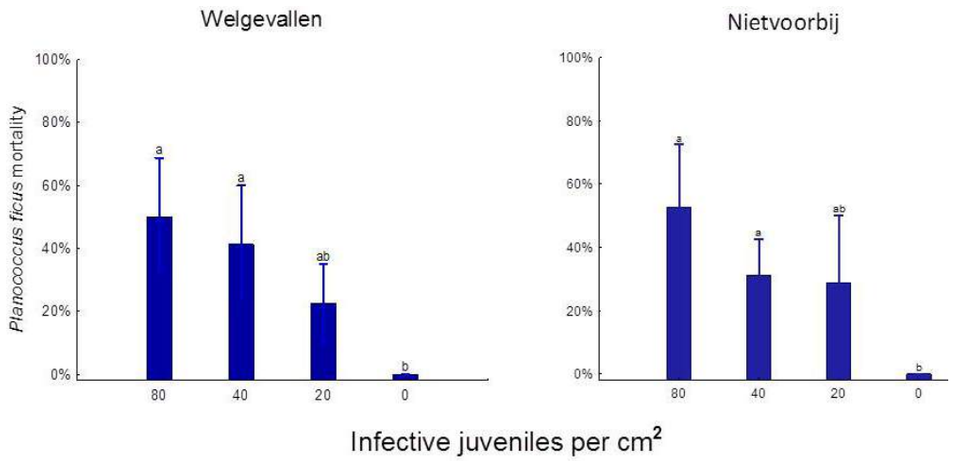

FIGURE 2. Percentage mortality of *Planococcus ficus* buried 15 cm beneath the soil in the Welgevallen and Nietvoorbij vineyard with a 48 hour exposure to *Steinernema yirgalemense* at concentrations of 80, 40, 20, and 0 infective juveniles/cm^2.

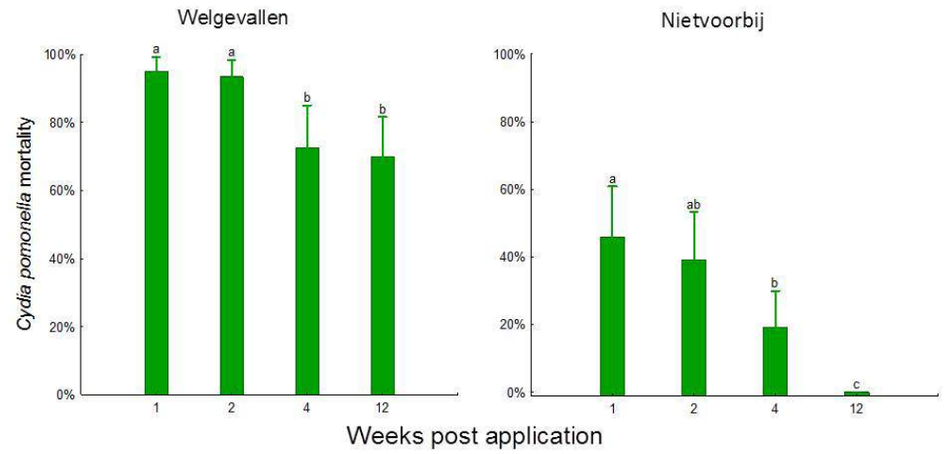

FIGURE 3. Percentage mortality of *Cydia pomonella* at the Welgevallen and Nietvoorbij vineyards, with the codling moth larvae buried 15 cm beneath the soil in the field and with a 5-day exposure to *Steinernema yirgalemense* after one, two, four, and 12 weeks post EPN application.

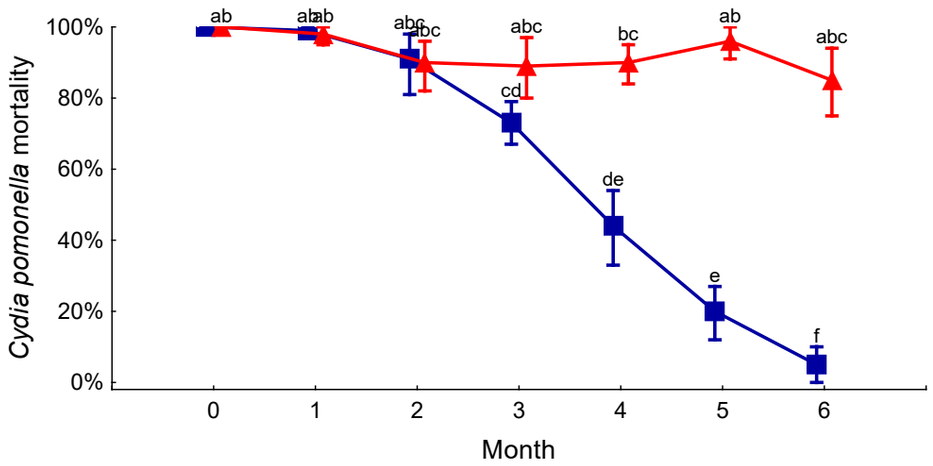

FIGURE 4. Percentage mortality of *Cydia pomonella* exposed to 10 infective juveniles/cm^2 *Heterorhabditis zealandica* (■) and *Steinernema yirgalemense* (▲) after six months persistence in sand in the laboratory.

and underground, on the roots of the vine. Their habitat should make them more vulnerable targets to applied EPN in the field than under controlled laboratory conditions, such as were present in the current experiment.

To test the ability of *S. yirgalemense* to persist in the soil after application, codling moth larvae were buried in the soil. This was as a result of the difficulties experienced when working with *P. ficus* (because of its small size and sensitivity to handling), which might otherwise have affected the data obtained. In contrast, codling moth larvae are known for their susceptibility to *S. yirgalemense.* Generally speaking, as soon as nematodes are applied to the soil, they are exposed to an

array of abiotic and biotic factors that cause nematode mortality. Over and above these many factors, some nematode species are known to be characteristically more persistent than are others.

The persistence of *S. yirgalemense* in the two vineyards that were studied in the current research differed dramatically, with steady persistence being obtained in the Welgevallen vineyard, and a drastic decrease being found in the Nietvoorbij vineyard over three months. The difference in persistence of *S. yirgalemense* could have been due to any of the many abiotic and biotic factors affecting the two differing vineyards. To hold one factor accountable for the low persistence in Block B would be almost impossible, as there might have been a multitude of factors at work at the time of the study. To make comparisons with, and to draw conclusions from, other persistence studies are also difficult, as different EPN species are usually studied under a variety of different conditions.

The decrease in the percentage of codling moth larvae mortality between two and six months as a result of *H. zealandica* infection, as was tested for in the laboratory, indicates a reduction in the number of infective juveniles persisting in the container. In contrast, *S. yirgalemense* steadily persisted at 12 weeks under laboratory conditions, from which its ability to persist can be seen as being superior to that of *H. zealandica.*

S. yirgalemense produced good results in the current soil field trials, in terms of the sensitivity of soil-dwelling stages of *P. ficus* as regarding their mortality immediately after application, and as regarding their field persistence. These findings place *S. yirgalemense* in a promising position as a potential biological control agent for the control of *P. ficus.*

SUMMARY

In South Africa, mealybugs are a serious problem in vineyards and control is mainly based on use of chemical insecticides. Insect-parasitic nematodes or entomopathogenic nematodes (EPNs) can potentially be used within an integrated pest management scheme to control *P. ficus*, the vine mealybug, which is mainly found above ground, but which can also occur on grapevine roots. When the EPN, *S. yirgalemense*, was applied to the soil of two vineyards by means of buried, pierced Eppendorf tubes containing mealybugs, mortalities of up to 50% were obtained. This control was obtained despite the tubes being buried to a depth of 15 cm and using a short exposure time of only 48 hours of the mealybugs to the nematodes in the field. In response to the testing that was undertaken for the persistence of *S. yirgalemense*, control was zero in one vineyard, while in another it was found to be 70%, 12 weeks after application. These studies showed that EPNs, and specifically *S. yirgalemense*, have promising potential for use as a biocontrol agent for *P. ficus* soil populations. Foliar applications require further investigation.

ACKNOWLEDGEMENTS

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Distribution and symptomatology of grapevine trunk disease pathogens

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KEYWORDS: Grapevine, trunk, disease, pathogen.

INTRODUCTION

Trunk disease pathogens associated with decline and dieback of grapevines include several pathogenic species. These pathogens often occur together in declining grapevines, with different pathogens often associated with the same type decay symptoms. Apart from overlapping symptomatology, pathogen distribution appears to be influenced by climate. In South Africa this information was largely unknown. The aim of this study was to study the influence of climate on the distribution of grapevine trunk pathogens and disease symptomatology.

MATERIALS AND METHODS

During 2003 and 2004, 15 wine and 15 table grape vineyards were surveyed in 11 production areas in the Western Cape, Northern Cape, Limpopo and Mpumalanga provinces of South Africa. Based on annual rainfall and the dominant rainfall season, production areas were grouped into summer, winter and marginal rainfall regions (Fig. 1).

In the Robertson, Vredendal, Malmesbury and Stellenbosch areas, Cabernet Sauvignon vineyards were sampled. In the Upington area, Ruby Cabernet vineyards were sampled. In the Trawal, Mookgopong, Modimolle, Groblersdal, Upington, De Doorns and Paarl areas, Dan-ben-Hannah vineyards were sampled. Vineyards eight years and older were sampled. In each vineyard, 20 visually healthy plants were selected randomly and sampled by removing the distal end of one cordon.

Isolations were made from 1 cm cross-sections of the cordons and the fungal cultures identified. The isolation frequency in each region was determined for each genus/species, as well as the symptom profile of the respective genera/species in each region.

RESULTS

SYMPTOM TYPES

Symptom types were classified as black streaking (Fig. 2a), brown streaking (Fig. 2b), wedge-shaped necrosis (Fig. 2c), brown internal necrosis (Fig. 2d), watery

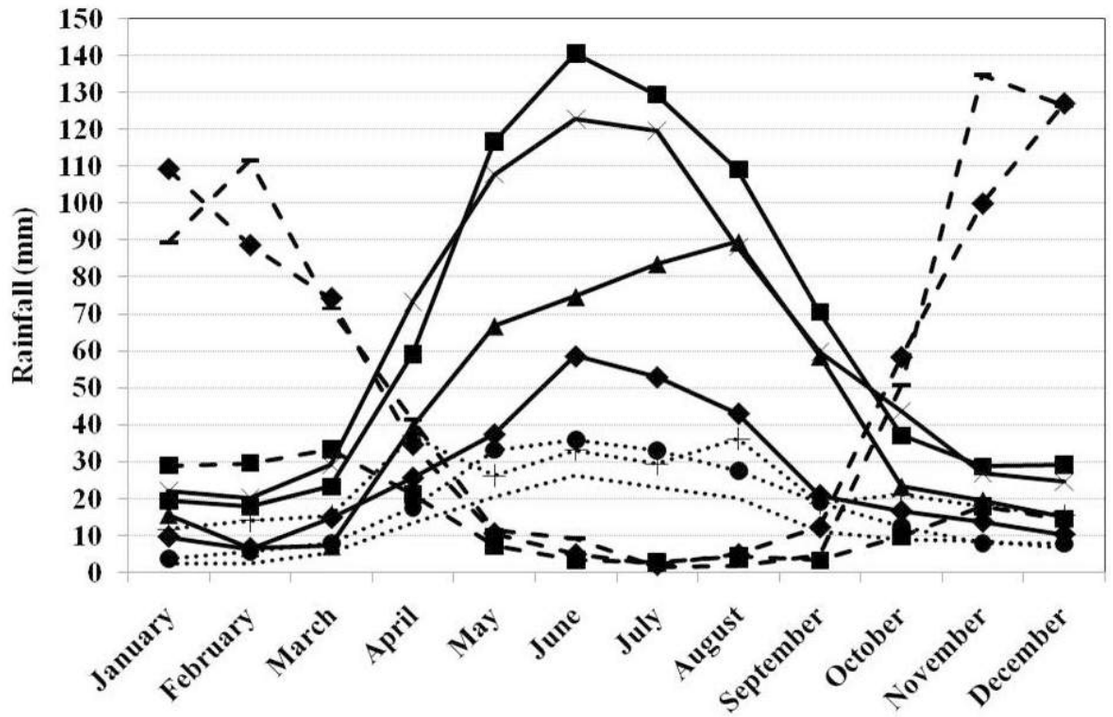

FIGURE 1. Long term average monthly rainfall patterns for De Doorns (-◆-), Paarl (-■-), Malmesbury (-▲-), Stellenbosch (-✕-), Trawal (-●-), Robertson (-+-), Vredendal (- -), Mookgopong (-—-), Modimolle (-•-) and Upington (-▪-), South Africa. Summer rainfall region included Upington, Mookgopong, Modimolle and Groblersdal. Winter rainfall region consisted of De Doorns, Malmesbury and Stellenbosch. Marginal rainfall region included Trawal, Vredendal and Robertson.

TABLE 1. Mean incidence of *Phaeomoniella chlamydospora*, *Eutypa lata* and species in the Botryosphaeriaceae, *Diaporthe* and *Phaeoacremonium* in the summer, marginal and winter rainfall regions of South Africa.

Region	Pathogen*				
	Pa. chlamydospora	***Phaeoacremonium* spp.**	***Diaporthe* spp.**	**Botryosphaeriaceae spp.**	***E. lata***
Summer	5.4 def	9.9 cd	0.2 gh	9.5 cde	0.0 h
Marginal	8.7 cdef	12.6 bc	4.5 efgh	16.0 b	0.0 h
Winter	24.5 a	16.0 b	4.3 fgh	5.2 defg	0.2 gh
LSD			5.04		

* Means followed by the same letter are not significantly different ($P = 0.05$).

TABLE 2. Mean isolation incidence of *Phaeomoniella chlamydospora* from the different symptom types in the summer, marginal and winter rainfall regions of South Africa.

Symptom type	Region*		
	Summer	Marginal	Winter
Brown internal necrosis	7.8 de	15.8 bcde	36.2 ab
Black streaking	19.9 bcde	20.9 bcde	33.7 abc
Brown streaking	0.0 e	3.2 e	27.9 abcd
Esca brown	6.0 de	4.0 e	29.7 abc
Esca yellow	1.0 e	2.7 e	16.9 bcde
Wedge-shaped necrosis	1.1 e	12.5 cde	19.9 bcde
Watery necrosis	0.0 e	0.0 e	42.9 a
Asymptomatic wood	0.0 e	0.8 e	0.4 e
LSD		21.88	

* Means followed by the same letter are not significantly different ($P = 0.05$).

TABLE 3. Mean isolation incidence of *Phaeomoniella chlamydospora*, *Eutypa lata* and species in the Botryosphaeriaceae, *Diaporthe* and *Phaeoacremonium* from wine and table grapes in South Africa.

Pathogen	Grapes*	
	Wine	Table
Botryosphaeriaceae spp.	12.7 b	6.9 c
Pa. chlamydospora	6.9 c	21.2 a
Phaeoacremonium spp.	22.5 a	3.5 cd
Diaporthe spp.	5.5 c	0.7 d
E. lata	0.2 d	0.0 d
LSD	4.08	

* Means followed by the same letter are not significantly different (P = 0.05).

TABLE 4. Mean isolation incidence of *Phaeoacremonium* spp. from the different symptom types in the summer, marginal and winter rainfall regions of South Africa.

Symptom type	Region*		
	Summer	Marginal	Winter
Brown internal necrosis	11.9 cde	9.6 cde	18.4 bcde
Black streaking	28.5 bc	36.6 b	27.5 bcd
Brown streaking	4.4 e	7.6 de	15.0 cde
Esca brown	0.0 e	5.0 e	19.5 bcde
Esca yellow	8.0 cde	0.0 e	1.9 e
Wedge-shaped necrosis	14.3 cde	3.8 e	26.9 bcd
Watery necrosis	0.0 e	60.0 a	15.0 cde
Asymptomatic wood	0.4 e	0.8 e	1.0 e
LSD		20.76	

* Means followed by the same letter are not significantly different (P = 0.05).

TABLE 5. Mean isolation incidence of *Diaporthe* spp. from the different symptom types in the summer, marginal and winter rainfall regions of South Africa.

Symptom type	Region*		
	Summer	Marginal	Winter
Brown internal necrosis	0.1 c	3.3 bc	6.7 bc
Black streaking	0.0 c	4.5 bc	3.5 bc
Brown streaking	0.0 c	1.8 c	1.0 c
Esca brown	0.0 c	0.0 c	1.8 c
Esca yellow	0.0 c	0.0 c	0.5 c
Wedge-shaped necrosis	1.4 c	21.8 a	14.2 ab
Watery necrosis	0.0 c	0.0 c	5.0 bc
Asymptomatic wood	0.0 c	0.0 c	0.6 c
LSD		11.80	

* Means followed by the same letter are not significantly different (P = 0.05).

TABLE 6. Mean isolation incidence of species in the Botryosphaeriaceae from the different symptom types in the summer, marginal and winter rainfall regions of South Africa.

Symptom type	Region*		
	Summer	Marginal	Winter
Brown internal necrosis	6.9 efg	37.7 abc	5.7 efg
Black streaking	2.9 g	1.6 g	0.6 g
Brown streaking	1.2 g	3.9 fg	0.0 g
Esca brown	14.7 defg	2.0 g	0.5 g
Esca yellow	22.4 cde	1.3 g	9.0 efg
Wedge-shaped necrosis	29.5 bcd	46.0 ab	22.0 cdef
Watery necrosis	0.0 g	50.0 a	0.0 g
Asymptomatic wood	1.7 g	0.6 g	0.8 g
LSD		18.17	

* Means followed by the same letter are not significantly different (P = 0.05).

necrosis (Fig. 2e) and esca-like brown and yellow soft wood rot (Fig. 2f). Black streaking, brown streaking and brown internal necrosis were most common on samples from the winter rainfall region followed by the marginal and summer rainfall regions. Esca-like brown soft wood rot had the highest incidence in the winter rainfall region followed in this case by the summer and marginal rainfall regions. Esca-like yellow soft wood rot, on the other hand, was most frequently observed from the summer rainfall region. Wedge-shaped and watery necrosis were the only symptom types that had their highest occurrence in the marginal rainfall region.

PATHOGENS AND ASSOCIATED SYMPTOM PROFILES

Phaeomoniella chlamydospora (13.9%) and *Phaeoacremonium* spp. (13.1%) (Petri disease) were most frequently isolated followed by Botryosphaeriaceae (9.8%) (Botryosphaeria dieback) and *Diaporthe* (3.2%) (Phomopsis dieback) species. *Eutypa lata* (Eutypa dieback/'tandpyn') was isolated only five times, in all cases in the winter rainfall region from wine grapes and from esca-like soft brown wood rot.

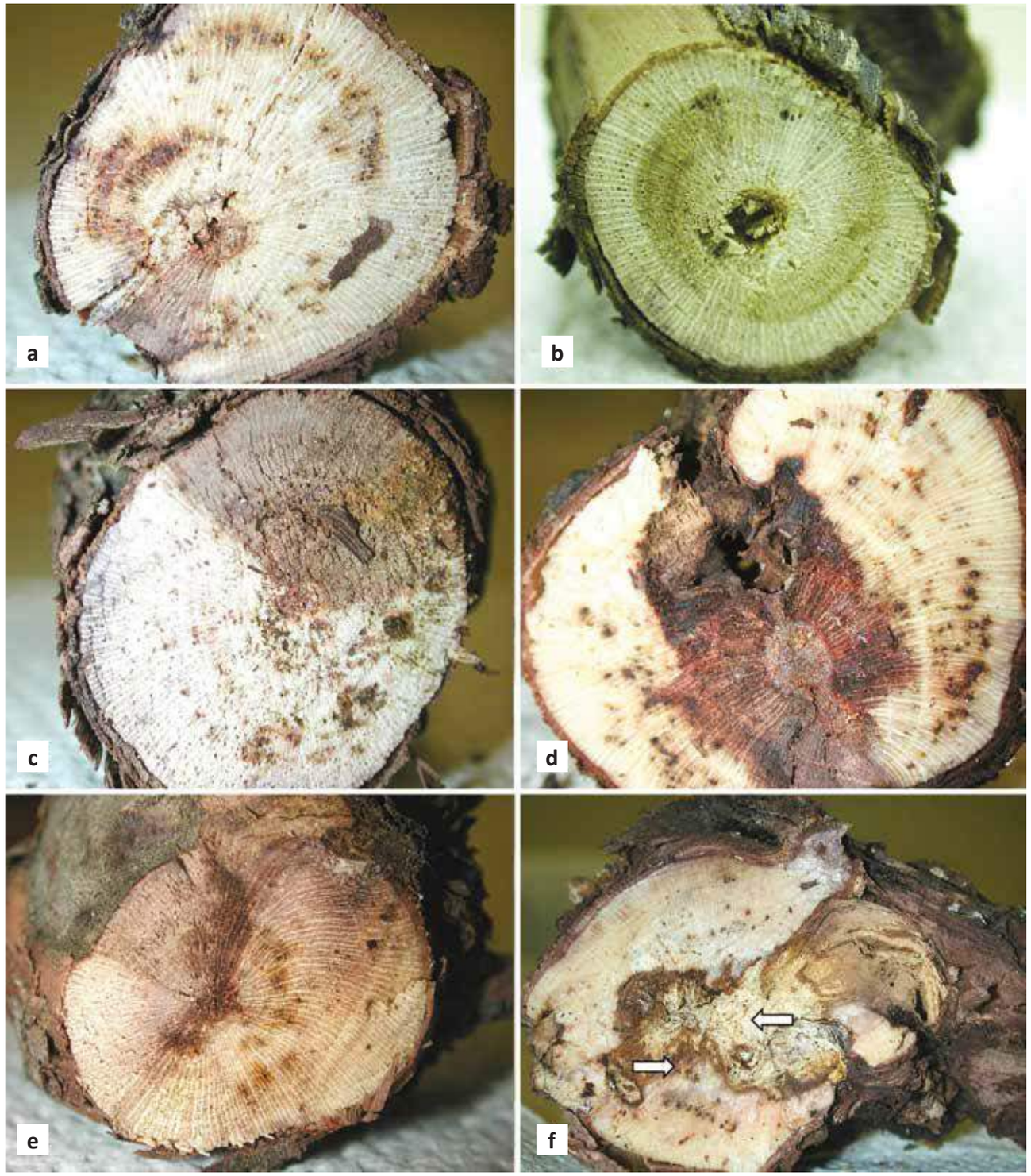

FIGURE 2. Cross sections made through cordon sections displaying various symptom types: a. black streaking, b. brown streaking, c. wedge-shaped necrosis, d. brown internal necrosis, e. watery necrosis, f. Esca-like brown (left) and yellow soft wood rot (right).

PHAEOMONIELLA CHLAMYDOSPORA

The incidence of *Pa. chlamydospora* was highest in the winter rainfall region (24.5%) followed by the marginal (8.7%) and summer rainfall (5.4%) regions (Table 1). The incidence of this pathogen in the winter rainfall region was furthermore significantly higher than the other trunk disease pathogens occurring in this region. *Phaeomoniella chlamydospora* had the widest symptom profile in the winter rainfall region (Table 2). In the marginal and summer rainfall regions, *Pa. chlamydospora* was mostly isolated from black streaking.

PHAEOACREMONIUM SPP.

The incidence of *Phaeoacremonium* spp. was the highest in the winter rainfall region (16.0%; Table 1). This group of pathogens also had a broad symptom profile in the winter rainfall region, isolated from all the symptom types (Table 4). In the marginal rainfall region, the highest incidence of *Phaeoacremonium* spp. was encountered from watery necrosis (60.0%), followed by black streaking (36.6%). Incidence of isolation from the remaining symptom types was relatively low (0-7.6%; Table 4). In the summer rainfall region, these pathogens were mostly isolated from black streaking, wedge-shaped necrosis, brown internal necrosis and esca-like yellow soft rot (28.5-8.0%) and at a low incidence from asymptomatic wood and brown streaking (0.4 - 4.4%; Table 4). All 159 *Phaeoacremonium* isolates were identified as *Pm. minimum.*

DIAPORTHE SPP.

Diaporthe spp. were equally common in the winter and marginal rainfall regions (4.3-4.5%) with a very low incidence in the summer rainfall region (0.2%; Table 1). *Diaporthe* spp. had a much broader symptom profile in the winter rainfall region; isolated with the highest incidence from wedge-shaped necrosis (14.2%) and then at incidences ranging between 0.6% and 6.7 % from all the other symptom types. In the marginal rainfall region, *Diaporthe* spp. were isolated from wedge-shaped necrosis (21.8%), brown internal necrosis and brown streaking (up to 4.5%) (Table 5). In the summer rainfall region, *Diaporthe* spp. were isolated from only wedge-shaped necrosis

and brown internal necrosis (Table 5). All 63 *Diaporthe* isolates were identified as *D. ampelina* (previously known as *Phomopsis viticola*).

BOTRYOSPHAERIACEAE

This is the only group of pathogens that occurred most frequently in the marginal rainfall region (16%; Table 1). These pathogens also had the broadest symptom profile in this region (Table 6). The symptom types in the marginal rainfall region yielding most isolates of Botryosphaeriaceae were watery necrosis (50%), wedge-shaped necrosis (46%) and brown internal necrosis (37.7%). In the summer rainfall region, isolates of the Botryosphaeriaceae were obtained from all the symptom types, except watery necrosis. Here, the highest incidence was from wedge-shaped necrosis (29.5%), esca-like yellow soft rot (22.4%), as well as esca-like brown wood rot (14.7%). The winter rainfall region displayed an even narrower symptom profile. In this region, this group of pathogens was not isolated from watery necrosis and brown streaking and seldom from black streaking, esca-like brown wood rot and asymptomatic wood. The highest incidence of isolation was from wedge-shaped necrosis (22%), followed by esca-like yellow soft rot (9%) and brown internal necrosis (5.7%). The majority of the isolates were identified as *D. seriata* followed by *Neofusicoccum parvum*, *N. australe Lasiodiplodia theobromae*, *L. crassispora*, *B. dothidea* and *N. vitifusiforme.*

Wine grapes yielded more *Phaeoacremonium* spp., *Diaporthe* spp. and Botryosphaeriaceae isolates, whereas table grapes yielded the most isolates of *Pa. chlamydospora* (Table 3). All of the fungi were also isolated from asymptomatic wood, although at low incidences.

CONCLUSIONS

Several trunk disease pathogens were obtained from asymptomatic tissue. This confirms their ability to survive endophytically in their host, causing disease when vines become predisposed by stress. The results of this study highlight the complex nature of grapevine trunk diseases. Apart from *E. lata* found only in the winter rainfall region, the different pathogens occurred in all the surveyed regions. However, the incidence and symptom profiles of the different pathogens varied greatly between regions. The predominant pathogens in the winter rainfall region were *Pa. chlamydospora* and *Pm. minimum* and both were isolated from all the symptom types. The Botryosphaeriaceae had a higher occurrence in the marginal rainfall region and was mostly associated with brown internal necrosis, wedge-shaped necrosis and watery necrosis. This indicates that climate influences the distribution and the symptomatology of the pathogens in a climatic region. The results furthermore showed that symptomatology overlap greatly between pathogens, making symptom-based diagnosis of these diseases and their aetiology unreliable. Therefore, management strategies for the different pathogens in a specific region should be aimed at the whole complex of trunk disease pathogens.

SUMMARY

Grapevine trunk diseases, caused by a range of pathogenic fungi, represent a serious impediment to wine and table grape production. Little was known, about the influence of climate on the disease symptomatology and of the different pathogens in a specific area. A survey was conducted in 30 wine and table grape vineyards in summer, marginal and winter rainfall regions of South Africa. The incidence of the different plant pathogenic fungal genera and species varied between regions, with overlapping symptom profiles that differed based on the climatic region. These findings suggest that the distribution and symptomatology of grapevine trunk pathogens are strongly influenced by climatic conditions in a specific production region.

ACKNOWLEDGEMENTS

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Molecular identification of the leafhopper species that vectors Aster yellows phytoplasma of grapevines in South Africa

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KEYWORDS: Leafhopper, Aster yellows, *Mgenia, M. angusta, M. fuscovaria.*

During a recent study it was shown that a leafhopper identified as *Mgenia fuscovaria* (no common name) (Figure 1) by Michael Stiller (Figure 2) of the ARC-Plant Protection Research Institute transmits "*Candidatus* Phytoplasma asteris", also called Aster yellows phytoplasma (AY), to grapevine (*Vitis vinifera* L.) (Krüger *et al.*, 2011) (Figure 2). AY is an organism only recently reported in South Africa (Engelbrecht *et al.*, 2010) (Figure 2) and causes a serious disease of grapevine (Figure 3). Within South Africa the disease is restricted to only some grapevine growing regions of the Western Cape and is a quarantine organism.

Various control measures, mainly prevention of the movement of planting material, vector control, and removal of infected vines are enforced to restrict the area affected by the disease locally. Delineating surveys are done regularly to monitor the spread of the disease. Very little is known of the epidemiology of the phytoplasma locally or of the biology of the vector. In order to conduct epidemiological studies and controlled transmission experiments in which nymphs are included, unequivocal identification of the species involved is required. This fact was reinforced with the recent finding of a second *Mgenia* species, identified as *Mgenia angusta* (Theron, 1984), in vineyards by Dr. A. de Klerk (unpublished data), formerly of the ARC-Nietvoorbij (Figure 2). It has not been determined yet whether *M. angusta* is also a vector of AY. Superficially *M. angusta* looks very similar to *M. fuscovaria* and only differs in a manner requiring microscopic examination. Even under the

(IMAGE: MICHAEL STILLER, ARC-PLANT PROTECTION RESEARCH INSTITUTE).

FIGURE 1. Close-up of *Mgenia fuscovaria*, a vector of Aster yellows phytoplasma in South Africa.

FIGURE 2. Researchers who have played an important role in understanding Aster yellows disease on grapevines in South Africa. Clockwise from top left: Michael Stiller (ARC-PPRI), Dr. André de Klerk (ARC-Nietvoorbij), Prof. Johan Burger (Stellenbosch University), Roleen Carstens (ARC-Nietvoorbij), Nicoleen Douglas-Smit (Pretoria University), Mandie Engelbrecht (Stellenbosch University), Jeff Joubert (VinPro) and Prof. Kerstin Krüger (Pretoria University).

(IMAGE: ROLEEN CARTENS ARC-NIETVOORBIJ).

FIGURE 3. Aster yellows disease on Chardonnay, Vredendal.

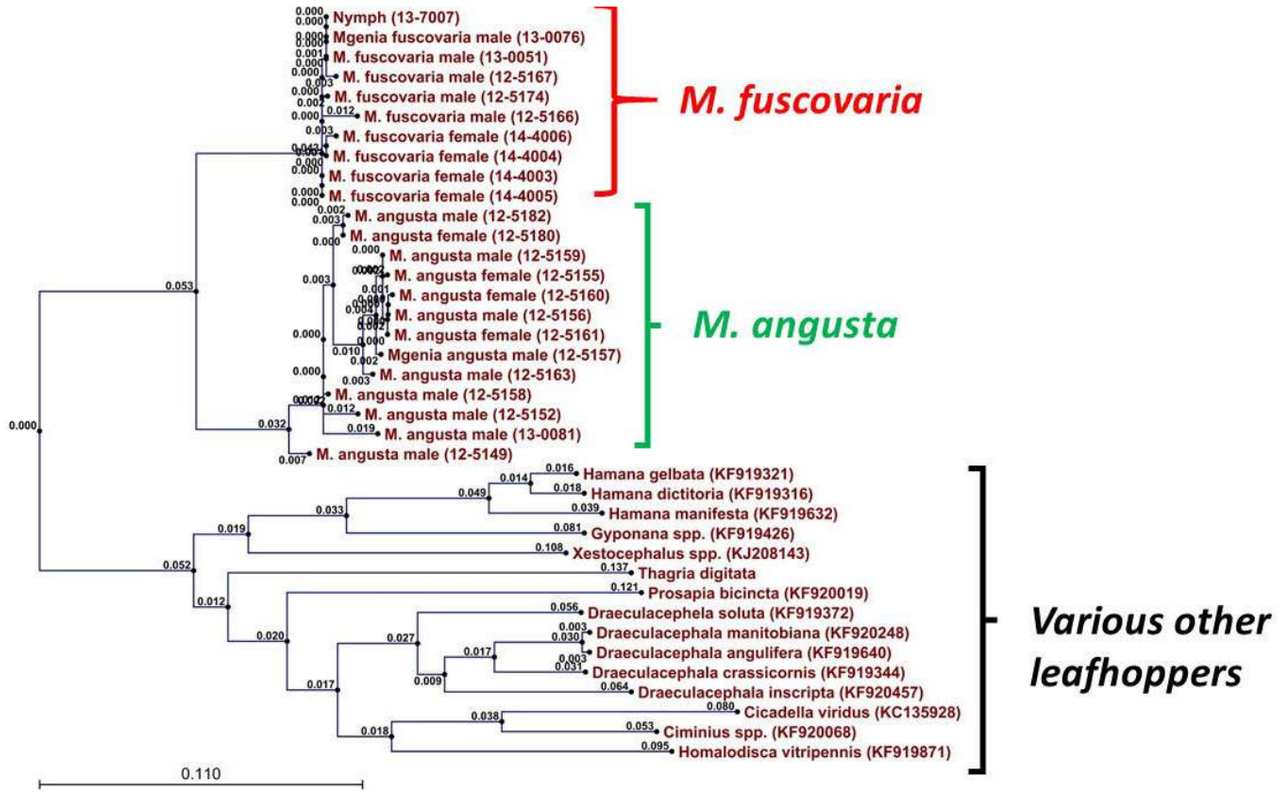

FIGURE 4. Phylogenetic tree of the mitochondrial cytochrome oxidase subunit 1 gene (mtCOI) of *Mgenia fuscovaria*, *M. angusta* and the closest leafhopper sequences currently available on the Genbank database.

microscope these differences are small and unlikely to be recognised by plant pathologists studying AY, and will be difficult to detect when testing large numbers of specimens. Nymphs of these species cannot be identified specifically as one of the two species.

In this study we have determined the nucleotide sequence of the mitochondrial cytochrome oxidase I gene (mtCOI), commonly used in insect barcoding, of a number of specimens of both *M. fuscovaria* and *M. angusta.* We have shown that the mtCOI sequences of males, females or nymphs specimens within a single species did not differ significantly, but that some nucleotide sequence differences were observed between *M. fuscovaria* and *M. angusta* specimens. The sequence is useful to differentiate *M. fuscovaria* and *M. angusta* individuals (see the two separate “branches” on the tree in Figure 4). These differences however were insufficient to allow the development of PCR systems specific to each species and currently we still need to sequence the PCR products to identify members of the two species.

ACKNOWLEDGEMENTS

I would like to thank Winetech, South Africa for funding; Dr. André de Klerk and Dr. Kerstin Krüger (Pretoria University) for collection of the majority of the samples, Gert and Inge Pietersen for doing the PCR reactions and sequences and Michael Stiller for morphological identification of specimens.

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Are mulches useful for Integrated Pest Management?

AUGUST 2016

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KEYWORDS: Mulches, IPM, arthropod, insect.

The degradation of sensitive agro-ecosystems coupled with the loss of biodiversity, and the enhancement of crop production, crop yield, as well as the reduction of crop production cost, have long been at odds, since chemical pesticides and crop enhancement products are among the major causes for biodiversity loss. That is, until the incorporation of Integrated Pest Management (IPM) methods into agricultural practices. IPM strives to optimise agricultural yields, minimise synthetic inputs, suppress pest populations to acceptable economic thresholds and boost ecosystem services by using biologically based pest management methods. This is achieved by promoting beneficial organisms and crop health. Beneficial organisms may promote crop health directly, via pollination, and indirectly via both biotic and abiotic means. Biotic benefits include the cultivation of habitats for natural enemies of crop pests and abiotic benefits are achieved by ecosystem engineers such as earthworms which aerate the soil.

Mulching is one such IPM method and was originally designed to benefit crop health via abiotic factors. Mulch consists of either woodchips, compost, straw, or black plastic, amongst others, which are applied to the soil surface surrounding the crops. The presence of mulch positively influences the crops by balancing soil humidity, temperature and structure. Mulches also increase the organic matter in soil which improves the crop's affinity for pest resistance due to improved soil microbial activity, soil nutrient balance and decreased nitrogen content. The lesser known positive influence of mulching may be seen in its effect on arthropod communities. There are indications that mulching may influence ovipositional preferences, host plant discrimination and location as demonstrated by the pest insects' ability to recognise crops as potential hosts, whereas the decreased soil

FIGURE 1. Vineyard with mulch on the vine row.

temperature may affect the completion of the life cycle of pest insects. There are many positive benefits that have been observed in mulched vineyards and orchards, such as an increase in natural enemies, though there have been a few negative effects too, which show that mulches have the potential to affect the arthropod composition in crop systems. This study therefore aimed to examine preliminary effects of various mulches on general soil-arthropod assemblages in South African vineyards in order to make recommendations for future research.

Four vineyards were sampled over one growing season in 2010 using pitfall traps, comparing plots with mulch layers (Fig. 1) and plots without mulch layers (clean cultivation). Mulches that were applied at the sites included woodchips, compost (comprising a dense layer of grape pomace) and straw. It was found that mulched sites had less pests and a greater arthropod diversity than non-mulched sites. Mulches could provide an ideal habitat for many insect species, improving the diversity of arthropods and thereby resulting in a more stable system. Springtails, which are common prey for many insect predators, as well as ants were also in higher abundance in non-mulched sites (control), which explains why predator populations were higher in non-mulched sites (Fig. 2). Ants have been shown to prefer bare soil in previous studies, as this provides optimal temperature conditions for their nests underground. Egg parasitoids, which are associated with predator roles, were also higher in mulched sites (Fig. 3). Mealybug parasitoids were found in the pitfall traps, which may show the role that mulches play as predator refugia and forage grounds on vineyard floors, though no significant difference was found between treatment (mulch) and control sites (non-mulch). The results further showed that there was a high degree of similarity between mulched sites, and that un-mulched sites also indicated a similar arthropod assemblage structure, which is a strong indicator that mulches do affect insect populations.

Pre-mulching sampling, compared to post-mulching sampling, would give a good idea of how mulching affects the arthropod assemblage and multi-season sampling would give an indication of seasonal influences. Although pitfall traps were adequate in this preliminary study, it was not ideal as an indicator of vine mealybug presence (a major vine pest) and the use of sticky traps and arboreal sampling would be recommended. A predator/prey interaction study would be useful to fully highlight mulch effects and benefits and this study suggests vine mealybug/ant/parasitoid tritrophic interaction as a model for future studies.

These preliminary results suggest that mulches do indeed affect arthropod assemblages; with higher arthropod diversity including more omnivorous species (generalist feeders) and fewer pest species and that they could therefore be useful for IPM.

ACKNOWLEDGEMENTS

This research formed part of an honours project conducted by Anna Baauw (Department of Conservation Ecology and Entomology, Stellenbosch University). The research was funded by Winetech and THRIP (Grant-specific unique reference number {UID} 71909).

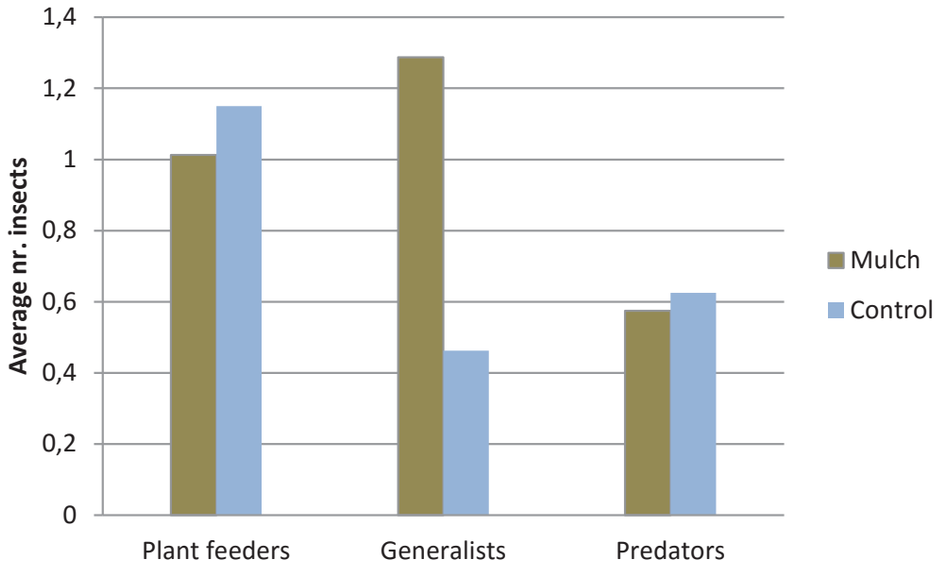

FIGURE 2. Average number of insects found in mulched plots versus control plots (clean cultivation) using pitfall traps during 2010. Plant feeders include potential pest species, generalists are omnivorous, while predators include predators and parasitic wasps.

FIGURE 3. Parasitic wasp (*Anagyrus* sp.), a natural enemy of the vine mealybug.

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New insect identification service for the fruit and wine industry – the key to good management

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KEYWORDS: Insect ID service, fruit and wine industry, IPM.

Correct insect pest identification is a crucial first step for the implementation of effective Integrated Pest Management (IPM) programs in the fruit and wine industries. IPM is based on the concept that targeted controls of specific pests will have less harmful side effects and will be more cost effective in the long run than application of general wide-ranging insecticides. Therefore, effective IPM requires sound knowledge of a pest's biology and correct identification of the pest species. Since 2014, the IPM research group at Stellenbosch University's Department of Conservation Ecology and Entomology has provided a user-pay insect ID service for the fruit and wine industry, and the general public.

Since 2008 when the service first started as a four year, industry funded project, the Insect ID service has processed over 500 samples submitted by a variety of users and industries. On average, 1.5 samples have been processed per week throughout the year, with peaks in the summertime when insects are more active and destructive to crops. Approximately half of all samples (47%) were submitted by non-wine fruit industries, particularly pome fruits and berries, while one-tenth were samples from the wine industry (Fig. 1). Samples which were submitted ranged from photographs of leaf or fruit damage to whole insects, larvae, pupae or adults, found in orchards or vineyards. Moths (Lepidoptera) were the most frequently identified pest insects, on various crops and weeds in vineyards/orchards (Fig. 2).

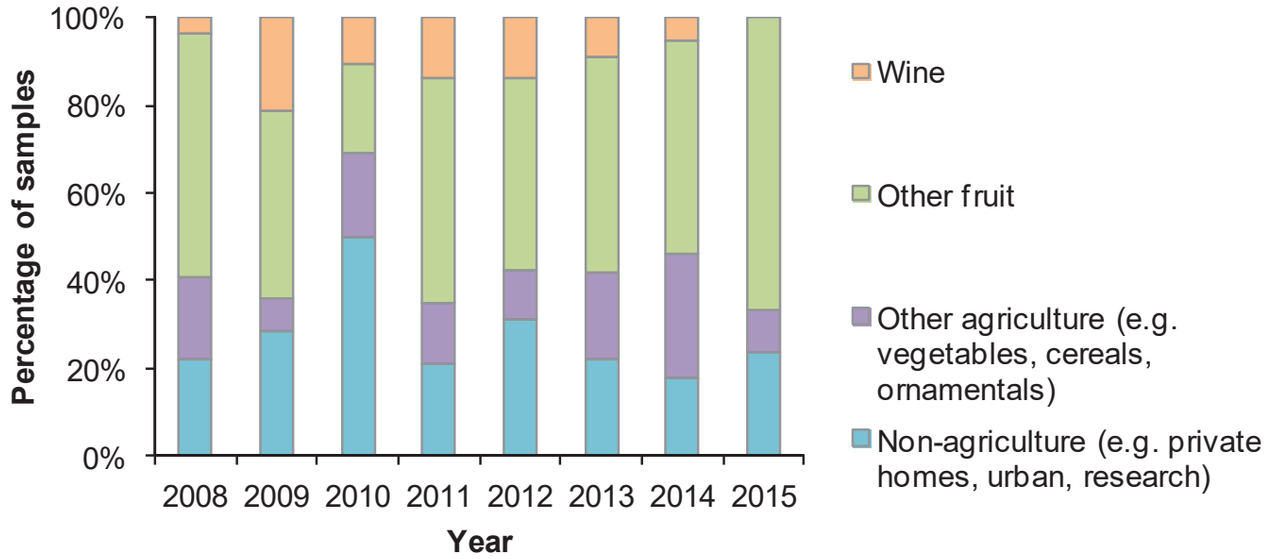

FIGURE 1. Percentage of samples submitted to Stellenbosch University's Insect ID service by commodity.

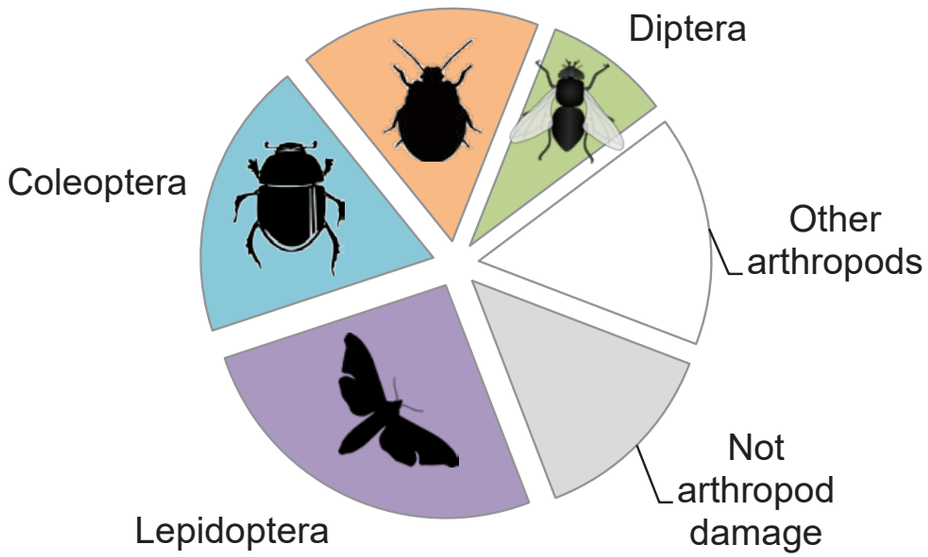

FIGURE 2. Distribution of samples submitted for insect ID over a four year period from 2008, by insect order. Lepidoptera (= moths and butterflies), Coleoptera (= beetles), Hemiptera (= sucking bugs, e.g. mealybugs and aphids), and Diptera (= flies).

Of the specimens submitted to the Insect ID service, the four most common insect orders were the moths and butterflies (order Lepidoptera, 26% of specimens), weevils and beetles (Coleoptera, 19%), true bugs, stinkbugs and mealybugs (Hemiptera, 17%), and flies (Diptera, 9%). The remaining specimens belonged to a variety of arthropod orders including mites, ants, cockroaches, silverfish and thrips (16% of specimens). A significant proportion of submitted samples consisted of damaged plant material which could not be attributed to mites, insects, or any other arthropods (13% of specimens).

Thirty-five pests are known to occur in South African grapevines (Allsopp *et al.*, 2015). Of the wine industry samples submitted to the Insect ID service, 39% belonged to one of those 35 species, and one submitted sample of Spotted Amber Ladybird is a known predator of mealybug and is, therefore, a beneficial insect. The remaining samples were most likely incidental collections of insects that make up the natural biodiversity within a wine production area.

The submission of these insects to the Insect ID service attests to the important role that the service plays for the fruit and wine industry. For example, moths and caterpillars can be notoriously hard to identify, even by seasoned entomologists. Ten moth species were submitted to the Insect ID service, six of which are listed as pests of grapevines:

- False Codling Moth (FCM),
- Trimen's False Tiger,
- Apple Trunk Borer,
- Large Striped Hawk Moth,
- Grapevine Hawk Moth, and
- Tomato Moth (see Fig. 3 for some examples).

FIGURE 3. Various species of insects causing damage on vines: a) Trimen's False Tiger, *Agoma trimenii* (Lepidoptera: Agaristidae) (Photo: Henk Geerstema); b) Tomato Moth larvae, *Spodoptera littoralis* (Lepidotera: Noctuidae); c) Adult Tomato Moth.

These six species require monitoring, in certain cases, more intense management, while the four other incidental species do not. In this case, submission of samples to the Insect ID service was able to provide an early identification of species and to distinguish between those species which are pests versus those which are not. This hopefully contributed to saving the grower time and money by allowing for the prediction of potential pest outbreaks early and to manage accordingly. The service therefore aims to help save costs associated with incorrect management for species which are not economic pests and would not cause significant damage, while also minimising the effects of such treatments on the environment.

To date, the Insect ID service has focused its efforts on developing a database of fruit pests of the Western Cape. Future efforts will focus on expanding the Insect ID service to provide a greater volume of targeted identification of pests and damage, and ultimately to promote IPM strategies as a viable alternative to traditional pest management techniques.

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This article originates from research funded by Winetech and the final report of project US E PA 01, "Generating a taxonomic database of fruit pests in the Western Cape", can be downloaded from http://www.sawislibrary.co.za/dbtextimages/AddisonP8.pdf.

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Susceptibility of grapevine pruning wounds to trunk pathogen infection

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KEYWORDS: *Eutypa, Phaeomoniella, Botryosphaeriaceae, Diaporthe, Phaeoacremonium.*

INTRODUCTION

Species within the Botryosphaeriaceae, *Diaporthe* and *Phaeoacremonium*, as well as *Phaeomoniella chlamydospora* and *Eutypa lata* have been shown to be part of a pathogen complex causing trunk diseases of grapevines. Pruning wounds are known infection portals for all of these pathogens. Several studies have indicated that air-borne inoculum of the respective pathogens is present in vineyards for long periods of time, especially when weather conditions are favourable for spore release and dispersal and therefore available for infection of susceptible wounds.

Due to the lack of curative control measures for trunk diseases, pruning wound protection is of critical importance. For the development of a successful pruning wound protection strategy, knowledge about the duration of pruning wound susceptibility to infection is important; especially to determine the timing of treatment and duration of protection required.

The aim of this study was therefore to determine the duration of pruning wound susceptibility to natural and induced infection by the major pathogens associated with grapevine trunk diseases. This knowledge would aid in the development of pruning wound protection strategies against trunk disease pathogens in South African vineyards.

MATERIALS AND METHODS

Pruning and inoculation

Grapevines in an 18-year-old vineyard, cv. Chenin blanc, in the Stellenbosch region were spur-pruned to three buds per cane at two stages [mid- (July) and late winter (August)] during the dormant seasons of 2004 and 2005. Individual pruning wounds were spray-inoculated with a spore suspension of either *E. lata*, *Pa. chlamydospora*, *Neofusicoccum australe* or *Diaporthe ampelina* (previously known as *Phomopsis viticola*) directly after pruning, and 1, 2, 3, 7, 10, 14, 17 and 21 days after pruning. Two non-inoculated treatments (sprayed with water or painted with wound sealant) were also included.

Trial evaluation and statistical analysis

Eight months after treatment, the distal internodes containing the treated pruning wounds were removed and taken to the laboratory for pathogen isolation. The occurrence of the inoculated pathogens in the tissues directly beneath the pruning wound scar was subsequently determined. This was done by removing tissue sections from the woody tissues and plating them out onto Petri dishes containing potato dextrose agar. Petri dishes were incubated at 25°C for two to four weeks before morphological identification of the isolated fungi. The incidence of the inoculated pathogens was subsequently calculated for the xylem and pith tissues and the whole pruning wound stub. Infections from aerial spores present in the vineyard were determined from the paint and water treatments. Lesions observed as vascular browning in the pith and xylem tissue of the split pruning wound stubs were also measured and recorded. The pathogen incidence data was subsequently subjected to appropriate statistical analyses.

Weather data

In the trial vineyard block a weather station recorded hourly measurement of rainfall, temperature, relative humidity and wind speed during the trial periods of 2004 and 2005.

RESULTS

Pathogen incidence in treated pruning wounds

Inoculated pruning wounds showed distinct dark brown to black streaking developing from the wound (Figure 1). Wounds inoculated directly after pruning in July 2004, yielded significantly lower mean pathogen incidences compared with wounds made in August 2004 (Figure 2). Results from both months in 2004 indicated a slow decline in pathogen incidence with increasing wound age at treatment (Figure 2, Table 1).

In 2005, wounds inoculated directly after pruning yielded similar pathogen incidences in wounds made in July compared to August (Figure 2, Table 1). Results

FIGURE 1. Pruning wounds of Chenin blanc showing distinct dark brown to black streaking developing from wounds (right hand side) inoculated with spore suspensions of: a. *Neofusicoccum australe*, b. *E. lata*; c. *Pa. chlamydospora* and d. *Diaporthe ampelina*.

indicated a more rapid decline in pathogen incidence with increasing wound age at treatment compared to 2004. The decline was also significantly more pronounced in wounds made in July 2005, compared with August 2005 (Figure 2).

When looking at individual pathogen groups' occurrence in 2004, the mean *Diaporthe* spp. incidence in inoculated pruning wounds increased slightly with increasing wound age at treatment. The mean pathogen incidence in wounds inoculated with *Pa. chlamydospora* remained almost constant with increasing wound age at treatment. Only in the case of pruning wounds inoculated with Botryosphaeriaceae spp. and *E. lata* did the mean pathogen incidence show a decline with increasing wound age at treatment (Figure 3). However, opposed to 2004, in 2005 all pathogens showed a decline in the mean pathogen incidence in inoculated pruning wounds (Figure 3). The mean pathogen incidence in *Diaporthe* spp. inoculated pruning wounds declined at the slowest rate with increasing wound age at treatment while the rate of decline in pruning wounds inoculated with *Pa. chlamydospora*, Botryosphaeriaceae spp. and *E. lata* were much faster (Figure 3).

Weather data

Average temperature for the 21 days after pruning in July 2004 was 13.1°C and for the same period after pruning in August 2004 was 13.5°C. This is in comparison with 14.8°C for the period after the July 2005 pruning and 14.6°C for the August 2005 pruning time. Rainfall recorded for the 21 days after the respective pruning times was 2.6 mm for July 2004, 1.4 mm for August 2004, 3.3 mm for July 2005 and 3.3 mm for August 2005.

DISCUSSION

Previous studies on the susceptibility of grapevine pruning wounds reported that wounds made early in the dormant season were more susceptible, and remained susceptible for longer, to infection by *E. lata*, *Pa. chlamydospora* and *Phaeoacremonium minimum* as opposed to pruning wounds made later in the dormant season. All of these studies also found that wound susceptibility declined with increasing wound age.

The present study is the first study comparing pruning wound susceptibility (at various wound ages) with different pruning times during the pruning season and to a wider variety of trunk disease pathogens. Results support the previous studies as pruning wound susceptibility declined with increasing wound age irrespective of the time of year pruning took place. However, differences in the rate of decline in wound susceptibility were noted between 2004 and 2005. The variation observed could be explained by the mechanisms involved in wound repair and the effect climatic conditions have on these mechanisms.

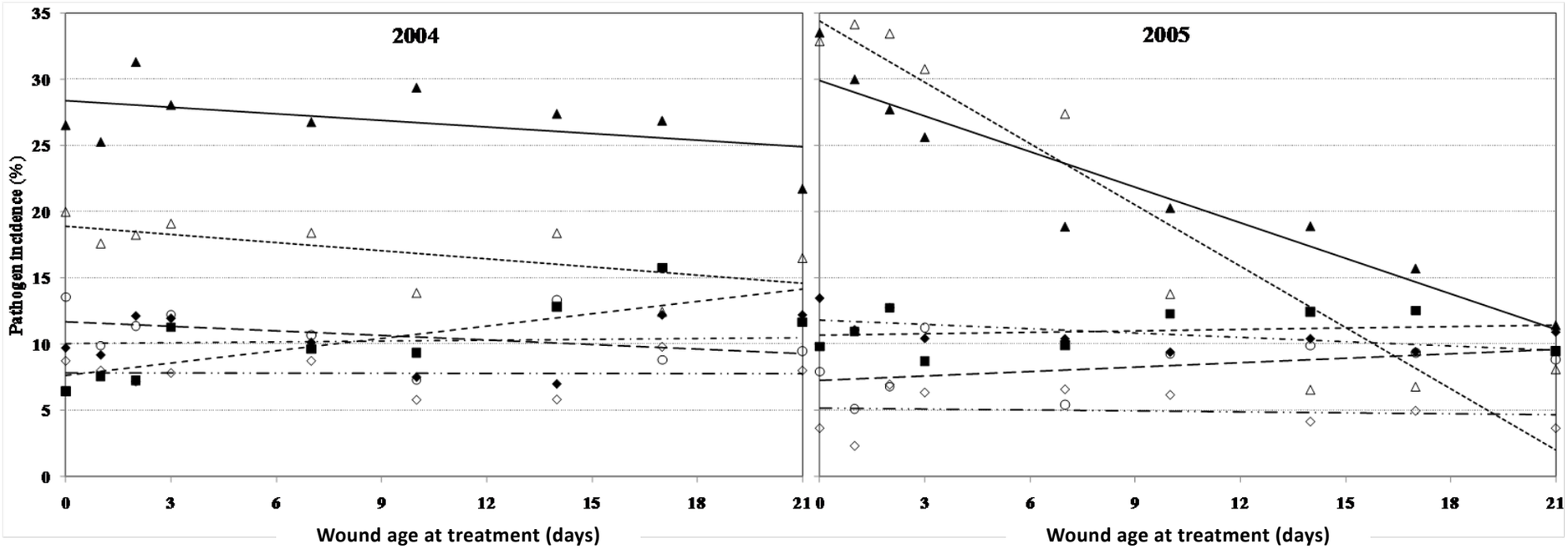


FIGURE 2. Linear regression lines [August inoculated (——); July inoculated (– – –); August painted (– –); July painted (– –); August water (– – –); July water (– – –)] fitted to the mean pathogen incidence [August inoculated (▲); July inoculated (Δ); August painted (■); July painted (○); August water (♦); July water (◊)] over wound age at treatment for the significant season x pruning time x wound age at treatment x treatment interaction observed in the 2004 and 2005 pruning seasons.

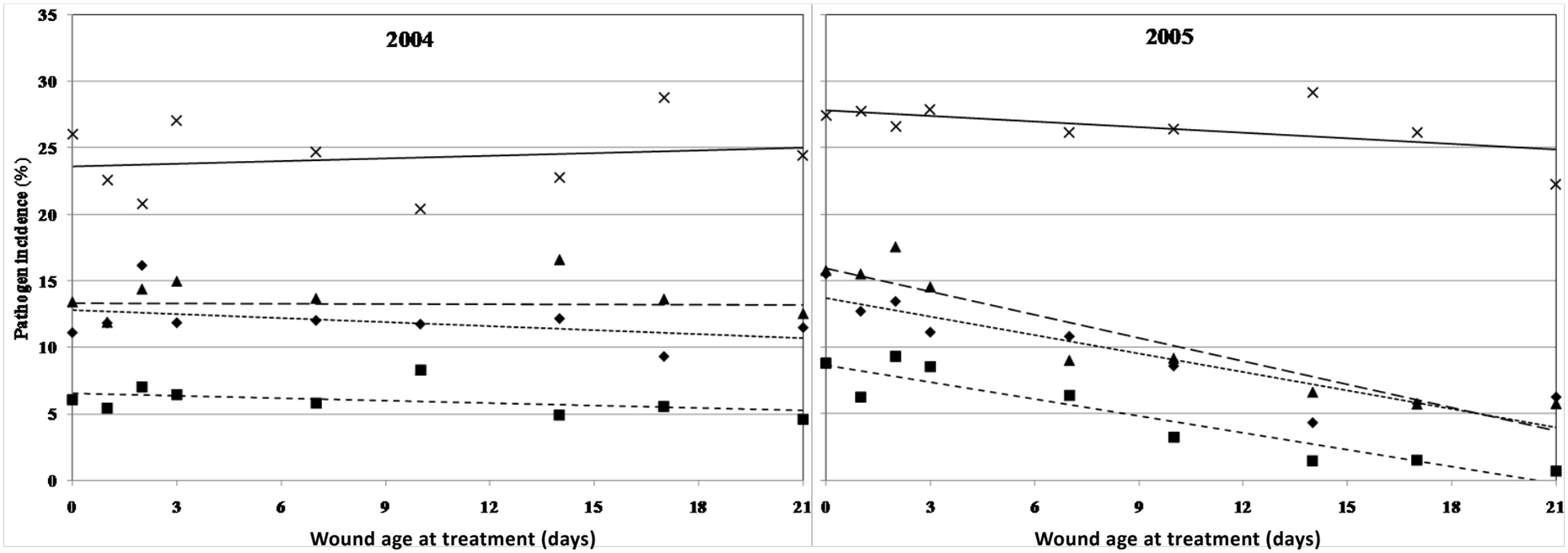


FIGURE 3. Linear regression lines [Botryosphaeriaceae spp. (----); E. lata (– – –); *Pa. chlamydospora* (– –); *Diaporthe spp.* fitted to the mean pathogen incidence [Botryosphaeriaceae spp. (♦), *E. lata* (■), *Pa. chlamydospora* (▲) and *D. ampelina* (✕)] over wound age at treatment for the significant year x pruning time x wound age at treatment interaction observed in the 2004 and 2005 pruning seasons.

TABLE 1. Mean incidence of *Eutypa lata*, *Phaeomoniella chlamydospora*, Botryosphaeriaceae and *Diaporthe* spp. in the xylem and pith tissues of pruning wounds that were made during July and August 2004 and 2005 and either painted, water treated or inoculated with *Eutypa lata*, *Phaeomoniella chlamydospora*, *Neofusicoccum australe* and *Diaporthe ampelina.*

Treatment	Pruning time					
	July			August		
	Inoculated*	Paint	Water	Inoculated	Paint	Water
Xylem						
Botryosphaeriaceae spp.	25.70d	7.79l-p	0.55pqr	33.29bc	7.17m-q	6.09pqr
E. lata	14.91hi	2.35rs	1.04s	20.34fg	0.41s	0.08s
Pa. chlamydospora	21.60ef	10.47j-o	3.86qrs	20.39fg	8.59k-p	7.89l-p
Diaporthe spp.	31.57bc	30.06bc	20.59fg	42.25a	35.63b	33.24bc
Pith						
Botryosphaeriaceae spp.	10.71j-n	1.15s	1.04s	10.51j-n	1.08s	0.56s
E. lata	10.72j-n	0.30s	0.00s	10.93j-m	0.07s	0.00s
Pa. chlamydospora	11.89ijk	7.03n-q	2.45rs	24.95de	14.20hij	12.73ij
Diaporthe spp.	11.37i-l	7.97l-p	6.63opq	17.47gh	13.88ij	12.88ij
LSD ($P < 0.05$)	3.877					

* Means followed by the same letter are not significantly different ($P < 0.05$).

At the trial site weather data showed that the average temperature and total rainfall for the 21 days after pruning in July and August 2005 was higher compared to the same periods for the July and August 2004 pruning times. Therefore, the pruning wound repair processes might have been slower in 2004 compared to 2005, due to the lower average temperature and total rainfall during the trial months of July and August. These higher temperatures and rainfall in 2005 could also have contributed to a faster colonisation of pruning wounds by naturally occurring epiphytes, therewith reducing pruning wound susceptibility.

Despite the variation observed between the two years, wounds remained susceptible to infection by all inoculated pathogens for at least three weeks. Pruning wounds that were made and inoculated during August in both years, generally had higher incidence of infection compared to the pruning wounds inoculated in July, which suggests that wounds made later in the dormant season are more susceptible to infection than wounds made earlier. This could also indicate that it is not necessarily the time of year that pruning is done that determines the period of wound susceptibility, but rather the climatic conditions experienced after pruning.

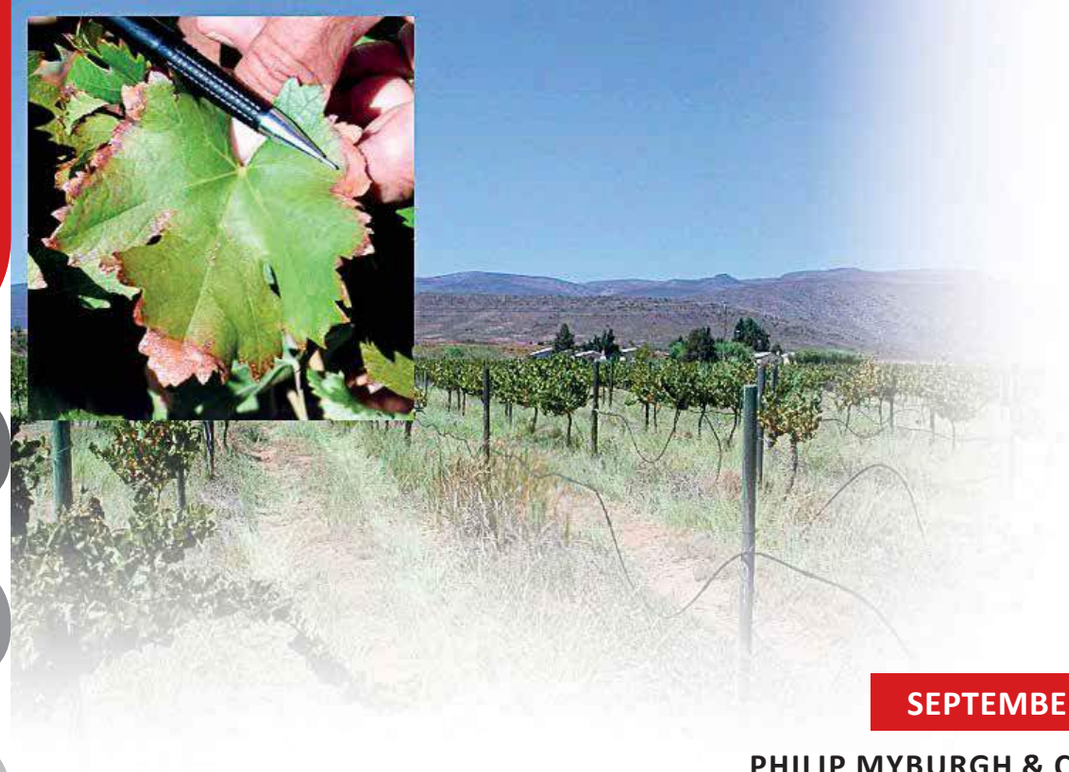
As in previous studies, pruning wounds remained susceptible to infection to all major trunk disease pathogens for an extended period after pruning, regardless of when pruning was done. It is therefore of utmost importance that grapevine pruning wounds should be protected for prolonged periods after pruning against infection by all pathogens implicated in the trunk disease complex by means of chemical and/or biological agents.

SUMMARY

Eutypa lata and *Phaeomoniella chlamydospora*, as well as several species in Botryosphaeriaceae, *Diaporthe* and *Phaeoacremonium* are trunk pathogens of grapevines that infect via pruning wounds. Duration of pruning wound susceptibility to some of these pathogens was largely unknown. Plants of the cv. Chenin blanc were pruned at two stages and spray-inoculated with spore suspensions of four important trunk pathogens directly after pruning, and 1, 2, 3, 7, 10, 14, 17 and 21 days after pruning. Results indicated that, irrespective of pathogen inoculated, pathogen incidence in the inoculated pruning wounds of both mid- and late winter declined with increasing wound age. Wounds remained susceptible for 3 or more weeks after pruning. Late winter (August) wounds were more susceptible to infection than wounds made earlier (July) in the season.

This article originates from research funded by Winetech and the final report of project US E PA 01, “Generating a taxonomic database of fruit pests in the Western Cape”, can be downloaded from http://www.sawislibrary.co.za/dbtextimages/AddisonP8.pdf.

For more information contact Lizel Mostert at lmost@sun.ac.za.



Vineyard responses to salinity-associated soil chemical properties

SEPTEMBER 2016

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KEYWORDS: Basic cations, EC_e, juice characteristics, pH, SAR, thresholds, toxicity, vegetative growth, yield.

INTRODUCTION

Soil and irrigation water of irrigated areas are becoming increasingly saline, particularly in semi-arid areas. In addition to the negative effects of high salt content, there is ample evidence that Na and Cl toxicity can decrease vineyard growth and yield (Moolman *et al*., 1999; De Clerque *et al*., 2001). Therefore, Na and Cl uptake and accumulation in grapevines should be as low as possible. Leaching of excessive or toxic ions from the root zone is not always a practical solution, particularly in soils with poor internal drainage or where the leachate can pollute natural resources. The objectives of the project were to determine (i) how salinity affects grapevines and (ii) how ion balances in soil, e.g. the sodium adsorption ratio (SAR), affect ion uptake and accumulation in grapevines.

EXPERIMENTAL BACKGROUND

The study was carried out in 13 full-bearing vineyards during the 2000/2001 season near Robertson in the Breede River Valley. Chardonnay and Ruby Cabernet, both grafted onto 110 R and 101-14 Mgt rootstocks were used for the study. Each vineyard had a poor performing patch that was presumably caused by high salinity or Na (Fig. 1). Soil samples were collected in the poor patches, as well as in the better parts. The $pH_{(KCl)}$, electrical conductivity (EC_e), water soluble K, Na, Ca, Mg, Cl and SO_4 in the 0 - 30 and 30 - 60 cm layers were determined by the soil laboratory at ARC Infruitec-Nietvoorbij. The adsorption ratios for sodium (SAR) and potassium (PAR) were calculated from the basic cations. Leaf and juice N, P, K, Na, Ca, Mg, Cl and SO_4 contents were determined at harvest by a commercial laboratory (Bemlab, Strand). Yield, number of bunches per grapevine and bunch mass were determined. Juice pH, TSS and TTA were determined by the winery at ARC Infruitec-Nietvoorbij. Cane mass per grapevine was measured at pruning in August 2001.

The boundary line approach was used to determine trends in vineyard reaction to salinity-associated soil properties. Boundary lines for a specific crop can be obtained by sampling the variation that occurs naturally under a range of conditions within a specific region (Walworth *et al.*, 1986). To obtain a boundary line, a scatter plot of plant response against a plant growth factor is created. In most cases, it would be possible to draw a line, or lines, which confines the data. Therefore, the boundary line describes the sole effect of a plant growth factor where no other limiting factors occur. More complete details of the experiment procedures were reported earlier (Myburgh & Howell, 2014).

RESULTS AND DISCUSSION

The following is only a summary of the boundary line trends. The actual boundary line plots were published earlier (Myburgh & Howell, 2014).

Leaf macro element content

Under the prevailing conditions, there was no relationship between leaf N and P and any of the measured soil variables (data not shown). Leaf K increased linearly with soil $pH_{(KCl)}$ to a maximum at $pH_{(KCl)}$ 7.5. This was followed by a linear decline (Table 1). Similarly, leaf Ca reached a maximum at $pH_{(KCl)}$ 7. Since leaf Ca decreased linearly as soil Na:Ca increased from 0.1 to 15, it suggested that high soil Na supressed Ca uptake and/or translocation to the leaves.

FIGURE 1. One of the poorly growing patches where soil chemical status and grapevine response were measured in the Robertson area. Insert shows leaf damage caused by high soil salinity.

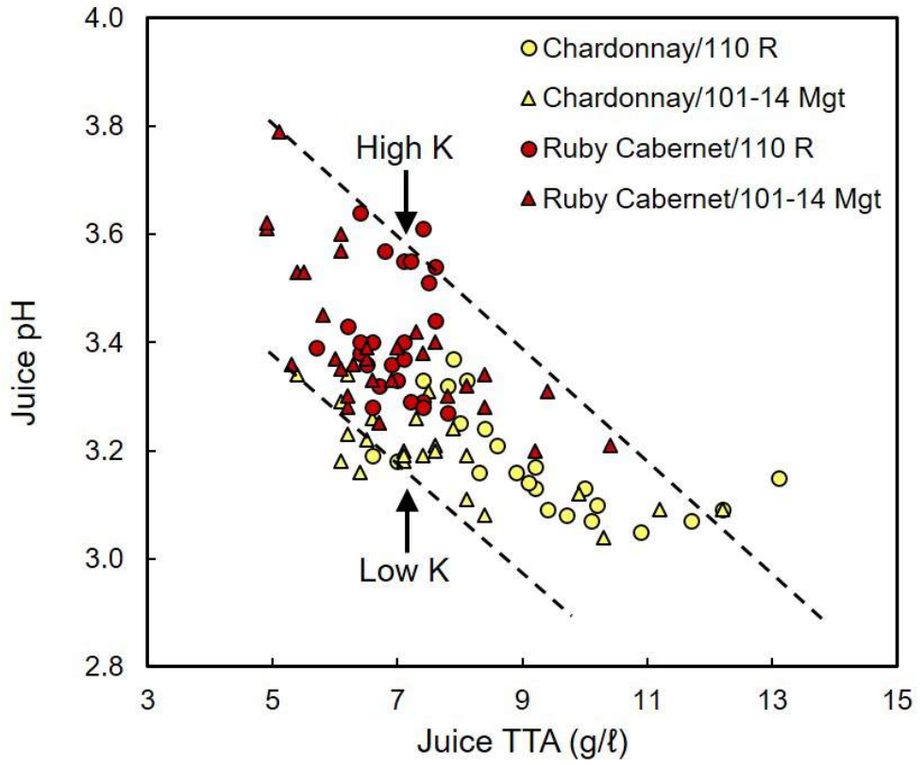

FIGURE 2. The relationship between juice pH and total titratable acidity (TTA) for four scion/rootstock combinations. Dashed lines indicate the boundary lines.

TABLE 1. Effect of soil chemical properties on cations and B in grapevine leaves. Values in brackets indicate the observed variation of the different variables.

Element	Soil property	Responses of element contents in leaves
Ca (0.9 to 3.2%)	Soil $pH_{(KCl)}$ (3.5 to 8.2)	Maximum leaf Ca occurred at $pH_{(KCl)}$ 7.
	Soil Na/Ca (0.1 to 15)	Leaf Ca decreased linearly as soil Na/Ca increased.
Mg (0.2 to 1.1%)	PAR(1) (0.1 to 1.7)	Leaf Mg decreased linearly as PAR increased.
	Soil SO_4 (1 to 200 mg/ℓ)	Leaf Mg decreased linearly as soil SO_4 increased.
K (0.1 to 2.5%)	Soil $pH_{(KCl)}$ (3.5 to 8.2)	Optimum leaf K reached at $pH_{(KCl)}$ 7.5.
	Soil SO_4 (1 to 200 mg/ℓ)	Leaf K decreased linearly as soil SO_4 increased.
Na (0.01 to 0.2%)	Soil K/Na (0.01 to 1.1)	Leaf Na decreased non-linearly as soil K/Na ratio increased, particularly when soil K/Na ratio is less than c. 0.15.
B (30 to 270 ppm)	Soil $pH_{(KCl)}$ (3.5 to 8.2)	Maximum leaf B reached at $pH_{(KCl)}$ 7.
	Soil Na (0.1 to 38 mg/ℓ)	Leaf B decreased non-linearly as soil Na increased.

(1) Potassium adsorption ratio.

TABLE 2. Effect of soil chemical properties and leaf element content on cane mass. Values in brackets indicate the observed ranges of the different variables.

Soil/Plant variable	Cane mass response (0.1 to 1.5 kg per grapevine)
Soil $pH_{(KCl)}$ (3.5 to 8.2)	Cane mass increased linearly as $pH_{(KCl)}$ increased. Chardonnay/110 R more sensitive than other combinations.
Soil EC_e[1] (0.1 to 7 dS/m)	Distinct threshold value could not be identified. Cane mass declined at a rate of 14% per dS/m.
Leaf Ca (0.9 to 3.3%)	Maximum cane mass reached at 2.4% leaf Ca.
Leaf K (0.1 to 2.6%)	Linear cane mass increase as leaf K increased. Cane mass of Chardonnay/110 R remained at c. 0.6 kg/vine at leaf K > 1.5%.
Leaf Na (0.01 to 0.2%)	Cane mass decreased non-linearly as leaf Na increased.
Leaf Cl (0.1 to 0.85%)	Cane mass decreased linearly as leaf Cl increased.

[1] Electrical conductivity of the saturated soil paste.

TABLE 3. Effect of soil chemical properties on yield. Values in brackets indicate the observed ranges of the different variables.

Soil variable	Yield response (2 to 25 ton/ha)
EC_e[1] (0.1 to 7 dS/m)	Yield showed a distinct threshold value at 1.8 dS/m. Yield declined linearly at a rate of 13% per dS/m above threshold.
Na (1 to 26 mg/ℓ)	Yield decreased linearly as soil Na increased.
SAR[2] (0 to 13)	Yield showed a distinct threshold value at SAR 3 and decreased linearly at a rate of 8% per SAR unit above this threshold.

[1] Electrical conductivity of the saturated soil paste.

[2] Sodium adsorption ratio.

TABLE 4. Effect of soil chemical properties and cane mass on juice Na. Values in brackets indicate the observed ranges of the different variables.

Soil/Plant variable	Juice Na response (1 to 130 mg/ℓ)
Soil (K + Ca/2):Na (0 to 12)	Juice Na increased non-linearly as soil (K + Ca/2):Na ratio decreased, particularly when it was lower than 2.
Cane mass (0.1 to 1.5 kg/vine)	Juice Na decreased non-linearly as cane mass increased.

Vegetative growth

Cane mass increased linearly as the soil $pH_{(KCl)}$ increased from 3.5 to 8.2 (Table 2). Under the prevailing conditions, vegetative growth of Chardonnay/110 R tended to be more sensitive for soil pH than the other scion/rootstock combinations. Cane mass did not show a distinct EC_e threshold value, but declined at a rate of 14% per dS/m (Table 2). Maximum cane mass occurred when leaf Ca was 2.4%. Cane mass increased linearly as leaf K increased. However, cane mass of Chardonnay/110 R remained constant at approximately 0.6 kg per grapevine when leaf K levels exceeded 1.5%. Cane mass decreased non-linearly as leaf Na increased from 0.01 to 0.2%. Cane mass also decreased as leaf Cl increased from 0.1 to 0.85%, but this trend was linear compared to the response to leaf Na. The foregoing trends clearly indicated the toxic effects of high Na and Cl on vegetative growth.

Yield

Large variation, from 2 to 25 ton/ha, occurred in the yield. Yield tended to remain constant up to an EC_e threshold value of 1.8 dS/m (Table 3). Above this threshold value, yield declined linearly at a rate of 13% per dS/m. Sodium had a negative effect on yield above a SAR threshold value of 3 (Table 3). Above the threshold value, yield decreased linearly as SAR increased to 13.

Juice TTA, pH and K

The Chardonnay juice generally had a higher TTA and lower pH than the Ruby Cabernet juice (Fig. 2). Under the prevailing conditions, juice pH increased as the TTA decreased. Near the upper boundary layer, juice K was higher than near the lower boundary line (Fig. 2). At a given TTA, a juice K increase from around 900 to 1 600 mg/ℓ, increased juice pH by approximately 0.4 units. The soil (K + Ca):Na should preferably be lower than two to reduce the risk of excessively high Na in juice and wine (Table 4). However, juice Na decreased as cane mass increased (Table 4), which indicated that vegetative growth might be a strong sink for Na.

CONCLUSIONS AND RECOMMENDATIONS

The boundary line approach can be useful to determine effects of plant growth factors on grapevine response. The selection of plots covered most of the possible variation in soil and vineyard conditions. Under the prevailing conditions, the four

scion/rootstock combinations responded almost similarly to the salinity-associated variables. Results confirmed that the ideal soil $pH_{(KCl)}$ for vineyards is around 6.0. The salinity threshold for vineyards in the Breede River Valley is between 0.7 and 1.5 dS/m. The SAR must be below 3, and the soluble Na content in the soil should not exceed 5 mg/kg, to reduce the Na toxicity risk. If gypsum is used to reduce soil Na, it must be applied judiciously to avoid soil SO_4 accumulation. The latter increases the risk of K and Mg deficiencies. Under the prevailing conditions, Cl and B toxicity probably contributed to reduced vegetative growth. Therefore, soil Cl and B must be kept as low as possible, but care should be taken that B is not reduced to deficient levels.

Serious saline or sodic soil conditions were the exception rather than the rule for vineyards in the Robertson area. Therefore, future research must be carried out in areas where the topography is such that over-irrigation of vineyards on higher land may cause salinisation of lower lying vineyards and/or natural water resources. Under such conditions, salinity could manifest on a more extensive scale. This could also increase the negative effects of salinity on the ion composition in grapevines, as well as on growth and yield.

SUMMARY

High levels of soil salinity or sodicity may have negative effects on grapevine growth, yield and quality. Soil chemical status and grapevine responses were measured in poor patches in 13 vineyards near Robertson in the Breede River Valley. Chardonnay and Ruby Cabernet, both grafted onto 110 R and 101-14 Mgt were used in the study. The boundary line concept was used to determine vineyard responses to salinity-associated soil properties. Under the prevailing conditions, the four scion-rootstock combinations responded almost similarly to the salinity-associated soil properties. Results confirmed that the ideal $pH_{(KCl)}$ for vineyard is 6. The salinity threshold for vineyards in the Breede River Valley must be between 0.7 and 1.5 dS/m (deciSiemens per metre) to avoid growth and yield losses. The SAR should be below 3, and the water soluble Na content in the soil should not exceed 5 mg/kg to reduce the Na toxicity risk. If gypsum is used to reduce soil Na, it must be applied judiciously to avoid soil SO_4 accumulation. The latter increases the risk of K and Mg deficiencies. Under the prevailing conditions, Cl and B toxicity apparently contributed to reduced vegetative growth. Therefore, soil Cl and B should be kept low. However, care should be taken that B is not reduced to levels that will cause deficiencies in vineyards.

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Herbicides and application techniques for control of leafroll infected full bearing grapevines (Phase 1): Vineyard with leafroll symptoms for four years or more

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KEYWORDS: Chemical control, foliar application, cut stump application, basal stem application, pruning wound application, grapevine growth.

INTRODUCTION

Grapevines infected with leafroll virus are increasing. As the virus may have a negative effect on grape quality and the productive lifespan of the vines, it is important to research methods by which the spread of the virus can be stopped and its occurrence effectively reduced. The danger of the virus being spread from infected vines to adjacent healthy vines by inter alia grapevine mealy bug [*Planococcus ficus* (Signoret)] necessitates fast and effective removal of these vines as a source of virus contamination. Unfortunately the mechanical removal of the grapevines leaves live roots in the soil on which mealy bug may survive to contaminate the newly planted grapevines replacing the affected vines. The aim of this study was to identify a herbicide or herbicides, combined with the correct application technique, that will kill leafroll infected full bearing grapevines that have shown leafroll symptoms for four years or more.

MATERIALS AND METHODS

Phase 1 of the study was executed in a 14-year-old Chardonnay/99 Richter vineyard which showed symptoms of being infected with leafroll virus for at least the past four seasons. The vineyard was established on a medium textured soil at Nietvoorbij Experiment farm in Stellenbosch. Fourteen treatments were applied post-harvest on 26 April 2006 and repeated 26 March 2007 (Table 1). Another 14 treatments were applied on 6 December 2006 and repeated on 12 December 2007 during grapevine berry set, as described in Table 1. The treatments were replicated three times in a randomised block design. Each replication plot consisted of six infected grapevines and the two adjacent working rows. A vine row functioned as a buffer zone between treatments situated in different working rows and two vines buffered treatment plots situated in the same vine row. The leafroll virus and mealy bug status of the grapevines were determined visually before the treatments were applied. The trial was repeated over two seasons as explained above, to accommodate the effect that seasonal climatic conditions could have on herbicide efficacy.

MEASUREMENTS

To determine the grapevine control efficacy, the vines were monitored visually for re-growth. The roots of one grapevine per treatment plot in which no re-growth occurred, were inspected visually to determine whether they are dead or alive. This was done by digging a profile pit 1.5 m long x 1.5 m wide x 1 m deep parallel to the vine row and by inspecting the four profile walls visually for any live roots. These measurements were done mid-October and mid-March, the two periods during which grapevine root growth is most active.

The effect of the different treatments on the occurrence of grapevine mealy bug on the grapevines, as well as the surrounding weeds and cover crops, was monitored visually once a month from August to May.

RESULTS AND DISCUSSION

Efficacy of herbicides applied post-harvest

2006/07 season

Only the foliar application (FA) of Brush Off® showed no above-ground re-growth up to one year after application (March 2007) (Table 2). However, active root growth was detected deeper than 10 cm in this treatment at this time. The live

TABLE 1. Treatments applied in Phase 1 during post-harvest and berry set.

Herbicide	Dosage	Application method
Roundup® (360 g/ℓ glyphosate)	5% solution	Foliar (FA)
MCPA 400 SL® (400 g/ℓ MCPA)	5% solution plus 0.05% Agral 90	FA
Brush Off® (200 g/ℓ metsulfuron methyl)	80 g/100 ℓ water plus 0.05% Agral 90	FA
Garlon 480 EC® (480 g/ℓ triclopyr)	0.5% solution plus 0.05% Actipron	FA
Confront 360 SL® (90 g/ℓ triclopyr & 270 g/ℓ clopyralid)	0.7% solution plus 0.5% Actipron	FA
Plenum 160 ME® (80 g/ℓ picloram & 80 g/ℓ fluroxypyr)	0.75% solution plus 0.5% Actipron	FA
Timbrel 360 SL® (360 g/ℓ triclopyr)	3% solution plus 0.5% Actipron	Cut stump (CS)
Confront 360 SL®	3% solution plus 0.5% Actipron	CS
Plenum 160 ME®	1.5% solution plus 0.5% Actipron	CS
Brush Off®	180 g/100 ℓ water plus 0.05% Agral 90	CS
Garlon 480 EC®	2% solution in diesel	Basal stem
Roundup®	5% solution	Fresh pruning wounds
MCPA 400 SL®	5% solution plus 0.05% Agral 90	Fresh pruning wounds
None (Control)	None (Control)	None

TABLE 2. The grapevine control efficacy of seven herbicides applied selectively by means of four application techniques after harvest in a Chardonnay/99 Richter vineyard as evaluated in March 2007, October 2007 and March 2008.

Application technique	Herbicide	Grapevines alive or showing re-growth (%)			
		Applied 26 April 2006			Applied 26 March 2007
		March 2007	October 2007	March 2008	March 2008
Foliar	Roundup® (360 g/ℓ glyphosate)	6	33	22	28
	MCPA 400 SL® (400 g/ℓ MCPA)	100	100	100	100
	Brush Off® (200 g/ℓ metsulfuron methyl)*	0[1]	0[2]	72[3]	0[1]
	Garlon 480 EC® (480 g/ℓ triclopyr)	100	39	28	44
	Confront 360 SL® (90 g/ℓ triclopyr & 270 g/ℓ clopyralid)*	100	89	94	0[1]
	Plenum 160 ME® (80 g/ℓ picloram & 80 g/ℓ fluroxypyr)*	6	22	33	0[1]
Cut stump	Timbrel 360 SL® (360 g/ℓ triclopyr)	28	28	67	67
	Confront 360 SL®	100	28	44	22
	Plenum 160 ME®	100	39	89	28
	Brush Off®	100	94	100	56
Basal stem	Garlon 480 EC®	33	17	28	6
Fresh pruning wounds	Roundup®	100	100	100	100
	MCPA 400 SL®	100	100	100	100
None	None (Control)	100	100	100	100

* Soil layer in which the roots were controlled: 1. 0-10 cm; 2. 0-35 cm; 3. 0-50 cm.

TABLE 3. The rainfall and temperature during the grapevine growing season (September to February) and post-harvest period (March to May) from April 2006 to March 2008.

Month	Rainfall (mm)			Average temperature (°C)		
	2006	2007	2008	2006	2007	2008
January	–	3	21	–	23.4	22.7
February	–	30	46	–	21.6	22.3
March	–	38	24	–	21.0	21.5
April	53	101	–	18.4	18.6	–
May	172	95	–	15.1	16.1	–
September	44	29	–	16.3	14.5	–
October	33	71	–	17.3	18.1	–
November	57	45	–	19.5	17.8	–
December	27	29	–	20.0	21.9	–

TABLE 4. The grapevine control efficacy of seven herbicides applied selectively by means of four application techniques during berry set in a Chardonnay/99 Richter vineyard as evaluated in March 2007 and March 2008.

Application technique	Herbicide	Grapevines alive or showing re-growth (%)			
		Applied 6 December 2006			Applied 12 December 2007
		March 2007	October 2007	March 2008	March 2008
Foliar	Roundup® (360 g/ℓ glyphosate)	11	6	6	44
	MCPA 400 SL® (400 g/ℓ MCPA)	100	100	100	100
	Brush Off® (200 g/ℓ metsulfuron methyl)	100	39	89	44
	Garlon 480 EC® (480 g/ℓ triclopyr)	0	0[1]	28[1]	0
	Confront 360 SL® (90 g/ℓ triclopyr & 270 g/ℓ clopyralid)	6	0[1]	61[1]	78
	Plenum 160 ME® (80 g/ℓ picloram & 80 g/ℓ fluroxypyr)	6	6	67	44
Cut stump	Timbrel 360 SL® (360 g/ℓ triclopyr)	0	0	39	0
	Confront 360 SL®	6	0	39	0
	Plenum 160 ME®	0	0	44	0
	Brush Off®	0	33	11	0
Basal stem	Garlon 480 EC®	22	22	22	0
Fresh pruning wounds	Roundup®	100	100	100	89
	MCPA 400 SL®	100	94	100	100
None	None (Control)	100	100	100	100

[1] Roots were controlled in the 0-10 cm soil layer.

roots in the deeper soil layers resulted in re-growth occurring 24 months after application (March 2008). The application date for the 2007/08 season was moved forward to 26 March (directly after harvest) to try and improve herbicide efficacy, as the average temperature in April is higher than that of May (Table 3).

2007/08 season

Brush Off® (FA), Confront 360 SL® (FA) and Plenum 160 ME® (FA) resulted in no re-growth for up to one year after application (March 2008) (Table 2). The efficacy of these treatments improved when applied directly after harvest, with the exception of Brush Off®. Visual evaluation of the roots in March 2008 confirmed the results of the 2006/07 season, with die-back of the roots in these treatments being limited to the 0-10 cm soil layer (Table 2).

Efficacy of herbicides applied during berry set

2006/07 season

The above-ground growth of the grapevines was controlled effectively by Garlon 480 EC® (FA), Timbrel 360 SL® applied to the cut stump of the grapevines (CS), Plenum 160 ME® (CS) and Brush Off® (CS) up to the end of March 2007 (twelve months after application) (Table 4). Root studies during March 2007 revealed that active root growth still occurred throughout the soil profile (data not shown). Although re-growth occurred after four months (March 2007) where Confront 360 SL® was applied (both CS and FA), the above-ground growth died 11 months after application (October 2007). However, active above-ground growth was observed in Brush Off® (CS) from 11 months after application (October 2007) onwards, while the same trend was observed in all the treatments 16 months after application (March 2008).

2007/08 season

In the case of Garlon 480 EC® (FA), Timbrel 360 SL® (CS), Plenum 160 ME® (CS) and Brush Off® (CS), the observations made four months after application (March 2007) was confirmed in March 2008 (Table 4). In contrast to the previous season, no re-growth was observed in the Confront 360 SL® (CS) and the basal stem treatment of Garlon 480 EC®. Similar to the previous season, active root growth still occurred in all the treatments after four months (data not shown).

Occurrence of grapevine mealy bug

In May 2006, 70% of the grapevines were infested with grapevine mealy bug, whereafter the above-ground level of infestation dropped to less than 1% where it stayed for the remainder of the study. No grapevine mealy bug was found in the soil, although the grapevine roots were still alive. This could be attributed to the fact that the farm practices to control mealy bug improved and that the natural enemies kept the infestation at acceptable levels.

SUMMARY

The mechanical removal of the grapevines leaves live roots in the soil on which grapevine mealy bug may survive to contaminate newly planted grapevines. The aim of this study was to identify a herbicide or herbicides, combined with the correct application technique, that will kill leafroll infected full bearing grapevines that have shown leafroll symptoms for four years or more. None of the herbicides or application techniques gave total control of both the scion and rootstock of the grapevines which showed leafroll symptoms for four years or more. Grapevines that are heavily infected or have been infected with leafroll virus for four seasons or more can, therefore, not be controlled by the herbicides, herbicide techniques and dosages tested in the trial.

Some of the herbicide/application technique combinations showed the potential to control both the scion and rootstock of the 15-year-old Chardonnay/99 Richter grapevines heavily infected with leafroll virus. In a follow-up trial (Phase 2), selected treatments were applied in full bearing grapevines which showed leafroll symptoms for two seasons or less. These results will be discussed in another article (Phase 2).

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Herbicides and application techniques for control of leafroll infected full bearing grapevines (Phase 2): Vineyard with leafroll symptoms for two years or less

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KEYWORDS: Chemical control, foliar application, cut stump application, basal stem application, grapevine growth, leafroll virus status.

INTRODUCTION

The mechanical removal of grapevines leaves live roots in the soil on which grapevine mealy bug [*Planococcus ficus* (Signoret)] may survive to contaminate the newly planted grapevines replacing the affected vines. The aim of this study was, therefore, to identify a combination of herbicide and application technique that will kill full bearing grapevines that have shown leafroll symptoms for two years or less.

Based on the results of Phase 1, and in liaison with the agrochemical companies which supplied the herbicides, the dosage of the most successful herbicides were increased in Phase 2 to determine whether full bearing grapevines that showed leafroll symptoms for two years or less can be controlled chemically. Care was taken not to exceed the dosages currently used to control exotic trees growing in river beds, agricultural land or forests.

MATERIALS AND METHODS

The study was executed in a six-year-old Chardonnay/Ramsey vineyard which showed symptoms of being infected with the leafroll virus for two years or less. The vineyard was established on a medium textured soil at Nietvoorbij Experiment farm in Stellenbosch. The treatments applied during the 2009/10 and 2010/11 seasons are described in Table 1. The treatments were replicated three times in a randomised block design. Each replication plot consisted of three infected grapevines that were buffered from the other treatments as described in the article reporting on Phase 1.

The grapevines that were controlled effectively above-ground and partially below-ground were removed during early June 2010 and early July 2011, and replaced by Cabernet/Richter 99 vines planted on 3 August 2010 and 30 August 2011, respectively. A red scion cultivar was used, as leafroll symptoms are much easier to identify on red grape cultivars.

MEASUREMENTS

Herbicide efficacy

The grapevines were monitored visually for re-growth. The roots of one grapevine per replication plot of a treatment in which no re-growth occurred, were inspected as described in the article reporting on Phase 1.

Occurrence of leafroll virus in newly planted grapevines

The new grapevines replacing the old ones were monitored visually for one (planted 30 August 2011) and two (planted 3 August 2010) growing seasons. Leaves were sampled from grapevines showing no visual symptoms during early April 2012. Indirect ELISA for specific or simultaneous detection of GLRaV-1, GLRaV-2 and GLRaV-3 in grapevines as developed by Dr. Goszczynski was done on these vines to verify that they were not contaminated with leafroll viruses.

TABLE 1. Treatments applied during 2009 and treatments applied during both 2009 and 2010.

Herbicide	Dosage	Application method	Time of application
Brush Off® (200 g/ℓ metsulfuron methyl)	80 g/100 ℓ water plus 0.05% Agral 90	Foliar	Post-harvest
Brush Off®	100 g/100 ℓ water plus 0.05% Agral 90	Foliar	Post-harvest
Garlon 480 EC® (480 g/ℓ triclopyr)	0.5% solution plus 0.5% Actipron	Foliar	Post-harvest
Garlon 480 EC®	0.75% solution plus 0.5% Actipron	Foliar	Post-harvest
Plenum 160 ME® (80 g/ℓ picloram & 80 g/ℓ fluroxypyr)	0.75% solution plus 0.5% Actipron	Foliar	Post-harvest
Garlon 480 EC®	2% solution in diesel	Basal stem	Post-harvest
Garlon 480 EC®	0.5% solution plus 0.5% Actipron	Foliar	Berry set
Garlon 480 EC®	0.75% solution plus 0.5% Actipron	Foliar	Berry set
Garlon 480 EC®	2% solution in diesel	Basal stem	Berry set
Brush Off®	180 g/100 ℓ water plus 0.05% Agral 90	Cut stump	Berry set
Brush Off®	200 g/100 ℓ water plus 0.05% Agral 90	Cut stump	Berry set
Timbrel 360 SL® (360 g/ℓ triclopyr)	3% solution plus 0.5% Actipron	Cut stump	Berry set
Timbrel 360 SL®	5% solution plus 0.5% Actipron	Cut stump	Berry set
Plenum 160 ME®	1.5% solution plus 0.5% Actipron	Cut stump	Berry set
Confront 360 SL® (90 g/ℓ triclopyr & 270 g/ℓ clopyralid)	3% solution plus 0.5% Actipron	Cut stump	Berry set
Confront 360 SL®	5% solution plus 0.5% Actipron	Cut stump	Berry set

TABLE 2. The effect of the most successful post-harvest (applied 30 March 2009) and berry set (applied 2 December 2009) treatments on the above-ground and root growth of seven-year-old Chardonnay/Ramsey grapevines, as evaluated visually during October 2009 and February 2010.

Herbicide	Dosage	Application method	October 2009		February 2010	
			Above-ground growth	Root growth	Above-ground growth	Root growth
Post-harvest application						
Garlon 480 EC® (480 g/ℓ triclopyr)	0.75% solution plus 0.5% Actipron	Foliar	Absent	Most of the roots dead to a depth of 10 cm	Absent	Most of the roots dead to a depth of 45 cm
Plenum 160 ME® (80 g/ℓ picloram & 80 g/ℓ fluroxypyr)	0.75% solution plus 0.5% Actipron	Foliar	Absent	Most of the roots dead to a depth of 10 cm	Absent	All the roots dead to a depth of 20 cm
Garlon 480 EC®	2% solution in diesel	Basal stem	Absent	Alive throughout soil profile	Absent	All the roots dead to a depth of 20 cm
Berry set application						
Garlon 480 EC®	2% solution in diesel	Basal stem	NA[1]	NA	Absent	All the roots dead to a depth of 20 cm

[1] Not applicable.

TABLE 3. The effect of the most successful post-harvest (applied 25 March 2010) and berry set (applied 1 December 2010) treatments, on the above-ground growth and root growth of seven year old Chardonnay/Ramsey grapevines, which was evaluated visually during November 2010 and February 2011.

Herbicide	Dosage	Application method	November 2010		February 2011	
			Above-ground growth	Root growth	Above-ground growth	Root growth
Post-harvest application						
Garlon 480 EC® (480 g/ℓ triclopyr)	0.75% solution plus 0.5% Actipron	Foliar	Absent	Most of the roots dead to depths varying from 5 cm to 20 cm	Absent	Most of the roots dead to a depth of 40 cm
Plenum 160 ME® (80 g/ℓ picloram & 80 g/ℓ fluroxypyr)	0.75% solution plus 0.5% Actipron	Foliar	Absent	Most of the roots dead to a depth of 10 cm and some to a depth of 40 cm	Absent	All of the roots dead to a depth of 20 cm and some to a depth of 50 cm
Garlon 480 EC®	2% solution in diesel	Basal stem	Absent	Alive throughout soil profile	Absent	All roots dead to a depth of 30 cm. Most roots dead in the 30-90 cm soil layer
Berry set application						
Garlon 480 EC®	2% solution in diesel	Basal stem	NA[1]	NA	Absent	50% of the roots dead to a depth of 15 cm

[1] Not applicable.

Herbicide phytotoxicity

The newly planted vines were monitored visually for the first two seasons after planting for any symptoms that may indicate herbicide uptake from the soil. Soil samples were taken from the 0-30 cm soil layer of each replication near the trunk of the grapevines planted end of August 2011. A bio-assay was done on these samples using tomato seedlings to determine whether herbicide residues were present in the soil.

RESULTS AND DISCUSSION

Herbicide efficacy

All the post-harvest applications made during 2009 and 2010 gave 100% control of the above-ground growth of the recently infected six-year-old Chardonnay/Ramsey grapevines during October 2009 and November 2010 (Table 2 and 3). However, only the grapevine roots of the foliar application (FA) of the 0.75% solutions of Garlon 480 EC® and Plenum 180 ME® caused the grapevine roots to start disintegrating during October 2009 and November 2010. These were the only treatments in which no above-ground growth occurred during February 2010 and February 2011 and in which the roots were also controlled partially.

The basal stem (BS) treatment with a 2% solution of Garlon 480 EC® was the only berry set treatment that controlled the above-ground growth and killed the grapevine roots to a depth of 20 cm (Table 2 and 3).

Occurrence of leafroll virus in newly planted grapevines

No visual symptoms indicating leafroll virus infection or herbicide phytotoxicity were detected on the grapevines planted 3 August 2010 and 30 August 2011. One year after these vines were established (vines planted 30 August 2011), one vine in Plenum 160 ME® (FA) tested positive for leafroll. Two years after these vines were established (vines planted 3 August 2010), one vine in 0.75% Garlon 480 EC® (FA) and 2% Garlon 480 EC® (BS) tested positive. In all three cases, the Chardonnay/Ramsey grapevines adjacent to the affected Cabernet/Richter 99 vines did not show leafroll symptoms when the new vines were planted, but showed visual symptoms during the April 2012 visual evaluation. It is possible that the full bearing Chardonnay/Ramsey vines could have facilitated the contamination of the newly planted grapevines.

SUMMARY

The mechanical removal of grapevines leaves live roots in the soil on which mealy bug may survive to contaminate the newly replanted grapevines. The aim of this study was, therefore, to identify a combination of herbicide and application techniques that will kill full bearing grapevines that have shown leafroll symptoms for two years or less.

The 2% Garlon 480 EC® in diesel applied post-harvest as a basal stem treatment took a full season (from March to March) to kill all roots to a depth of 20 cm or 30 cm. Depending on the season, most roots were also controlled in the 30-90 cm soil layer. A post-harvest foliar application of Plenum 160 ME® may also be considered as, within the above-mentioned time frame, the grapevine roots were controlled to a depth of 20 cm or 30 cm with some roots being controlled as deep as 50 cm, depending on the season.

Research that is currently being executed, will determine to which depth the mealy bug can survive on grapevine roots in the soil in the absence of above-ground growth.

The current results were submitted to the agrochemical company that holds the patent to the above-mentioned two herbicides to help facilitate registration of these chemicals for the control of leafroll infected grapevines.

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The authors thank the ARC and Winetech for financial support. The following chemical companies for supplying the herbicides and technical advice: Dow AgroSciences for supplying Confront 360 SL®, Garlon 480 EC®, Plenum 160 ME®, Timbrel 360 SL®; E I Du Pont de Nemours & Co for supplying Brush Off®.

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Cabernet Sauvignon responses to irrigation using winery wastewater

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KEYWORDS: Chemical oxygen demand, grapevines, off-flavours, water quality, wine.

INTRODUCTION

Wineries produce large volumes of poor quality wastewater, and approximately 3 to 5 m^3 is produced per tonne of grapes crushed (Mosse *et al.*, 2011). Scarce irrigation water could be restricted further in future irrigation water allocations (Petrie *et al.*, 2004). Where wineries are surrounded by vineyards, irrigation with diluted winery wastewater could be used instead of water from natural resources. Currently, the Department of Water and Sanitation is drafting new General Authorisations for wineries. The objective of this study was to determine the effect of using diluted winery wastewater for vineyard irrigation on growth, yield, juice and wine quality in order to make recommendations for the refinement of the General Authorisations.

EXPERIMENTAL BACKGROUND

The field experiment was carried out with micro-sprinkler irrigated Cabernet Sauvignon/99 R near Rawsonville in the Breede River Valley region from the 2009/10 to 2012/13 seasons. In 2009/10, treatments were only applied after harvest due to late infrastructure completion. Nine treatments were applied as indicated in Table 1. Irrigation with winery wastewater diluted to 100, 250, 500, 1 000, 1 500, 2 000, 2 500 and 3 000 mg/ℓ chemical oxygen

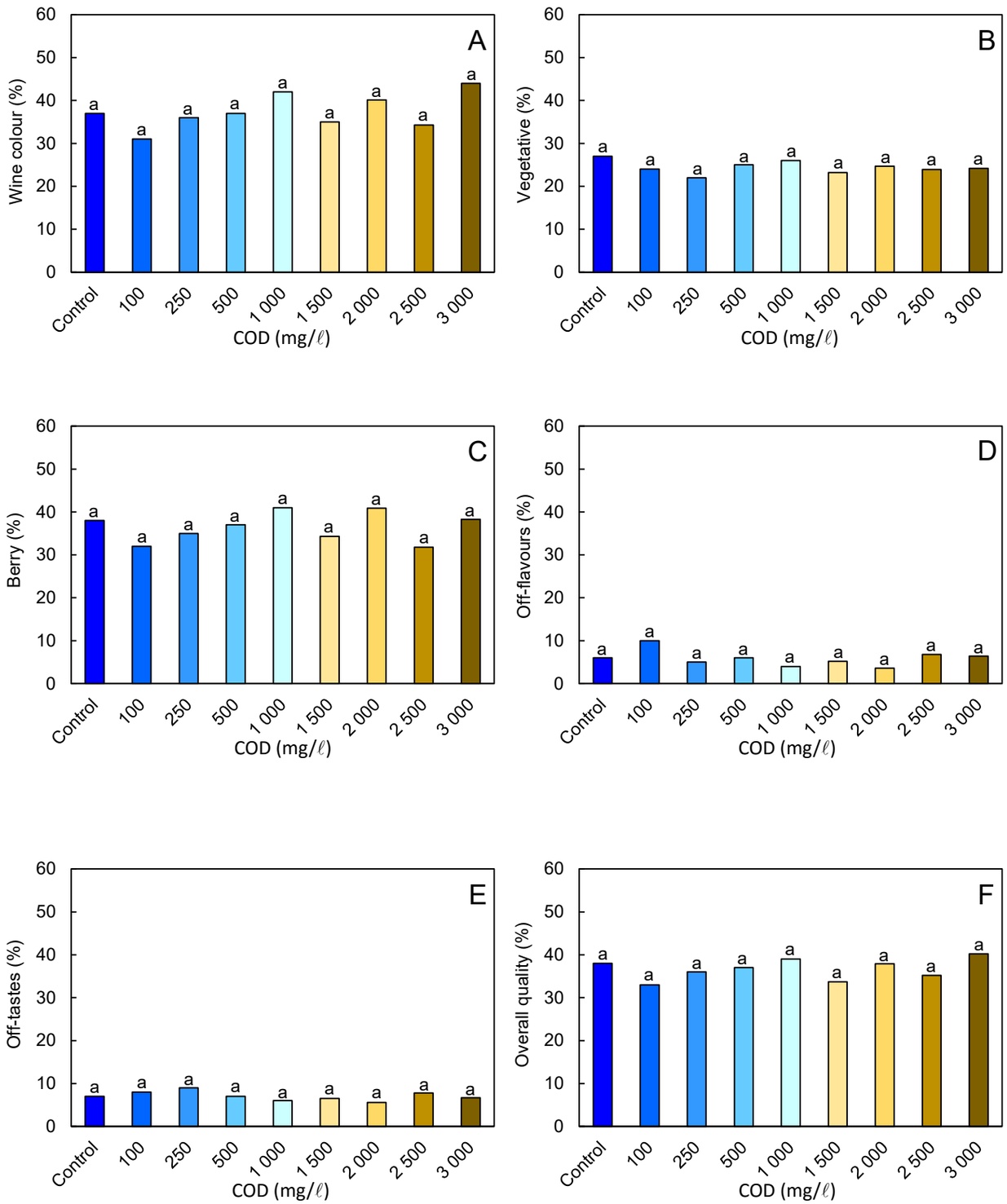

FIGURE 1. The effect of irrigation using diluted winery wastewater on (A) colour, (B) vegetative character, (C) berry character, (D) off-flavours, (E) off-tastes and (F) overall quality of Cabernet Sauvignon wines. Data are means for three seasons.

demand (COD) was compared to irrigation with river water. Treatments were replicated three times in a randomised block design. In addition to the normal winter oats cover crop, an interception crop of pearl millet was cultivated in summer. Details of the irrigation infrastructure, dilution procedures and water quality was reported previously (Myburgh & Howell, 2014).

RESULTS AND DISCUSSION

Plant water constraints

Since midday stem water potential ranged between -0.57 MPa and -0.65 MPa, grapevines experienced no to low water constraints (Myburgh *et al.*, 2016). Furthermore, irrigation using diluted winery wastewater, regardless of the level of dilution, clearly had no effect on the grapevine water status compared to where grapevines were irrigated using river water (Table 1). Given the low levels of water constraints, poor wine quality would be expected (Myburgh *et al.*, 2016).

Vegetative growth

Irrigation using diluted winery wastewater did not affect vegetative growth compared to river water (Table 1). This was to be expected, since irrigation using diluted winery wastewater did not affect grapevine water status, as well as leaf and shoot chemical status (Myburgh & Howell, 2014). Furthermore, the N load in the wastewater was inadequate to supply the grapevine's N requirement. It must be noted that winery wastewater diluted up to 3 000 mg/ℓ COD did not pose any salinity hazard to grapevine growth. Cane mass was comparable to values reported for Cabernet Sauvignon in the Breede River Valley (Roux, 2005) and the Lower Olifants River Valley (Bruwer, 2010), but was substantially higher than non-irrigated grapevines in the Swartland region (Mehmel, 2010). Based on the foregoing, the interception crop did not seem to have negative effects on grapevine vegetative growth.

Yield

Irrigation using diluted winery wastewater did not affect grapevine fertility, i.e. number of bunches per grapevine (data not shown). In the 2010/11 and 2011/12 seasons, treatments had no effect on berry size development (Schoeman, 2012), and was probably due to a lack of differences in grapevine water status. Irrigation using diluted winery wastewater had no effect on berry mass, bunch mass or yield compared to the river water (Table 1). Grape yield was comparable to approximately 15 t/

TABLE 1. Effect of irrigation using diluted winery wastewater on stem water potential (SWP), growth, yield and juice characteristics of Cabernet Sauvignon/99 R near Rawsonville. Data are means of three seasons.

Measured variable	River water (control)	Level of COD[(1)] in diluted winery wastewater (mg/ℓ)							
		100	250	500	1 000	1 500	2 000	2 500	3 000
SWP (MPa)	-0.62	-0.57	-0.62	-0.63	-0.65	-0.60	-0.59	-0.64	-0.64
Cane mass (t/ha)	2.61	2.72	2.59	2.63	2.49	2.31	2.24	2.47	2.56
Berry mass (g)	1.35	1.41	1.34	1.33	1.31	1.38	1.33	1.4	1.30
Bunch mass (g)	155	157	156	160	154	162	146	163	146
Yield (t/ha)	14.9	14.8	15.2	15.6	15.5	14.4	13.3	16.2	14.1
Juice sugar (°B)	23.2	23.0	23.1	23.4	23.8	23.1	23.1	23.2	23.9
Juice acidity (g/ℓ)	5.22	5.32	5.01	4.97	4.91	5.03	5.37	5.13	4.83
Juice pH	3.57	3.59	3.55	3.60	3.63	3.63	3.64	3.67	3.70

(1) Chemical oxygen demand.

ha reported for irrigated Cabernet Sauvignon (Roux, 2005), but substantially higher than non-irrigated ones (Mehmel, 2010). The relatively high yield confirmed that grapevines experienced low levels of water constraints (Myburgh *et al.*, 2016).

Juice characteristics

In 2010/11 and 2011/12, sugar loading into the berries and acid breakdown during ripening were not affected by irrigation using diluted winery wastewater compared to the river water (Schoeman, 2012). The fact that the rate of berry ripening was comparable to the river water control indicated that the winery wastewater had no effect on the physiological functioning of the grapevines, irrespective of the level of dilution. Consequently, there were no differences in juice sugar and acidity at harvest (Table 1). However, juice pH increased with an increase in COD level of the irrigation water. This could have negative consequences on fermentation and microbial stability and colour of wine. More details regarding this aspect will be given in a subsequent article.

Wine characteristics

Analysis confirmed that no wines contained pathogenic bacteria and were safe for sensorial evaluation (data not shown). Wine colour (Fig. 1A), as well as vegetative and berry character (Fig. 1B & C) were not affected by the use of diluted winery wastewater for irrigation. There were no differences in wastewater associated off-flavours and tastes compared to the river water control (Fig. 1D & E), thereby confirming that no contaminants were transferred from the wastewater into the wines. However, in a parallel study where bunches were deliberately sprayed with diluted winery wastewater, a winery wastewater-like odour was detected in the wines, and their spicy character reduced (Schoeman, 2012). This highlights the importance of avoiding contact between grapes and winery wastewater. There were no differences in the overall quality of the wines (Fig. 1F). The low quality, i.e. less than 40%, can be ascribed to the low water constraints as discussed above.

CONCLUSIONS AND RECOMMENDATIONS

Under the prevailing conditions, using diluted winery wastewater for vineyard irrigation did not affect vegetative growth or yield. The grapevines did not respond to level of COD per se. This indicated that sufficient aeration occurred between irrigations which allowed organic carbon breakdown. Although salinity and sodicity levels in the diluted winery wastewater were below the thresholds where growth and yield reductions are expected for grapevines, it should be monitored frequently. The low salinity and sodicity levels in the diluted winery wastewater could be a further explanation why the grapevines did not respond negatively to the wastewater irrigation. A summer interception crop did not have any negative effects on grapevine growth, yield and quality. With the exception of pH, irrigation using diluted winery wastewater did not have detrimental effects on juice and wine characteristics. However, the relatively large irrigation volumes applied during berry ripening resulted in poor wine quality. To improve wine quality, winery wastewater should rather be applied over larger areas to obtain sufficient depletion of plant available water between irrigations. However, this will require additional expensive infrastructure. Although wine sensorial characteristics were not affected, off-odours due to direct contact with winery wastewater may reduce wine quality. In heavier soils, regions with lower winter rainfall, situations where the winery wastewater contains more K or where no interception crop is grown, grapevine responses may be more pronounced.

Based on the project results, the following criteria should be considered for possible amendments to the General Authorisations for wineries when using diluted wastewater for irrigation of vineyards:

- The COD must be diluted to 3 000 mg/ℓ or less, preferably to less than 2 000 mg/ℓ to avoid unpleasant odours in the vineyard while irrigations are applied.
- The electrical conductivity (EC_{iw}) must be less than 0.75 dS/m.

- The sodium adsorption ratio (SAR_{iw}) must be less than 5.
- The soil must have a low cation exchange capacity.
- The internal drainage in the root zone must be unrestricted.
- The irrigation water must not percolate beyond the root depth.
- Only micro-sprinklers should be used, since drippers have narrow flow paths and/or small orifices, and are more susceptible to clogging.
- The irrigation must be applied with micro-sprinklers in such a way that the bunches are not wetted.
- At least 50% plant available water (PAW) depletion should be allowed between irrigations to allow sufficient aeration for oxidation of organic material applied via the irrigation water.
- The irrigation frequency and volumes (schedule) should enhance, rather than negate, wine quality characteristics.
- A summer interception crop, e.g. pearl millet, should be sown in early January to ensure that its growth peaks when the winery wastewater is applied, and that the species does not complete its life cycle too early.
- Since a summer interception crop may increase the evapotranspiration (ET) of vineyards substantially if growing conditions are favourable, it could induce competition for water between grapevines and cover crop. However, this can be avoided by timely slashing of the interception crop, which will also minimise possible competition for N and P.

SUMMARY

The effect of irrigation using winery wastewater diluted to eight different levels of chemical oxygen demand (COD) with river water on grapevine responses were compared to irrigation using river water in a temperate climate with hot, dry summers. The field trial was carried out with Cabernet Sauvignon/99 R near Rawsonville in the Breede River Valley region from the 2009/10 to 2012/13 seasons. Under the prevailing conditions, i.e. Mediterranean climate and sandy soil, plant water status did not respond to irrigation with diluted winery wastewater, irrespective of the level of dilution. Irrigation using diluted winery wastewater did not affect vegetative growth, yield or juice characteristics, except that of juice pH or wine sensorial characteristics. The latter practice did not induce off-flavours or tastes in the wines. In heavier soils or in regions with lower winter rainfall, situations where the winery wastewater contains more K or where no pearl millet interception crop is cultivated during summer, grapevine responses may be more pronounced.

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Pome and stone fruit trees harbour grapevine trunk disease pathogens

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KEYWORDS: Pome fruit trees, stone fruit trees, grapevine trunk disease, trunk disease pathogens.

INTRODUCTION

Grapevine trunk diseases are caused by various fungal groups. Botryosphaeria dieback is caused by species of the Botryosphaeriaceae, Petri disease by a combination of *Phaeomoniella chlamydospora* and *Phaeoacremonium* species and esca ('beroerte') by the aforementioned pathogens together with wood rotting Basidiomycetes. Eutypa dieback ('tandpyn') is caused by species of the Diatrypaceae with *Eutypa lata* being the most well known. Phomopsis dieback is caused by different *Diaporthe* species (previously known as *Phomopsis*).

Some of the fungal pathogens associated with grapevine trunk diseases have been associated with dieback or decline symptoms of various woody hosts. Economically important agricultural crops include pome fruit trees, stone fruit trees, olive trees, date palms and kiwifruit vines. However, very little is known about the trunk pathogens present on pome and stone fruit trees.

In South Africa, pome and stone fruit trees are often planted in close proximity of vineyards (Figure 1). Pome fruit trees are in close proximity of vineyards in the Grabouw/Villiersdorp and Wolseley/Ceres areas. Stone fruit trees are close to

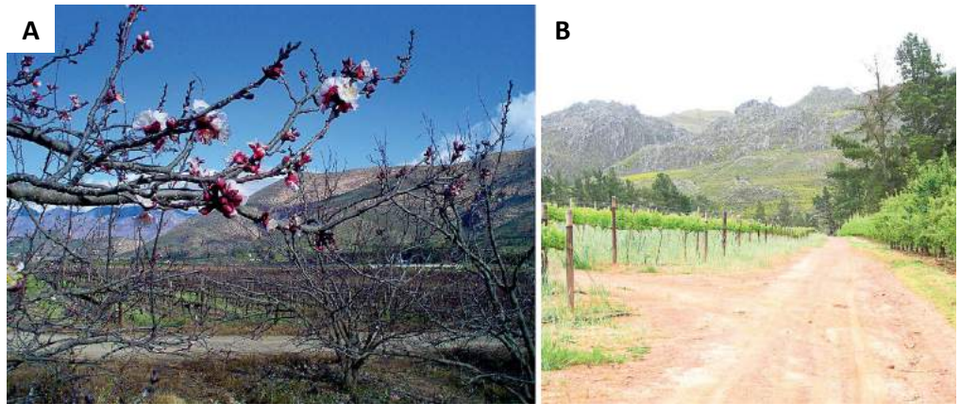

FIGURE 1. Vineyards planted next to a) an apricot orchard in Montagu and b) an apple orchard in Villiersdorp.

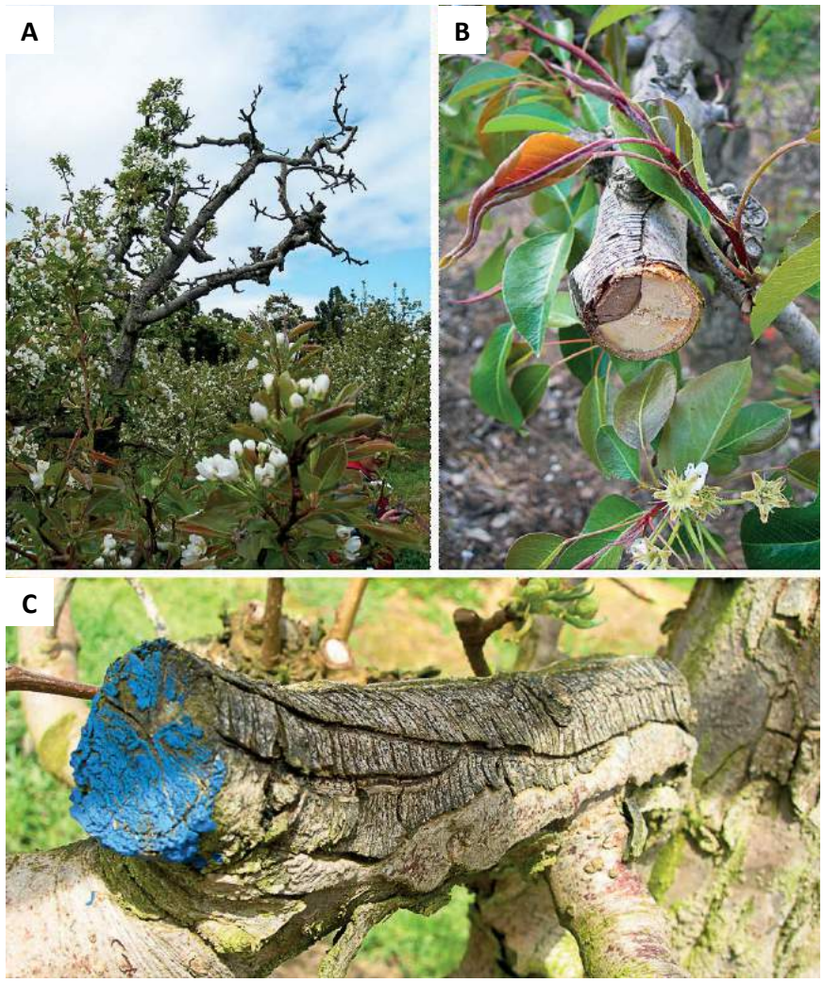

FIGURE 2. Outward symptoms observed on pome fruit trees included: a) dieback, b) a canker showing wedge-shaped dark brown internal necrosis and c) a canker that developed from a pruning wound.

vineyards in different parts of the winter rainfall area of the Western Cape, as well as summer rainfall areas in the Limpopo Province. To investigate if pome and stone fruit trees can act as an inoculum source of grapevine trunk disease pathogens, a survey was conducted to sample trees from orchards close to vineyards showing dieback or cankers (Figure 2).

MATERIAL AND METHODS

Apple (cv. Granny Smith) and pear (cv. Packham's Triumph) trees older than 15 years were sampled in 2006 and 2007 in Grabouw, Vyeboom, Villiersdorp, Wolseley and Ceres. Plum, peach, nectarine and apricot orchards in high (Stellenbosch, Paarl and Franschhoek) and low (Robertson, Bonnievale and Montagu) winter rainfall areas in the Western Cape Province and in summer rainfall areas in the Limpopo Province (Mookgopong and Modimolle) were sampled in 2004 and 2005. Isolates were made from fungi developing from surface sterilised wood pieces and from sporulating structures on the bark surface. Fungal species were identified according to cultural and morphological characters and DNA sequences.

The pathogenicity of selected species and isolates were determined on detached shoots. For isolates obtained from pome fruit trees, detached woody shoots of grapevine (cv. Sauvignon blanc), pear (cv. Packham's Triumph) and apple (cv. Granny Smith) were used. Three fungal groups identified from stone fruit isolations were tested in separate pathogenicity assays. Isolates were inoculated onto detached nectarine (cv. Alpine) and plum (cv. Ruby Nel) shoots; detached apricot (cv. Belida) and plum (cv. Southern Bell) shoots; or detached apricot (cv. Belida), peach (cv. San Pedro) and plum (cv. Southern Bell) shoots. Shoots were inoculated with colonised agar plugs and lesions measured after 14 days of incubation.

RESULTS

Survey of pome fruit orchards

Six different internal symptom types similar to those occurring in grapevines were identified from pear and apple samples (Figure 3). These were brown vascular streaking, black vascular streaking, wedge-shaped necrosis, watery necrosis, brown internal necrosis and soft rot.

The fungal species isolated from symptomatic wood included: four species of Botryosphaeriaceae, four species of *Phaeoacremonium*, two species of *Diaporthe*, *Eutypa lata* (Diatrypaceae), two species of *Didymosphaeria* and *Pyrenochaeta*-like fungi (Table 1). Of these the predominant species were *Diplodia seriata* and *Phaeoacremonium minimum.*

Survey of stone fruit orchards

In cross-section, the symptomatic wood had either irregularly shaped or V-shaped necrotic lesions and was situated close to old pruning wounds and/or cankers, sometimes also associated with gummosis (Figure 4). In plum wood, lesions from which *Phaeoacremonium* species were isolated were often reddish brown in the centre and greenish towards the margin.

A wide diversity of fungal species were isolated from stone fruit trees and 22 new species were described (Table 2). The fungal species identified included: nine species within the Botryosphaeriaceae (three new species), 14 species of

FIGURE 3. Internal symptom types associated with trunk disease on pome fruit trees: a) watery necrosis, b) soft rot indicated by arrow, c) brown internal necrosis, d) black/brown streaking, e) brown streaking and f) wedge-shaped necrosis.

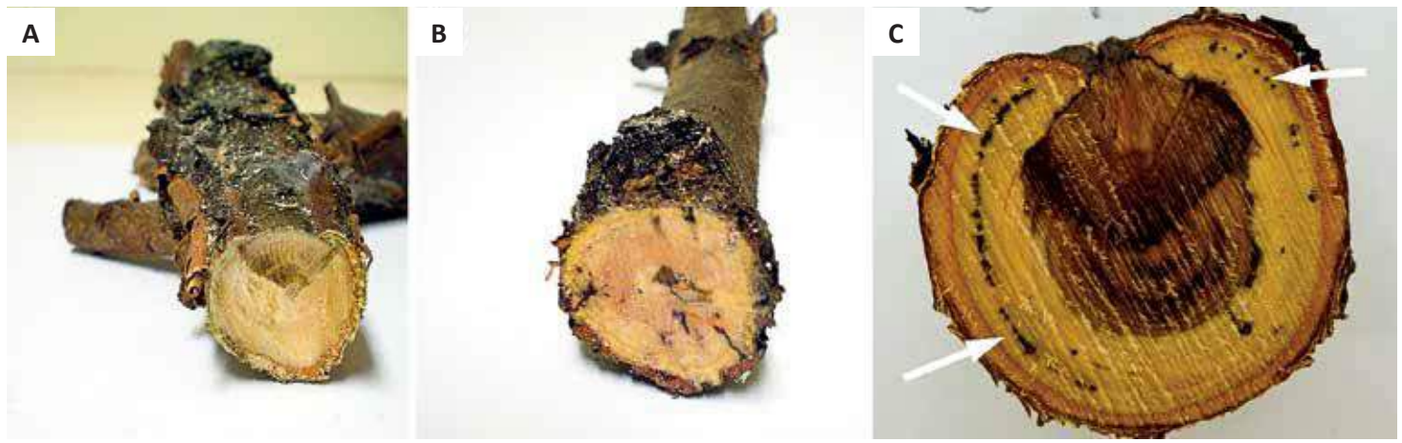

FIGURE 4. Internal symptoms observed on stone fruit trees included: a) wedge-shaped necrosis, b) black/brown streaking and c) central brown internal necrosis together with black/brown streaking (indicated by arrows). (Photos: Providence Moyo.)

TABLE 1. Fungal species isolated from dieback and canker symptoms sampled from pome fruit trees.

Species	Number of isolates	Host	Area
Botryosphaeriaceae			
Diplodia seriata[1]	82	Apple, pear	Elgin, Vyeboom, Villiersdorp, Ceres, Wolseley
Neofusicoccum australe[1]	3	Pear	Vyeboom
Neofusicoccum vitifusiforme[1]	2	Apple, pear	Ceres
Diplodia malorum	6	Pear	Wolseley, Elgin, Ceres
Phaeoacremonium			
Phaeoacremonium iranianum[1]	1	Pear	Wolseley
Phaeoacremonium fraxinopennsylvanicum[1]	2	Pear	Villiersdorp, Ceres
Phaeoacremonium minimum[1]	32	Apple, pear	Elgin, Villiersdorp, Vyeboom, Ceres, Wolseley
Phaeoacremonium viticola[1]	2	Pear	Villiersdorp, Vyeboom
Diaporthe			
Diaporthe sp. 7[1]	2	Pear	Villiersdorp, Vyeboom
Diaporthe foeniculina[1]	1	Apple	Elgin
Eutypa			
Eutypa lata[1]	6	Apple, pear	Elgin, Vyeboom, Wolseley
Didymosphaeriaceae			
Didymosphaeria rubi-ulmifolii s.l.	2	Pear	Ceres, Wolseley
Didymosphaeria variabile	2	Apple	Elgin
Other			
Pyrenochaeta-like sp.	6	Apple	Elgin

[1] Fungal species also reported from grapevines.

Phaeoacremonium (five new species), at least five *Diaporthe* species, *Eutypa lata* (Diatrypaceae), two species of *Didymosphaeria* (one new species), one new species of *Paraconiothyrium*, three new species of Calosphaeriaceae, a new genus, *Collophora*, with three species, five species of Phaeomoniellales (four new species) and three species of *Coniochaeta* (two new species). The predominant fungal species were *Diplodia seriata*, *Collophora rubra*, *Collophora paarla*, *Phaeoacremoniunium scolyti*, *Didymosphaeria variable* and *Celeloriella prunicola.*

Multiple trunk disease pathogens were often isolated in different combinations from symptomatic tissues. A specific symptoms type could not be ascribed to any single pathogen. On stone fruit trees from many specimens (mostly peach and nectarine), especially *Collophora rubra*, was isolated as the only fungus.

PATHOGENICITY TESTS

Pome fruit

Isolates obtained from pome fruit trees that were inoculated on grapevine shoots showed that *Neofusicoccum australe* was the most virulent species. The *Pyrenochaeta*-like sp., *Didymosphaeria variabile*, *Diplodia seriata* and *P. fraxinopennsylvanicum*, could also be considered pathogenic, since their lesion lengths were significantly longer than the negative controls. On pear, the most virulent species were *Diplodia malorum* and *Neofusicoccum australe.* The lesion lengths of *Diplodia seriata* and *Eutypa lata* were also significantly different from the negative controls. On apple, *Neofusicoccum australe* and *Phaeoacremonium iranianum* formed the longest lesions and were the most virulent species. *Diplodia malorum*, *Phaeoacremonium minimum*, *Neofusicoccum vitifusiforme*, *Diplodia seriata, Phaeoacremonium fraxinopennsylvanicum*, *Eutypa lata* and *Didymosphaeria rubi-ulmifolii* s.l. could be considered pathogenic with lesion lengths significantly longer than the negative controls.

Stone fruit

Pathogenicity tests for stone fruit isolates were conducted only for species from the Botryosphaeriaceae, *Coniochaeta/Collophora* and *Phaeoacremonium.* All Botryosphaeriaceae species tested, except *Dothiorella viticola*, caused lesions

TABLE 2. Fungal species isolated from dieback and canker symptoms sampled from stone fruit trees.

Species	Number of isolates	Host	Area
Botryosphaeriaceae			
Aplosporella prunicola[2]	2	Nectarine	Modimolle
Diplodia seriata[1]	43	Peach, plum, apricot, nectarine	Bonnievale, Modimolle, Mookgopong, Montagu, Robertson, Paarl
Diplodia africana[2]	3	Peach	Paarl
Diplodia mutila[1]	1	Plum	Paarl
Diplodia pinea	4	Peach	Paarl
Dothiorelle viticola[1]	2	Plum, nectarine	Paarl, Modimolle
Lasiodiplodia plurivora[1, 2]	1	Plum	Stellenbosch
Neofusicoccum australe[1]	6	Peach, apricot, plum	Paarl, Bonnievale, Stellenbosch
Neofusicoccum vitifusiforme[1]	7	Peach, plum	Paarl, Mookgopong, Modimolle
Phaeoacremonium			
Phaeoacremonium africanum[2]	3	Apricot	Montagu
Phaeoacremonium australiense[1]	5	Plum	Paarl, Stellenbosch
Phaeoacremonium fuscum[2]	2	Plum	Mookgopong
Phaeoacremonium fraxinopennsylvanicum[1]	2	Plum	Franschhoek
Phaeoacremonium griseo-olivaceum[2]	1	Apricot	Mookgopong
Phaeoacremonium griseorubrum[1]	2	Plum	Paarl
Phaeoacremonium iranianum[1]	3	Apricot	Robertson, Montagu
Phaeoacremonium pallidum[2]	2	Apricot	Bonnievale
Phaeoacremonium prunicola[2]	2	Plum	Mookgopong
Phaeoacremonium minimum[1]	6	Apricot, plum	Robertson, Bonnievale, Paarl
Phaeoacremonium parasiticum[1]	1	Apricot	Montagu
Phaeoacremonium scolyti[1]	10	Peach, apricot, plum	Paarl, Mookgopong, Stellenbosch, Modimolle, Robertson, Bonnievale
Phaeoacremonium subulatum[1]	1	Apricot	Robertson
Phaeoacremonium viticola[1]	2	Plum	Paarl, Franschhoek
Diaporthe			
Diaporthe spp.[3]	24	Apricot, plum, peach	Paarl, Mookgopong, Montagu, Bonnievale
Diatrypaceae			
Eutypa lata[1]	4	Apricot, plum	Montagu, Robertson, Bonnievale, Franschhoek, Stellenbosch
Calosphaeriaceae			
Calosphaeria africana[2]	2	Apricot	Robertson
Jattaea prunicola[2]	3	Plum	Franschhoek
Jattaea mookgoponga[2]	2	Nectarine	Mookgopong
Didymosphaeriaceae			
Paraconiothyrium africanum[2]	1	Peach	Paarl
Didymosphaeria rubi-ulmifolii s.l.	7	Plum, peach, nectarine	Paarl, Mookgopong
Didymosphaeria variabile[2]	10	Plum, peach	Stellenbosch, Paarl
Collophora			
Collophora africana[2]	5	Plum	Paarl, Franschhoek
Collophora paarla[2]	12	Plum, peach	Paarl, Mookgopong, Modimolle
Collophora rubra[2]	24	Almond, peach, plum, nectarine	Paarl, Mookgopong, Modimolle, Tulbagh
Coniochaeta			
Coniochaeta africana[2]	1	Plum	Mookgopong
Coniochaeta prunicola[2]	3	Plum, apricot	Mookgopong, Robertson
Coniochaeta velutina	4	Peach, plum	Mookgopong, Robertson
Phaeomoniellales			
Aequabiliella effusa[2]	1	Plum	Paarl
Celeloriella dura[2]	1	Plum	Mookgopong
Celeloriella prunicola[2]	10	Plum	Mookgopong, Paarl
Minutiella tardicola[2]	1	Apricot	Robertson
Neophaeomoniella zymoides	1	Plum	Mookgopong

[1] Fungal species also reported from grapevines.

[2] New fungal species described.

[3] Unpublished data.

on nectarine shoots, and all species except *Diplodia viticola* and *Diplodia pinea* caused lesions on plum shoots that were significantly longer than the controls. All *Phaeoacremonium* species, except *Phaeoacremonium fuscum* and *Phaeoacremonium pallidum*, caused lesions in the xylem of plum shoots that were significantly longer than the controls. Five species caused lesions on apricot shoots that were significantly longer than the controls: *Phaeoacremonium parasiticum*, *Phaeoacremonium iranianum*, *Phaeoacremonium subulatum*, *Phaeoacremonium griseorubrum* and *Phaeoacremonium africanum. Collophora africana*, *Collophora rubra*, *Coniochaeta prunicola* and *Celeloriella dura* caused lesions on the xylem of apricot shoots that were significantly longer than the negative controls. On peach shoots, *Collophora paarla*, *Coniochaeta africana* and *Neophaeomoniella zymoides* caused lesions that were significantly longer than the negative controls. Only *Collophora paarla* caused lesions on plum shoots that were significantly longer than those caused by the negative controls.

DISCUSSION

The results of the isolations confirmed that pome and stone fruit trees are hosts to known grapevine trunk pathogens of the Botryosphaeriaceae, Diatrypaceae, *Diaporthe* and *Phaeoacremonium.* Most of the species from these fungal groups have been isolated from grapevines and are known as grapevine trunk disease pathogens (Table 1 and 2). The new species in the Botryosphaeriaceae and of *Phaeoacremonium* need to be taken note of and could in future also be found on grapevines.

The Botryosphaeriaceae species that was most frequently associated with symptomatic wood of pome and stone fruit trees was *Diplodia seriata.* This species has also been found to be the dominant species on grapevine wood in South Africa and is a known pathogen of grapevines. *Neofusicoccum australe* is one of the most pathogenic Botryosphaeriaceae on grapevine, now also found on pear, peach, apricot and plum trees in this study. *Phaeoacremonium minimum* was the dominant *Phaeoacremonium* species from pome fruit trees and *Phaeoacremonium scolyti* from stone fruit trees. Interestingly, *Phaeoacremonium minimum* is also the *Phaeoacremonium* species found most commonly associated with Petri disease and esca of grapevines. *Eutypa lata* was isolated for the first time from pome fruit trees in South Africa, whereas this pathogen is a known canker pathogen of stone fruit trees and grapevines. Interestingly, *Diaporthe ampelina* (previously known as *Phomopsis viticola*, causal agent of Phomopsis cane and leaf blight/'streepvlek' and Phomopsis dieback) have not been found on pome and stone fruit trees.

Several genera were found that are less known as trunk pathogens and have so far not been found on grapevines. For example, *Didymosphaeria* species were found both on pome and stone fruit trees. In the pathogenicity assay *Didymosphaeria variable* was shown to be also pathogenic to detached grapevine shoots. The unidentified *Pyrenochaeta*-like species isolated from apple trees caused significant lesions on grapevine, which might be an indication that this fungus could be a possible future grapevine trunk pathogen. Genera found only on stone fruit wood include *Calosphaeria*, *Collophora* (new genus), *Coniochaeta*, *Jattaea* and species originally described as *Phaeomoniella* species that have recently been transferred to other genera (*Aequabiliella*, *Celeloriella*, *Minutiella* and *Neophaeomoniella*). Unfortunately pathogenicity tests of these genera were not conducted on grapevines, but the results on detached stone fruit shoots provide an indication of their relevance as trunk pathogens. Three of the species of *Collophora* (*Collophora africana*, *Collophora rubra* and *Collophora paarla*), two species of *Coniochaeta* (*Coniochaeta Africana* and *Coniochaeta prunicola*), *Celeloriella dura* and *Neophaeomoniella zymoides* caused lesions that were significantly longer than the negative controls. Of these fungi, *Collophora rubra* was the most frequently isolated species, indicating the importance of this newly found species on stone fruit trees. As the pathogenicity assays were done on detached shoots, they provide a preliminary indication of pathogenicity. Further studies are necessary to investigate the impact of the species on tree health.

Results of this project confirmed the presence of several major grapevine trunk pathogens in pome and stone fruit wood in South Africa. Trunk disease pathogens usually form fruiting structures on dead branches. The removal and destruction of dead/diseased branches in fruit orchards is highly recommended to minimise the formation of inoculum of trunk disease pathogens and would also prevent the spread of trunk diseases.

SUMMARY

Grapevines are often grown in close proximity to stone and pome fruit trees. Fungal pathogens causing Botryosphaeria dieback, esca ('beroerte'), Eutypa dieback ('tandpyn'), Petri disease and Phomopsis dieback of grapevines were isolated from dieback and canker symptoms of pome and stone fruit trees. Therefore, pome and stone fruit orchards should be considered as potential inoculum sources of grapevine trunk disease pathogens. The removal and destruction of dead branches in fruit orchards is highly recommended to minimise the development of fruiting structures of trunk disease pathogens and would also prevent the spread of trunk diseases to nearby vineyards.

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Grape associated fungal communities are influenced by farming practices

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KEYWORDS: Farming systems, yeast diversity, metagenomics.

INTRODUCTION

Grape berries harbour a complex microbial community comprising yeasts, bacteria and filamentous fungi that inhabit the skin surface and endosphere of the berry. The filamentous fungi are not known to contribute to wine quality and therefore receive less attention from the research community. In contrast, yeasts and bacteria play a crucial role and have a direct influence on wine quality (Barata *et al.*, 2012). The microbial population is spatially distributed over the grape berries and grape bunches and display temporal fluctuations over the course of the grape berry development (Prakitchaiwattana *et al.*, 2004). Indeed, several studies have shown that after *veraíson* the yeast community associated with undamaged/healthy berries closely resembles that of plant leaves, which is dominated by basidiomycetous yeast species of the genera *Cryptococcus*, *Rhodotorula*, *Sporobolomyces* and *Rhodosporidium*, as well as the ascomycetous yeast-like fungus, *Aureobasidium pullulans.* At full ripeness, the fermentative yeasts of the genera *Candida*, *Hanseniaspora*, *Lachancea*, *Torulaspora*, *Pichia* and Saccharomyces (albeit at a relatively lower level) become more abundant (Prakitchaiwattana *et al.*, 2004; Renouf *et al.*, 2005). Yeasts are the main drivers of the primary fermentation and are the main producers of the organic acids, esters and higher alcohols which are the main compounds that form the fermentation bouquet.

Several studies have shown that the microbial community structure associated with grape berries responds to biotic and abiotic factors including pesticides. A concerted shift from high chemical inputs on vineyards in the past two decades has stimulated interest in understanding the effect of farming practices on the vineyard ecosystem, most importantly the microbial community structure. Studies comparing organic farming with integrated pest management, conventional farming generally report higher microbial diversity on organic farms

TABLE 1. Spray programme for the biodynamic, conventional and integrated vineyard from leaf-fall until full bloom.

Spray	Biodynamic		Conventional		Integrated	
	Product	Dosage (g/ha)	Product	Dosage	Product	Dosage
1	Nordox Striker	390 450	Folpan Kumulus	312,5 g/ha 2,5 kg/ha	Hyperphos Dithane Kumulus Acrobat	2 kg/ha 600 g/ha 3 kg/ha 1 kg/ha
2	Nordox Kumulus	390 600	Folpan Kumulus Rootex	312,5 g/ha 2,5 kg/ha 750 mℓ/ha	Kumulus Acrobat	3 kg/ha 1 kg/ha
3	Nordox Kumulus	225 600	Dithane Kumulus Rootex	1 kg/ha 3 kg/ha 750 mℓ/ha	Kumulus Acrobat Talendo	3 kg/ha 1,5 kg/ha 1,5 kg/ha
4	Nordox Kumulus	390 600	Acrobat Talendo	1 kg/ha 25 g/ha	Hyperphos Curzate	3 kg/ha 1,5 kg/ha
5	Nordox Kumulus	390 600	Acrobat Talendo	1,5 kg/ha 25 g/ha	Talendo Hyperphos	1,5 kg/ha 4 kg/ha
6	Nordox Kumulus Lime	390 600 500	Cungfu Kumulus	605 g/ha 3 kg/ha	Curzate Kumulus	2 kg/ha 3 kg/ha
7	Nordox Kumulus Lime	390 600 500	Dithane Topaz	2 kg/ha 30 g/ha	Hyperphos Dithane Stroby	4 kg/ha 1,2 kg/ha 150 g/ha
8			Dithane Topaz	2 kg/ha 30 g/ha	Dithane Stroby	1,2 kg/ha 150 g/ha

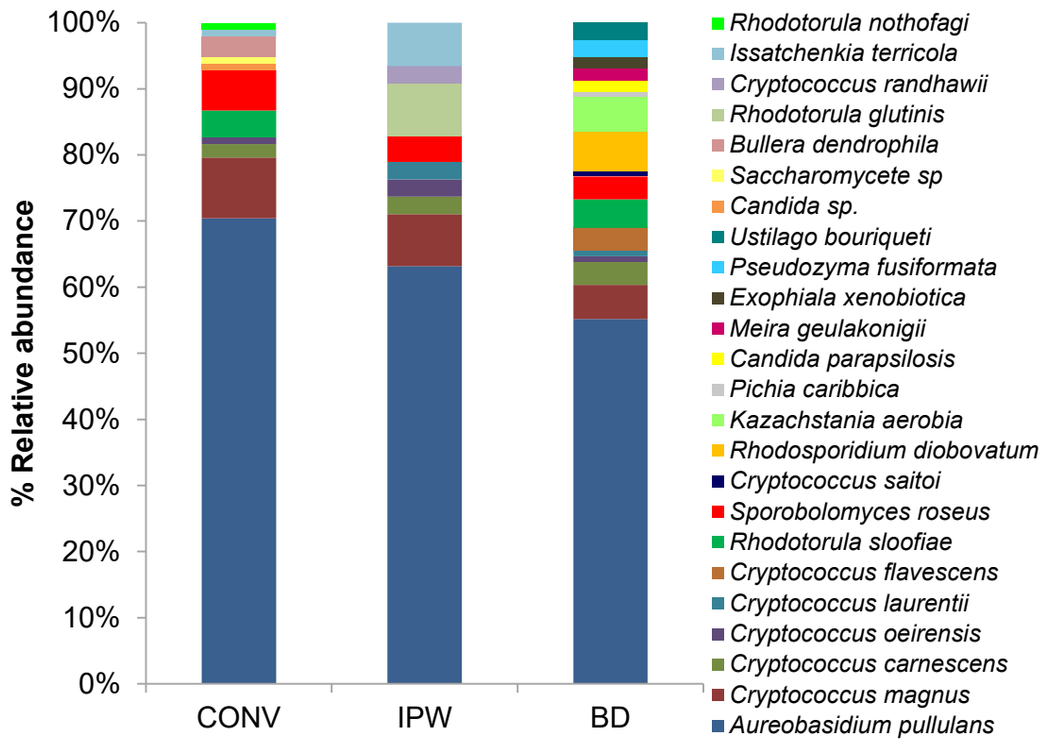

FIGURE 1. Yeast species associated with Cabernet Sauvignon grapes in the biodynamic (BD), integrated (IPM) and conventional (CONV) vineyard.

(Cordero-Bueso *et al*., 2011; Milanović *et al*., 2013. However, only a few of such studies have been performed and the research findings are not always consistent across the different studies due to differences in sampling strategies.

Our study evaluated the fungal diversity in three adjacent vineyards situated in the Polkadraai area in Stellenbosch. The aim was to assess the influence of farming practices on the grape mycobiota on grape berry surfaces and in grape must. We employed culture-dependent and culture-independent techniques for an in-depth analysis of the entire fungal community.

MATERIALS AND METHODS

Cabernet Sauvignon grapes were obtained from three vineyards employing distinct farming practices. Each vineyard has been managed consistently and over a long period through strongly divergent farming methods, referred to as “conventional” (33° 57’41.50” S, 18° 45’11.87” E, elevation 179 m), “integrated production” (33° 57’40.65” S 18° 45’08.23” E, elevation 184 m) and “biodynamic” (33° 57’39.33” S 18° 45’13.46” E, elevation 183 m). A summary of the vineyard treatments applied in 2011 provides an example of the typical chemical applications (Table 1). The conventional and biodynamic vineyard has the same Cabernet Sauvignon rootstock (R101-14), while the integrated vineyard has rootstock R110-CS23A.

Wallerstein Laboratory (WL) Nutrient agar supplemented with appropriate antibiotics was used to isolate and enumerate yeast species by direct plating of serially diluted grape must samples. Molecular identification of the yeast isolates involved extracting the genomic DNA (gDNA) from isolates, PCR amplification of the internal transcribed spacer (ITS1-5.8S-ITS2 rRNA) region, digesting the PCR amplicons with restriction enzymes (*Cfo*I, *Hae*III and *Hin*fI) in order to group isolates based on their respective banding patterns and sequencing of the representative PCR amplicons from each group.

For in-depth metagenomics analysis, microbial cells were collected from freshly crushed grape must by centrifugation and community genomic DNA was extracted by mechanical lysis in conjunction with an SDS-based lysis buffer. The ITS1-5.8S rRNA-ITS2 genes were amplified and sequenced using the Illumina MiSeq High throughput sequencing platform. The sequences obtained were processed using the Illumina pipeline on the MetaGenomics Rapid Annotation using Subsystems Technology server to assess quality and compare the sequences with those present in different sequence databases in order to identify the fungi they represented.

RESULTS AND DISCUSSION

Analysis of the yeast community present on the surfaces of sound ripe grapes that had seemingly not been in contact with over-ripe or damaged grapes showed that

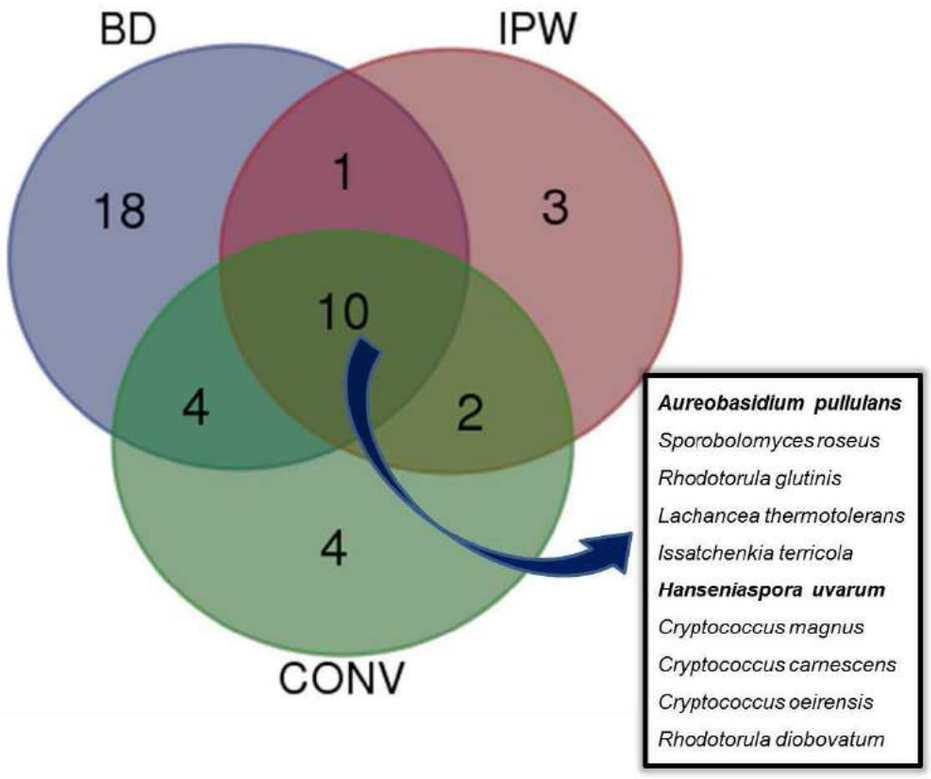

FIGURE 2. A Venn diagram showing the distribution of yeasts associated with Cabernet Sauvignon grapes and grape must from the biodynamic (BD), integrated (IPW) and conventional (CONV) vineyard.

oxidative basidiomycetous yeasts, as well as the yeast-like fungus *Aureobasidium pullulans* are dominant on the berry surface. The grapes from the biodynamic vineyard were also found to carry unique yeasts such as *Meira geulakonigii* (known to have antagonistic activities against mites), *Pseudozyma fusiformata (*which produces anti-fungal glycolipids), as well as *Exophiala* sp. and *Ustilago* sp. This suggests that farming systems that do not regularly apply chemical pesticides promote the development of diverse microbial communities that may include natural pests of potential grapevine pathogens (Setati *et al*., 2012). This suggests to some extent that the reduction in pesticide application creates conducive conditions that support the establishment of balanced ecosystems.

Analysis of the yeast diversity in grape must revealed a community mainly comprising oxidative and fermentative ascomycetous yeasts. These included common yeasts such as *Metschnikowia pulcherrima*, *Hanseniaspora uvarum*, *Lachancea thermotolerans*, *Starmerella bacillaris*, *Torulaspora delbrueckii*, as well as *Saccharomyces cerevisiae.* In addition, uncommon yeasts such as *Kazachstania aerobia* were detected in the must from the biodynamic vineyard (Bagheri *et al*., 2015). Overall, the yeast community in the must varied from year to year, however, *A. pullulans*, *H. uvarum*, *M. pulcherrima* and *Issatchenkia terricola* were frequently encountered (Figure 2). Our data showed that although there are

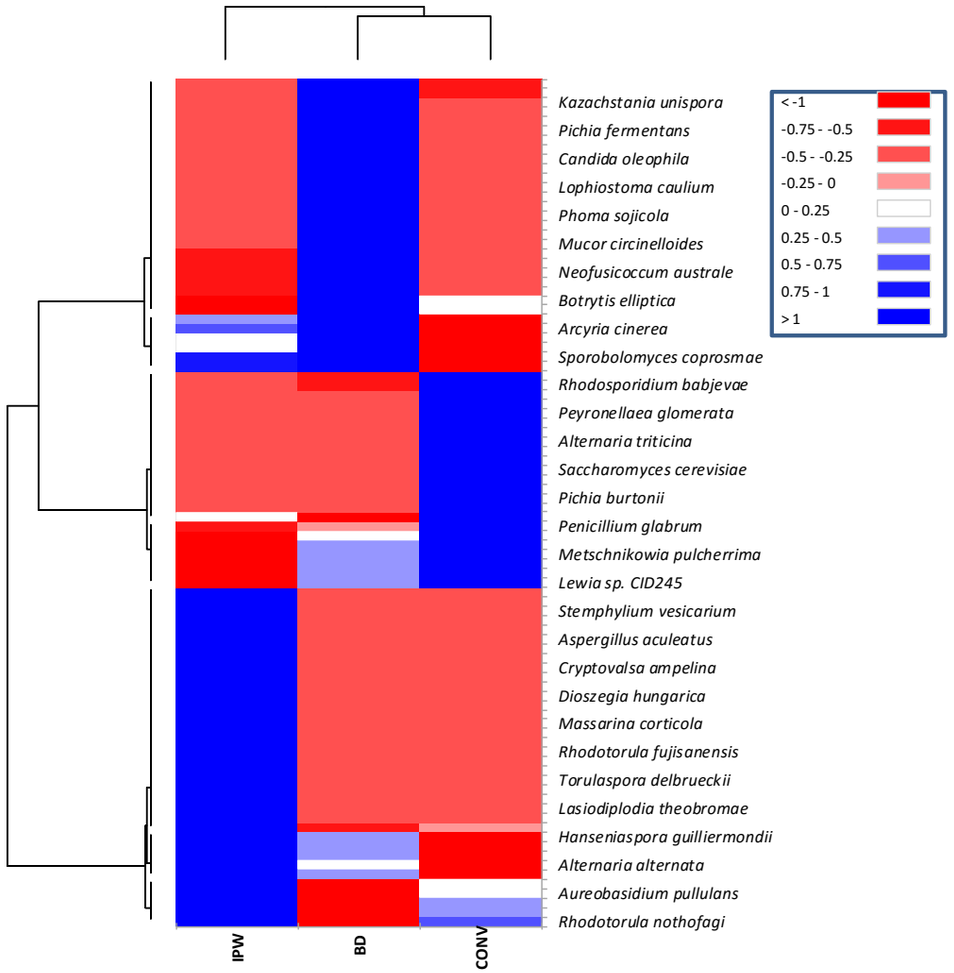

FIGURE 3. A heat map showing the presence and abundance of yeasts and filamentous fungi in the grape must derived from the integrated (IPW), biodynamic (BD) and conventional (CONV) vineyard as detected through direct sequencing of the ITS1-5.8S rRNA-ITS2 genes.

common yeasts present in the three vineyards, only *H. uvarum* and *A. pullulans* were isolated in all three of them in consecutive years, while other species were encountered fortuitously in years and samples where they were present in higher concentrations.

An in-depth analysis of the entire fungal community using high-throughput sequencing of the ITS1-5.8S rRNA-ITS2 gene pool derived directly from the grape must without prior cultivation of the microorganisms, revealed more fungal diversity in the three vineyards including yeasts and filamentous fungi (Figure 3). Similar to the culture-based method, the data showed that the whole fungal community structure associated with grapes and grape must responded to farming practices since there were obvious differences in the diversity and abundances of the fungal species in the three vineyards.

Overall, our data showed that the three vineyards that employ different agronomic practices harboured different fungal communities. Similar to other studies performed on organic and conventional vineyards, the grapes derived from the vineyard which applies less chemical pesticides (i.e. the biodynamic vineyard) was found to have more fungal diversity (Cordero-Bueso *et al*, 2011; Pancher *et al*., 2012. Furthermore, our study also showed that there was contribution of the vineyard fauna and flora to the grape fungal diversity. For instance, our metagenomic data revealed fungi such as *Aschochyta* spp. which are known to be associated with turf grasses, wheat, barley, rye and oats; plants commonly used as cover crops in the vineyard. A comparison of the fungal diversity in the three South African vineyards to those derived from different vineyards and different grape cultivars across California in the United States of America revealed very low similarity (Setati *et al*., 2015). This suggests that South African winemakers should harness the natural biodiversity and apply methods that would promote the persistence of indigenous yeasts in fermentation in order to drive unique chemical signatures that would distinguish wines on the bases of their origin.

CONCLUSION

Our study showed that South African vineyards harbour unique diverse fungal communities which are significantly distinct from vineyards on other continents. This diversity is strongly affected by farming practices including pesticide applications, as well as the types of cover crops used. A better understanding of these influences may help winemakers employ strategies that will harness the natural biodiversity and most importantly promote the establishment of desirable microbiota.

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Effect of irrigation with diluted winery wastewater on Cabernet Sauvignon juice and wine pH

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KEYWORDS: Cabernet Sauvignon, potassium, sodium, micro-sprinklers, sandy soil.

INTRODUCTION

Approximately 3 to 5 m^3 of poor quality wastewater is produced per tonne of grapes crushed by wineries (Mosse *et al.*, 2011). Winery wastewater contains high levels of potassium (K) and sodium (Na) which originate from cleaning products, grape lees and spillage from the grape fermentation process (Laurenson *et al.*, 2012; Conradie *et al.*, 2014). Where wineries are surrounded by vineyards, irrigation with winery wastewater could be an alternative to fresh water abstracted from natural resources. However, winemakers are generally reluctant to dispose of winery wastewater for vineyard irrigation due to high Na and K (Kumar *et al.*, 2014).

Potassium is the predominant cation involved in the pH balance of grape juice or wine, and there is a good relationship between pH and K (Kodur, 2011 and references therein). During winemaking, high wine K increases the precipitation of tartaric acid, consequently reducing free tartaric acid (Kodur, 2011). High wine K makes pH adjustment difficult and expensive (Kumar *et al.*, 2014). High juice K can also reduce the tartaric/malic acid ratio which is undesirable for high wine quality (Mpelasoka *et al.*, 2003). Elevated berry K

will influence the effect of other cations (Boulton, 1980; Kumar *et al.*, 2014) and is thought to have a negative impact on fermentation and microbial activity, as well as wine properties such as taste, bitterness and sourness.

According to Jackson and Lombard (1993), high levels of K in the juice are not only associated with high pH, but also poor colour of red wines. Although high K levels in the plant are correlated with high soil K concentrations, soil K effects on juice levels are small unless excessive K is applied. Although application of wastewater with high K levels will increase soil fertility, long-term application may cause accumulation of soil K (Kumar *et al.*, 2014). This could decrease soil hydraulic conductivity (Arienzo *et al.*, 2009). Currently, the Department of Water and Sanitation is drafting new General Authorisations for winery wastewater. The objective of this study was to determine the effect of using diluted winery wastewater for vineyard irrigation on K, Na and pH in juice and wine in order to make recommendations for possible adaptations to the General Authorisations.

EXPERIMENTAL BACKGROUND

The field experiment was carried out with micro-sprinkler irrigated Cabernet Sauvignon/99 R in a sandy soil near Rawsonville in the Breede River Valley region from 2009/10 until 2012/13. Irrigation of grapevines using winery wastewater diluted to 100, 250, 500, 1 000, 1 500, 2 000, 2 500 and 3 000 mg/ℓ chemical oxygen demand (COD) was compared to a control of irrigation using river water. A cover crop was cultivated in summer to intercept salts applied via the winery wastewater. More complete details on the wine preparation and analyses methods have been published by the authors (Myburgh & Howell, 2014).

RESULTS AND DISCUSSION

Juice

In general, juice K and Na were within the recommended levels (Wooldridge *et al.*, 2010). Juice K tended to increase with an increase in COD level of the diluted winery wastewater (Table 1). Juice K also tended to increase where undiluted winery wastewater was used for vineyard irrigation (Kumar *et al.*, 2014). It has also been reported that irrigation using artificial winery wastewater containing wine and high K levels produced juice with lower K compared to wastewater containing high K and Na (Mosse *et al.*, 2013). This indicated that the presence of wine in the artificial winery wastewater prevented an increase in juice K. In another study, juice K increased where 45 kg/ha K was applied as vineyard fertiliser compared to where no K was applied (Conradie & Saayman, 1989). However, there were no further increases in juice K when the fertiliser application was 90 kg/ha. Another study indicated that when 450 kg/ha K was applied to grapevines, there was an increase in juice K (Morris & Cawthon, 1982). Juice K of Concord grapevines also increased when K application increased from none, to 225, 450 or 900 kg/ha K (Morris *et al.*, 1980).

The juice K increase was strongly related to the amounts of K applied via the diluted winery wastewater (Fig. 1A). Juice pH increased with an increase in COD level of the diluted winery wastewater (Table 1). As a result, juice pH increased linearly with juice K (Fig. 1B). This agreed with a previous study where juice pH also increased where juice K increased due to K fertilisation (Morris *et al.*, 1980; Morris & Cawthon 1982). It should be noted that even when 900 kg/ha K was applied to grapevines, juice pH did not exceed 3.57 (Morris *et al.*, 1980).

TABLE 1. Effect of irrigation using winery wastewater diluted to different levels of COD on juice and wine K, Na and pH of Cabernet Sauvignon/99 R near Rawsonville. Data are means of three seasons.

Measured variable	River water (control)	Level of COD[(1)] in diluted winery wastewater (mg/ℓ)							
		100	250	500	1 000	1 500	2 000	2 500	3 000
Juice K (mg/ℓ)	1 814	1 931	1 856	2 020	2 126	2 158	2 226	2 158	2 345
Juice Na (mg/ℓ)	18.4	18.5	17.2	19.0	18.9	18.3	18.0	19.5	18.1
Juice pH	3.57	3.59	3.55	3.60	3.63	3.63	3.64	3.67	3.70
Wine K (mg/ℓ)	1 106	1 163	1 168	1 188	1 266	1 350	1 362	1 380	1 410
Wine Na (mg/ℓ)	22.9	21.9	19.9	20.9	21.8	20.6	19.8	20.7	22.1
Wine pH	3.99	4.02	4.04	4.11	4.13	4.15	4.18	4.18	4.18

(1) Chemical oxygen demand.

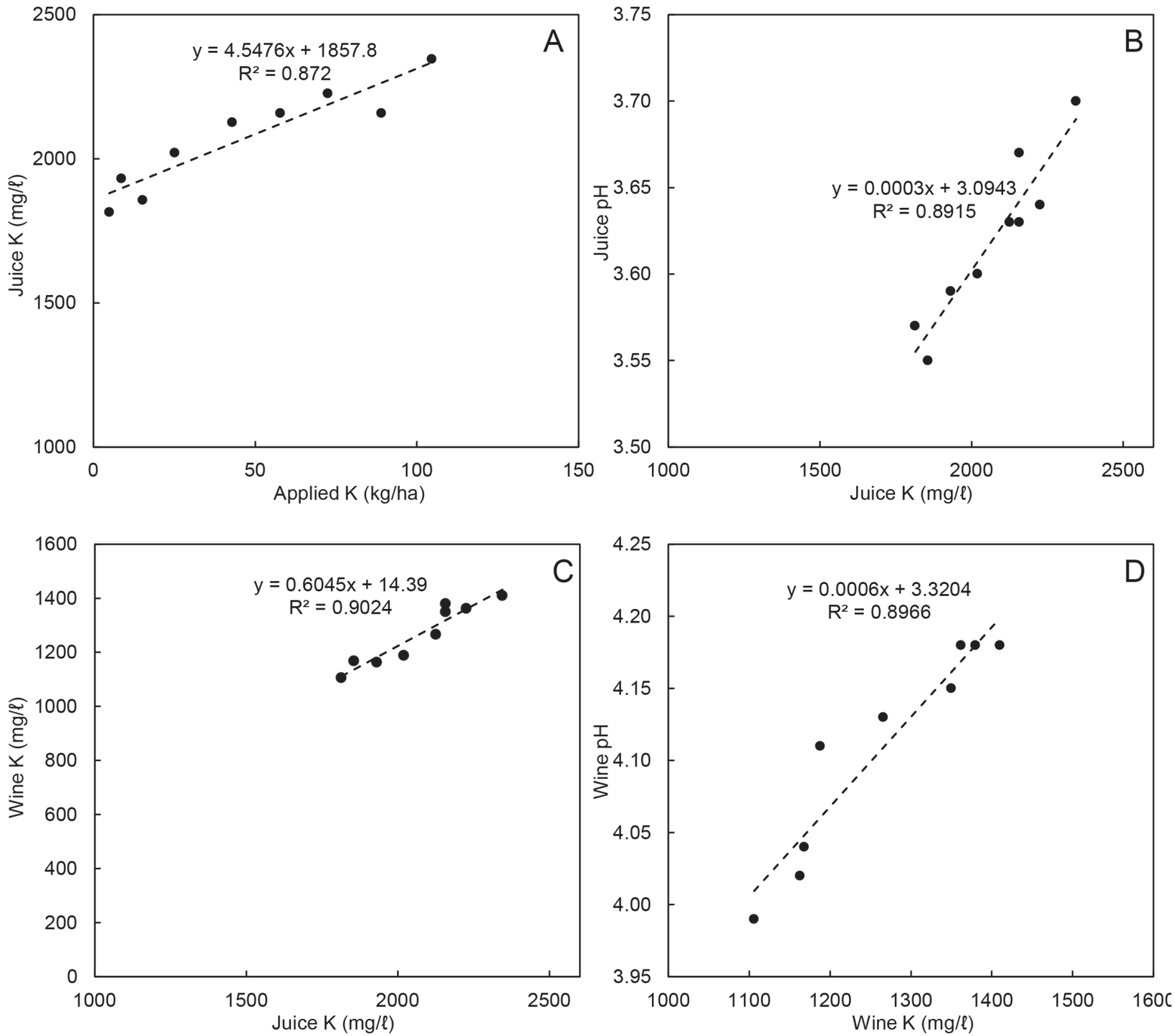

FIGURE 1. Effects of (A) K applied via the irrigation water on juice K, (B) juice K on juice pH, (C) juice K on wine K and (D) wine K on wine pH where Cabernet Sauvignon were irrigated with diluted winery wastewater. Data are means for three seasons.

Wine

Wine K tended to increase with an increase in COD level of the diluted winery wastewater (Table 1). Furthermore, wine K was linearly related to juice K (Fig. 1C). A similar trend was reported by Walker and Blackmore (2012). Wine pH tended to increase with an increase in COD level of the diluted winery wastewater (Table 1). Although wine pH was strongly affected by wine K (Fig. 1D), the pH increase did not have any negative effect on sensorially determined wine colour (Myburgh & Howell, 2014).

There were no consistent trends with regard to juice and wine Na (Table 1). However, a previous study showed that juice Na was higher where Na-based wastewater was used compared to artificial winery wastewaters with high and low K, respectively (Mosse *et al.*, 2013). Wine Na was appreciably lower than 40 mg/ℓ to 190 mg/ℓ (Moolman *et al*., 1998) and 78 mg/ℓ to 533 mg/ℓ reported for Colombar and Shiraz wines, respectively (Walker *et al.*, 2003). It is important to note that, throughout the three seasons, wine Na was considerably lower than the legal limit of 100 mg/ℓ Na for South African wines (Department of Water Affairs and Forestry, 1996).

CONCLUSIONS AND RECOMMENDATIONS

Irrigation of vineyards with winery wastewater increased juice K, which in turn increased juice pH. The same increases occurred in wine K and pH. Under the prevailing conditions, the increase in juice and wine pH did not affect wine sensorial properties. However, this does not rule out the possibility that negative juice and wine responses may be more pronounced where (i) salts accumulate more readily in heavier soils, (ii) low winter rainfall limits the leaching of salts or (iii) no cover crop is cultivated during summer to intercept salts.

Based on the project results, the following criteria should be considered for possible amendments to the General Authorisations for wineries when using diluted wastewater for irrigation of vineyards:

- The COD must be diluted to 3 000 mg/ℓ or less, preferably to less than 2 000 mg/ℓ to avoid unpleasant odours in the vineyard while irrigations are applied.
- The electrical conductivity (EC_{iw}) must be less than 0.75 dS/m.
- The sodium adsorption ratio (SAR_{iw}) must be less than 5.
- The soil must have a low cation exchange capacity.
- The internal drainage in the root zone must be unrestricted.
- The irrigation water must not percolate beyond the root depth.
- Only micro-sprinklers should be used, since drippers have narrow flow paths and/or small orifices, and are more susceptible to clogging.
- The irrigation must be applied with micro-sprinklers in such a way that the bunches are not wetted.
- At least 50% plant available water (PAW) depletion should be allowed between irrigations to allow sufficient aeration for oxidation of organic material applied via the irrigation water.
- The irrigation frequency and volumes (schedule) should enhance, rather than negate, wine quality characteristics.
- A summer interception crop, e.g. pearl millet, should be sown in early January to ensure that its growth peaks when the winery wastewater is applied, and that the species does not complete its life cycle too early.
- Since a summer interception crop may increase the evapotranspiration (ET) of vineyards substantially if growing conditions are favourable, it could induce competition for water between grapevines and cover crop. However, this can be avoided by timely slashing of the interception crop, which will also minimise possible competition for N and P.

SUMMARY

The effect of irrigation using winery wastewater on grapevines was compared to irrigation using river water in a temperate climate with hot, dry summers. Cabernet Sauvignon/99 R was irrigated using winery wastewater diluted to eight different levels of chemical oxygen demand (COD) with river water. The field trial was carried out near Rawsonville in the Breede River Valley region from 2009/10 until 2012/13. Under the prevailing conditions, juice K increased linearly with the amount of K applied *via* the diluted winery wastewater prior to harvest. The increase in juice K increased juice pH. Wine K increased as juice K increased. Although wine pH increased with wine K, it did not impact negatively on wine colour. It is noteworthy that wine Na did not exceed the legal limits. However, the foregoing does not rule out the possibility that negative juice and wine responses could occur (i) where salts accumulate more readily in heavier soils, (ii) where low winter rainfall limit leaching of salts or (iii) where no cover crop is cultivated during summer to intercept salts.

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Soil preparation with special emphasis on the re-compaction of vineyard soils

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KEYWORDS: Vineyard soils, re-compaction, soil strength, bulk density.

INTRODUCTION

The deep preparation of vineyard soils has a long history in South Africa. Abraham Perold (1926) had already discussed the pros and cons of "delving vs. ploughing" and based his recommendations on soil type. Deep soil preparation has however, only been scientifically grounded with the visit of H. Schulte-Karring (1976) and publishing of the results of soil preparation trials at Robertson and Stellenbosch (Claassen, Van Zyl & Kleynhans, 1973; Saayman & Van Huyssteen, 1980; Saayman, 1982). Since then deep soil preparation on wine farms has become an essential practice aiming to break up compact soil layers, as well as abrupt transitions between soil horizons down to a depth of 1 000 mm. Soil preparation also provides an ideal (and often the only) opportunity to rectify unfavourable soil chemical conditions such as high acidity and low phosphate content.

Soil layers with a high density limit root penetration forces of roots to only utilise the overlying soil (Schulte-Karring, 1976; Saayman, 1982). The inability of roots to penetrate into compacted subsoil layers is related to the lack of macro pores in these layers (Van Huyssteen, 1989). Roots cannot penetrate a pore which has a smaller diameter than the root tip (Wiersum, 1957). A root cannot decrease its diameter, but will rather thicken when the growing tip experiences resistance. The presence of a good distribution of macro pores is consequently a prerequisite for good root distribution in soil. Various researchers also found a positive linear relationship between rooting depth and aboveground grapevine performance (Saayman & Van Huyssteen, 1980; Van Huyssteen, 1982, Myburgh *et al.*, 1996).

In the past the results of different cultivation practices have been characterised by measuring bulk density, soil strength, shear strength, water infiltration and soil water hydraulic conductivity. Impediment of grapevine root growth was successfully characterised by Leopoldt van Huyssteen (1983) in terms of bulk density and soil strength. Soil strength was measured by a constant-speed penetrometer. In a wide variety of soil types, a critical, albeit poorly defined, penetrometer resistance of 2 000-2 500 kPa was found above which root penetration is drastically impeded (Zimmerman & Kardos, 1961; Taylor & Gardner, 1963; Taylor & Burnett, 1964; Greacen *et al.*, 1969; Bar-Yosef & Lambert, 1981).

The current study was conducted at three locations in the Western Cape in order to determine i) whether the positive effect of deep soil preparation on the soil physical condition is sustainable in the long term and ii) which methods best indicate the physical differences between different soil preparation practices.

MATERIAL AND METHODS

Nietvoorbij

Measurements to determine the degree of re-compaction were conducted in an old soil-volume experiment where soil preparation was done in 1984, i.e. 26 years previously. In this 1984-experiment a series of soil-volumes were created using an excavator on a Cartref soil of granitic origin. After mixing the excavated soil with calcitic lime and superphosphate, the soil was backfilled into the open trenches (Myburgh *et al.*, 1996). The treatments thus created, appear in Table 1. In the current study only eight of the 10 treatments were included; the 400 mm irrigated and 1 200 mm irrigated treatments were excluded.

TABLE 1. Soil preparation treatments (1984) applied in the soil-volume trial at Nietvoorbij (Myburgh *et al.*, 1996).

Soil preparation treatments	
Dryland	**Irrigated**
Control, ploughed to 200 mm	
Ridges, 400 mm high	
Trenches, 400 mm deep	Trenches, 400 mm deep
Trenches, 600 mm deep	
Trenches, 800 mm deep	Trenches, 800 mm deep
Trenches, 1 000 mm deep	
Trenches, 1 200 mm deep	Trenches, 1 200 mm deep

This sandy clay loam soil contained 50% gravel and had a high bulk density in its natural state. After soil preparation, the soil was left to consolidate for a year and then it was planted to Pinot noir/99 Richter. At that stage soil bulk density was a favourable 1 550 kg/m^3 compared to 1 750 kg/m^3 of the control. Penetrometer studies at that stage, i.e. a year after soil preparation, also showed that the soil was effectively loosened to the pre-determined depths. No vehicle traffic was allowed in the vineyard after planting.

Broodkraal

The study at Broodkraal near Piketberg investigated selected physical properties of a Tukulu soil on three plots that represented the following soil treatments: i) control (natural state), i.e. undisturbed soil next to the other two treatments on the same soil type, ii) newly prepared soil (soil prepared in 2010 and measurements taken in 2010), and iii) soil one year after soil preparation (soil prepared in 2009, but measurements taken in 2010). The last-mentioned treatment was prepared and planted to Thompson Seedless/ Ramsey in 2009. Soil preparation consisted of cross ripping to a depth of 1 000-1 200 mm followed by construction of ridges.

Kanonkop

The result of two soil preparation methods were compared in 2010, i.e. six years after execution in 2004. The soil was classified as Tukulu and Oakleaf forms. The first method involved 600 mm deep ripping followed by delve ploughing, while the second treatment consisted of ripping (600 mm depth) followed by tilling with a soil mix implement (Fig. 1).

FIGURE 1. Soil mix implement used for soil preparation at Kanonkop.

The soil mix implement was specifically developed to work between vine rows. A rotating cylinder of 800 mm diameter equipped with tungsten tines that give it extra reach, creates the loosening action of the implement. Both soil preparation methods aimed to loosen the soil down to a depth of 1 000 mm. Land prepared by these two methods was planted to Cabernet Sauvignon/99 Richter in 2004. Due to the nature of the treatments, they could not be replicated, but comprised large areas of land adjacent to each other. Undisturbed soil of the same soil family in an adjacent vineyard served as a control.

Soil physical measurements

Soil bulk density was determined in triplicate at various depths of the different treatment plots. A pocket vane tester was used for determining shear strength (Fig. 2). This instrument was placed against the profile wall, the vanes pushed into the soil and the instrument slowly turned clockwise until the soil fails. Since soil strength is a highly variable property sensitive to changes in soil water content, all shear strength measurements were executed at field capacity after rain on the same day in a specific trial.

Soil strength was measured by a constant speed penetrometer equipped with a rod that can reach a depth of 800 mm, and a conic tip (30° angle) with a base area of 1.3 cm^2. This particular instrument can take measurements at 10 mm intervals and to a maximum resistance of 5 000 kPa. Soil water content during penetrometer measurements was close to field capacity. Penetrometer measurements were taken at 10 randomly positions on each treatment plot of the soil-volume experiment at Nietvoorbij. Due to equipment breakdown, none of the other two trial sites could be included in the penetrometer study.

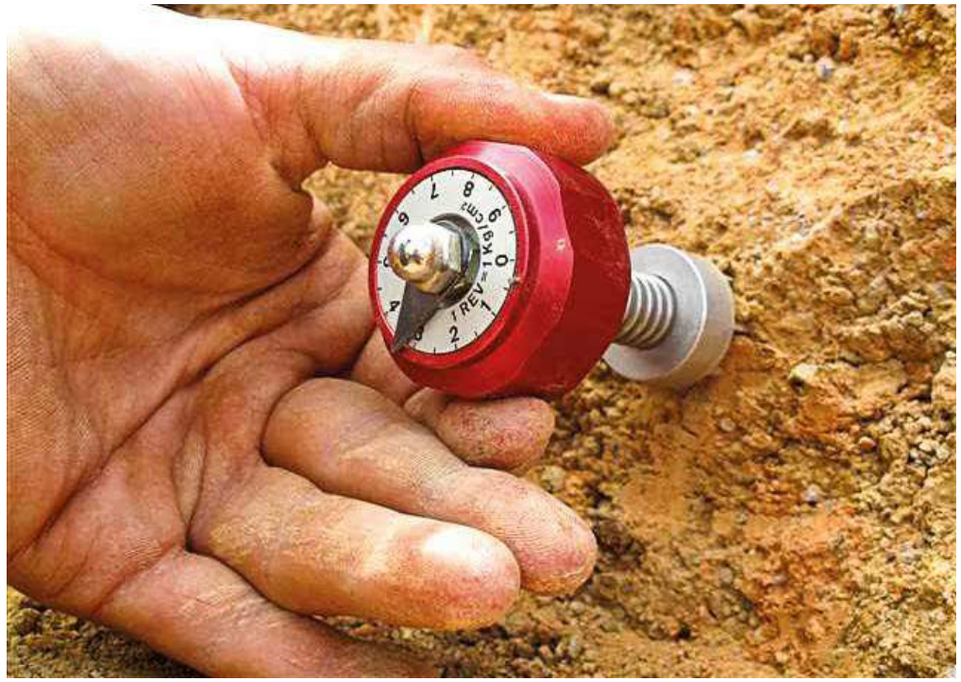

FIGURE 2. Measurement of soil shear strength using a pocket vane tester.

A double-ring infiltrometer (Fig. 3) was used for measurement of saturated hydraulic conductivity from the soil surface. The apparatus consists of two vertical cylinders filled with water and two double rings which are placed on the soil. The infiltrometer is mounted in such a way that one cylinder supplies water to the outer ring and the other cylinder supplies water to the inner ring. Water from the outer ring cancels lateral water flow from the inner ring and measurements are only taken of the latter. Measurements were done in duplicate on all treatments of the three trial sites, namely Nietvoorbij, Broodkraal and Kanonkop.

FIGURE 3. Double-ring infiltrometer for determination of saturated hydraulic conductivity.

A mini disc infiltrometer was used to measure unsaturated flow in the soil. Measurements were taken at 150 mm increments from the soil surface down steps in the profile wall. Infiltration rate was determined at a tension of 0.1 kPa on loose soils, while 0.05 kPa was used on compact soil to make provision for the slow water infiltration under those conditions. Measurements on each treatment plot at both Nietvoorbij and Kanonkop were replicated at two sites, but Broodkraal was not included in these measurements.

Root studies were conducted at one grapevine per treatment at Nietvoorbij and Kanonkop, using the profile wall method. In addition to mapping their positions, roots were also classified in different diameter classes of fine (<2 mm), medium (2-5 mm), coarse (5-10 mm) and thick (>10 mm).

RESULTS AND DISCUSSION

Twenty six years after soil preparation, the beneficial effect of this action could still be detected on most of the treatment plots at Nietvoorbij by the majority of soil physical determination methods. Penetrometer readings were 1 969 kPa (2 000-2 500 kPa is accepted as the upper boundary above which no root penetration can occur) on average in the 200-600 mm soil layer in comparison with 3 538 kPa at the same depth in the control. The abrupt increase in soil strength below the working depth of the excavator could also still be measured. Despite the lasting difference between loosened and undisturbed soil, serious re-compaction had taken place and it was clear that deep soil preparation would be necessary before a new vineyard could be planted.

The penetrometer results also corresponded well with the compaction pattern indicated by the bulk density values. Current results confirmed the findings of previous studies, namely that penetrometer resistance is a sensitive, accurate and quick method to measure soil strength. Automated constant speed penetrometers are however expensive, but as an alternative a steel rod can be pushed manually into a wet soil to discover compacted layers. Although such a crude method will not give quantitative values, together with experience it can be used to give a fair indication of soil compaction.

Measurement of shear strength on the three test sites using the pocket vane tester gave variable results. This instrument did not perform well on the gravelly Cartref soils at Nietvoorbij, but on the fine textured soils of Kanonkop and Broodkraal it could successfully distinguish between the undisturbed controls and their matching deep-tilled treatments. Shear strength could even detect re-compaction in the deep-tilled soil at Broodkraal after one year (Fig. 4).

Saturated, as well as unsaturated, hydraulic conductivity showed limited differences among soil preparation treatments at Nietvoorbij. High values of unsaturated hydraulic conductivity were caused by preferential flow since the apparatus is particularly sensitive to changes in soil macro porosity. Results obtained with saturated hydraulic conductivity and bulk density measurements did not agree consistently. Bulk density and penetrometer resistance were more sensitive to soil preparation effects than saturated hydraulic conductivity. More differences between treatments would probably have been found if infiltration time had been increased to three hours and even longer.

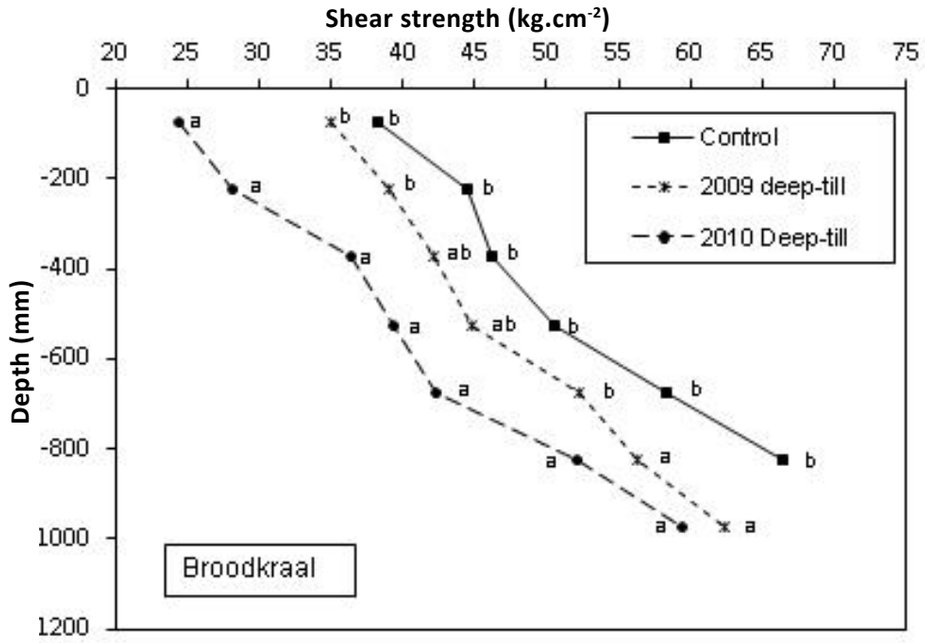

FIGURE 4. Shear strength on treatment plots at Broodkraal.

Root distribution correlated well with depth of soil preparation at the two sites where root studies were conducted. Despite serious re-compaction at Nietvoorbij, grapevine root distribution in the soil-volume trial still reflected the positive effect of deep soil preparation 26 years after it had been carried out (Fig. 5 and 6). An explanation for this long-term benefit of soil preparation is the fact that roots can explore the full potential rooting depth within two years after planting thus establishing the root frame very early, i.e. before re-compaction normally takes place. Thereafter rooting depth remains constant, but rooting density increases with time. Although a very reliable indicator of soil conditions, root mapping is tedious and time consuming, but well worth the effort. In fact, root distribution remains the ultimate indicator of soil conditions for grapevine growth.

In the case study at Broodkraal, bulk density not only showed the positive effect of soil preparation, but also quantified soil re-compaction that took place within one year after deep tilling (Fig. 7). Such re-compaction is a natural process due to, among other factors, soil wetting and the weight of the soil mass itself. Despite re-compaction, the soil physical condition (bulk density values <1 650 kg/m^3) would still allow grapevine root penetration.

At Kanonkop, five years after the soil was deep-tilled, all the soil physical determination methods, including saturated and unsaturated hydraulic conductivity, clearly indicated the positive effect of two soil preparation methods compared to an undisturbed control. The delve plough and the soil mix implement both gave similar results (Fig. 8) and this result was confirmed by the excellent root distribution on the respective treatment plots. The first abrupt decrease in root penetration occurred at 1.0 m in the rip/soil mix combination and at 1.1 m in the rip/delve plough treatment.

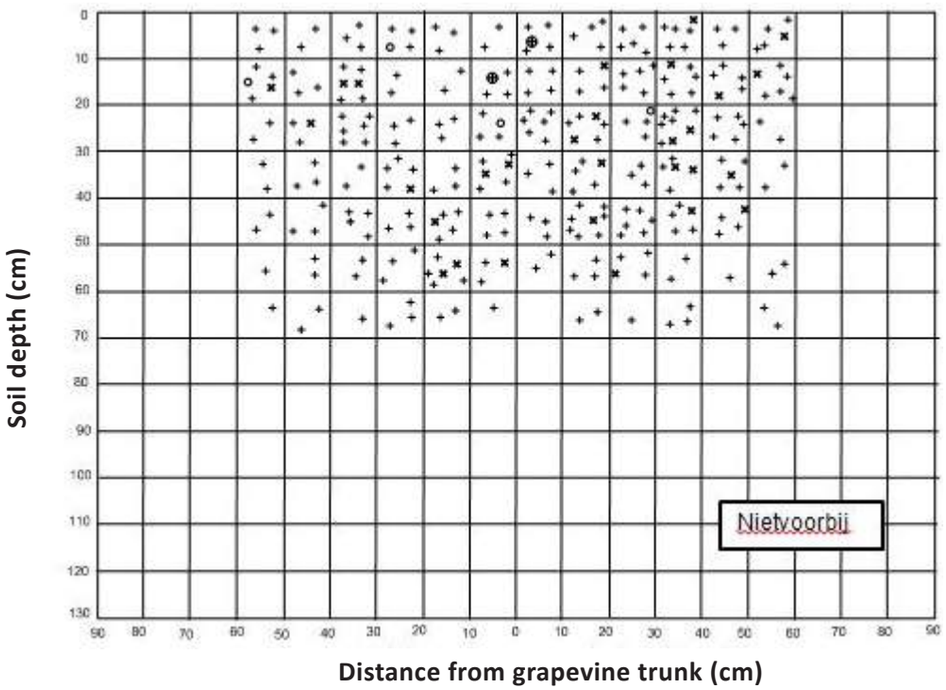

FIGURE 5. Root distribution on a control plot at Nietvoorbij.

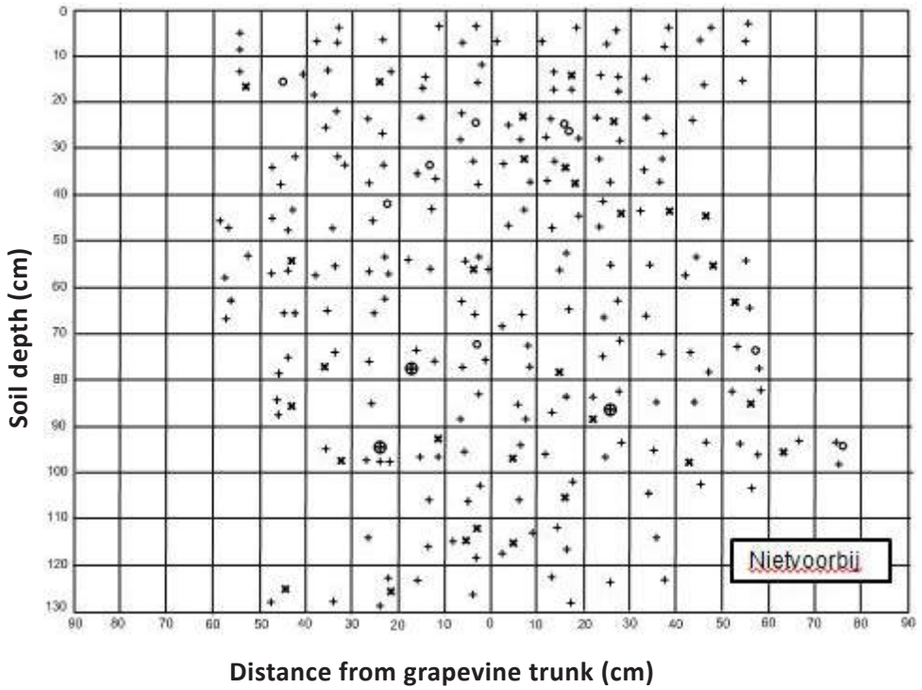

FIGURE 6. Root distribution on a deep-tilled (120 cm) plot at Nietvoorbij.

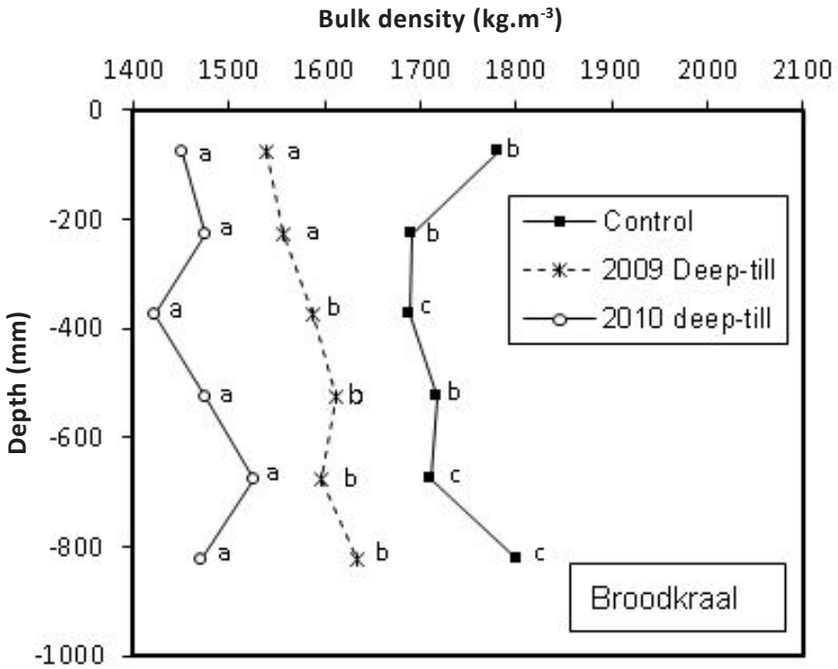

FIGURE 7. Bulk density on treatment plots at Broodkraal.

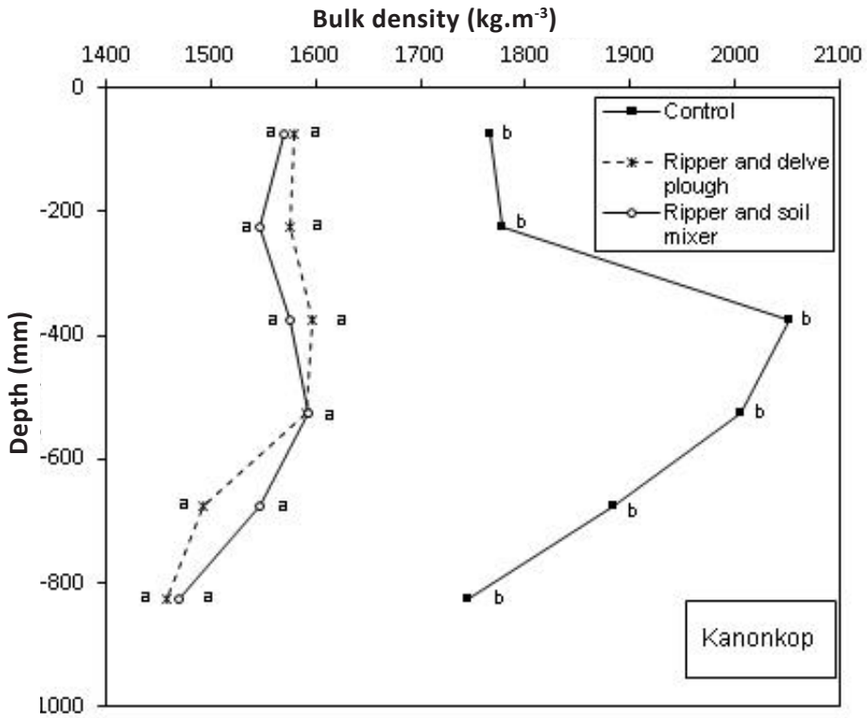

FIGURE 8. Bulk density on treatment plots at Kanonkop.

In summary it can be concluded that significant re-compaction can already occur one year after soil preparation and it increases with time. Deep soil preparation will therefore normally be necessary when an existing vineyard has to be replaced after 20-25 years. There are various instruments that can be used successfully to determine soil compaction. Determination of soil strength using a penetrometer is probably the easiest, quickest and most accurate. Due to the high cost and unavailability of constant-speed penetrometers, the pocket vane tester or a simple steel rod with handle are alternatives that can be used to detect compact soil layers.

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A method for a brief estimation of the complexity of virus infections of grapevines

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KEYWORDS: RT-PCR, SSCP, grapevine leafroll, GLR, rugose wood, RW, diseases of grapevines.

Grapevine leafroll (GLR) and rugose wood (RW) are the most widely spread diseases of grapevines in vineyards worldwide (Naidu *et al.*, 2014; Minafra, 2000). The diseases are easily transmitted between plants by common vineyards pests like mealybug and scale insects. GLR disease is of great concern to grapevine industries, because it delays maturation, and decreases the sugar content of berries, which negatively influences the quality of produced wine. RW diseases cause abnormal differentiation of cambium cells to xylem and phloem tissue, which leads to various externally visible modifications of the grapevine trunk. The RW diseases affect graft takes and are responsible for reduced vigour of grapevines, which ultimately results in lower productivity and longevity of vineyards.

After many years of investigations of the aetiologies of GLR and RW, it is a widely accepted conclusion that viruses of the families *Closteroviridae* and *Betaflexiviridae*, respectively, are causing these diseases. The investigation is still ongoing since these families are composed of many virus species infecting grapevines (Table 1), and every one of them shows high genetic heterogeneity. The study is difficult, since GLR- and RW-affected grapevines are usually infected with a mixture of virus species, and genetic variants of viruses (Table 2). RT-nested PCR amplifications of viruses using degenerate, *Closteroviridae*- and *Betaflexiviridae*-specific oligonucleotide primers, and analysis of the amplicons using a single strand conformation polymorphism (SSCP) technique is the method of choice for a brief estimation of the complexity of virus infections of grapevines.

TABLE 1. The viruses associated with grapevine leafroll (GLR) and rugose wood (RW) diseases of grapevines.

Disease	Associated virus	Taxonomic classification (family, genus)*
Grapevine leafroll (GLR)	GLRaV-1	*Closteroviridae*, *Ampelovirus* (subgroup 1)
	GLRaV-2	*Closteroviridae*, *Closterovirus*
	GLRaV-3	*Closteroviridae*, *Ampelovirus* (subgroup 1)
	GLRaV-4	*Closteroviridae*, *Ampelovirus* (subgroup 2)
	GLRaV-5	*Closteroviridae*, *Ampelovirus* (subgroup 2)
	GLRaV-6	*Closteroviridae*, *Ampelovirus* (subgroup 2)
	GLRaV-9	*Closteroviridae*, *Ampelovirus* (subgroup 2)
	GLRaV-10	*Closteroviridae*, *Ampelovirus* (subgroup 2)
	GLRaV-11	*Closteroviridae*, *Ampelovirus* (subgroup 2)
Rugose wood (RW)	GVA	*Betaflexiviridae*, *Vitivirus*
	GVB	*Betaflexiviridae*, *Vitivirus*
	GVD	*Betaflexiviridae*, *Vitivirus*
	GVE	*Betaflexiviridae*, *Vitivirus*
	GVF	*Betaflexiviridae*, *Vitivirus*
	GRSPaV	*Betaflexiviridae*, *Foveavirus*

* *Ampeloviruses* of subgroup 2, according to recent molecular analysis, should be regarded as divergent genetic variants of the first discovered *Ampelovirus*, GLRaV-4 (Martelli *et al.*, 2012).

TABLE 2. Viruses associated with grapevine leafroll (GLR) and rugose wood (RW), which were detected in various grapevines affected by these diseases.

Number	Grapevine	Disease	Virus status*
1	Waltham Cross	GLR	GLRaV-1,-2,-3,-5,-10
2	Pinot Noir	GLR	GLRaV-1,-2,-3
3	Chasselas	GLR	GLRaV-2,-3,-5,-6
4	Barlinka	GLR	GLRaV-3,-4
5	Ohanez	GLR	GLRaV-3,-5,-6,-11
6	Black Spanish	GLR	GLRaV-1,-2,-3,-4,-5
7	Shiraz	GLR	GLRaV-2,-3,-4
8	LN33 (93/953)	RW (CB)	GRSPaV, GVB (3 variants)
9	LN33 (93/955)	RW (CB)	GRSPaV, GVB (2 variants)
10	LN33 (248)	RW (CB)	GRSPaV, GVB (1 variant)
11	LN33 (I327)	CB-free	GRSPaV, GVB (1 variant)

* Refers only to viruses associated with the diseases specified in this table.

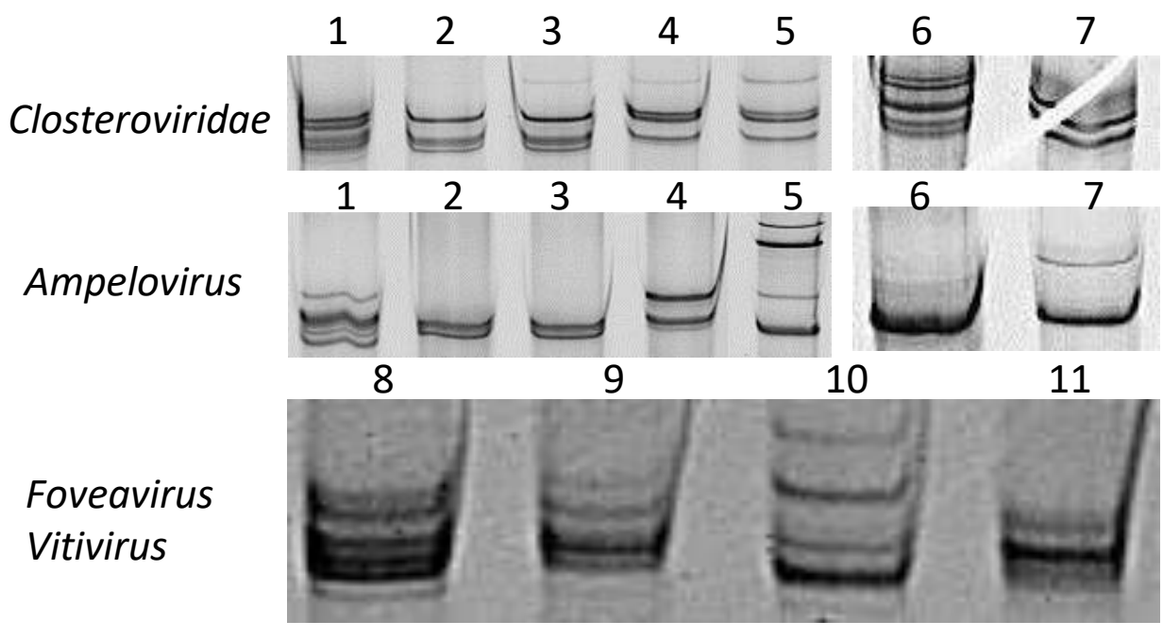

FIGURE 1. Results of SSCP analysis of amplicons generated in RT-nested PCR based on degenerate primers specific to viruses of the family *Closteroviridae* and genuses *Ampelovirus*, *Foveavirus* and *Vitivirus.* Numbers 1-11 refer to grapevines shown in Table 2.

Degenerate primers for the specific and simultaneous RT-nested PCR amplifications of members of these two virus families associated with GLR and RW diseases were designed using the amino acid sequence data of conserved (the same or very similar for different virus-species of a family) stretches of the sequences of virus-encoded proteins (Dovas & Katis, 2003; Maliogka *et al.*, 2008). SSCP technique utilises the phenomenon of different migration of single strands of DNA in polyacrylamide gels, which depends on the nucleotide sequence of the strands (Orita *et al.*, 1989). To separate DNA strands, a very easy to prepare reagent is added to a sample of a PCR amplicon, followed by incubation of the mixture at 99°C for 10 minutes, and electrophoresis in polyacrylamide gels for one hour at 200V. Results of the analysis of virus infection of various GLR- and RW-affected grapevines using this method are shown in Figure 1.

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2 Oenology

Sensory evaluation of wine (Part 1)

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KEYWORDS: Sensory evaluation, Descriptive analysis, Sorting, Projective mapping, Napping, CATA.

Today, there is little doubt in the wine industry that personnel, who have been trained to manage sensory evaluation of wine at their respective cellars, add great value to the winery's quality control activities, as well as aid with product development. The purpose of this article is to provide a short and graphic overview of the main wine sensory evaluation methods, as well as some guidelines for people in the industry, on what factors should be taken into account when choosing an appropriate method. This article (Part 1), is the first in a series of four and provides information about Descriptive analysis (DA), the classic method where each wine is profiled in detail, as well as information about alternative rapid methods. Part 2 focusses on the Sorting method, Part 3 on Projective mapping and Napping®, and Part 4 on the Check-all-that-apply (CATA) method. This series is concluded in Part 4 by a summary where the methods are compared in terms of their practical requirements. These include the number of panellists required, time required for the completion of the tasting by the panel and difficulty of the tasks, amongst others.

On the research side of sensory evaluation, the main focus currently being investigated worldwide (in food and wine), is methodology. The question: "What is the best method to do sensory tests on wine?" is often asked by the industry.

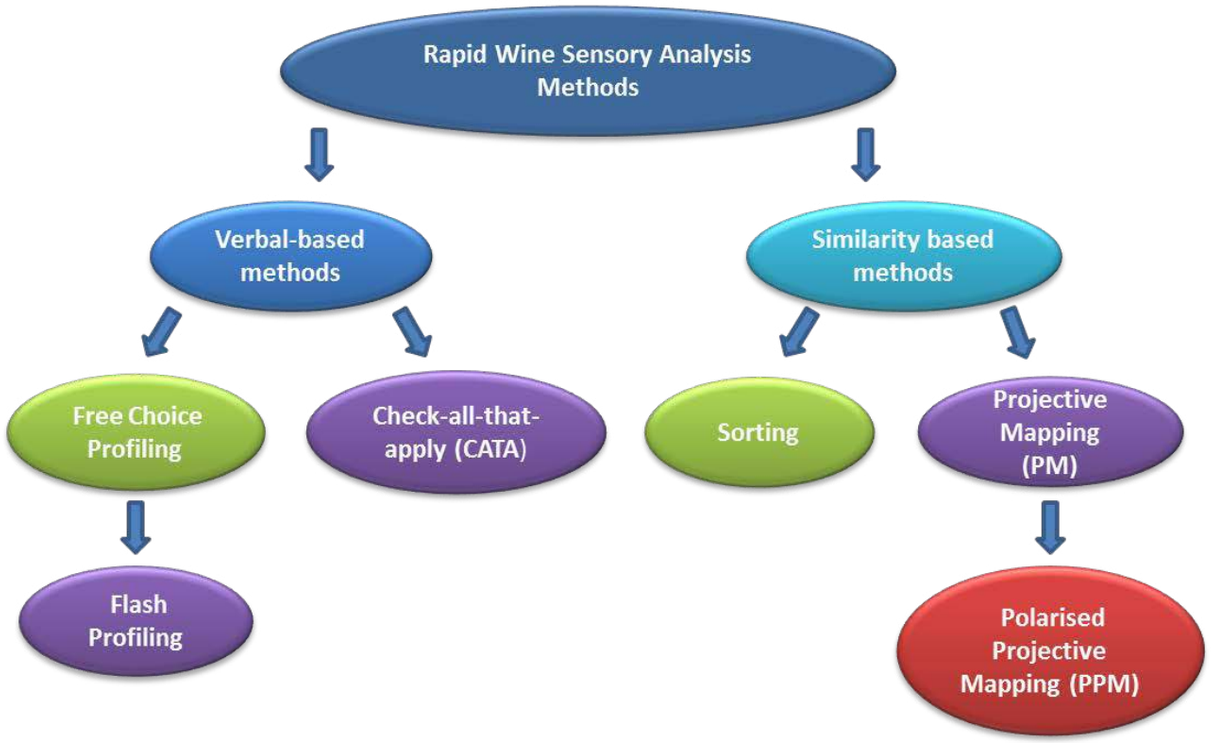

FIGURE 1. Rapid wine sensory analysis methods. The simplest methods are shown in green, the slightly more complex methods in purple, and the method which is the most involved and requires the most preparation, in red.

Time is money, and it is no wonder that the current objectives of sensory research are to find cost-effective and less time consuming methods that are sustainable in the long term. Development of new sensory methods, as well as optimisation of existing ones, is also one of the focus areas of the sensory research currently being done at the Institute for Wine Biotechnology and Department of Viticulture and Oenology (IWBT-DVO) and the Institute for Grape and Wine Sciences (IGWS), Stellenbosch University (SU).

When we speak of sensory testing methods this includes not only the evaluation of aroma and taste of the wine, but also the handling and statistical analysis of the measurements, or data. One of the main aims is to make the statistical procedures as user friendly as possible and to present the information in simple graphic ways, so that it can easily be interpreted. In short, the expression fit-for-purpose is especially applicable to wine sensory evaluation. There is a myriad of methods in literature that are available for the ordinary user, a situation that can become very confusing to interpret.

COMPILING A PANEL

With sensory analysis the “instrument” is a human panellist. Different panels can be formed by people with different levels of knowledge and experience of the product to be tested. The following three types of panels are generally used:

- Ordinary (non-product expert) consumers;
- Wine experts (e.g. winemakers or experts from the wine industry); and
- Trained professional sensory tasters (not necessarily wine experts).

METHODS FOR SENSORY EVALUATION

In terms of the method, the whole set of wines can be profiled in depth, capturing intensity ratings for each characteristic on a line scale. Other more rapid methods are based on only evaluating similarities and differences between wines in a set. It is important to choose the right method, and factors to be considered include the number of panellists, their availability, and type of panel that is required, the method to be used and choice of data analysis techniques. With data analysis it is important to consider which analysis you can do yourself and, which methods of analyses will require support from experts. The most important aspect is that the exact purpose of the sensory evaluation be clarified, this makes the choice of panel and test method easier. Some of the useful methods that can be evaluated for their efficiency by the industry are discussed in the following sections, as well as in the series of three articles that follow on this review.

DESCRIPTIVE ANALYSIS FOR COMPLETE SENSORY PROFILING OF WINE

Descriptive analysis (DA) is the most well-known and regularly used method of sensory analysis. DA is used to identify sensory characteristics of a product and score their intensities. A well-trained panel is essential for quality results. The standard procedure requires that a panel of between eight and 12 judges is trained to identify and score the intensities of aromas and tastes. For this reason no more than eight to 10 wines are normally included per test. Panel training is followed by the testing stage, in which the same set of wines used for training is scored by the panel. Data generated with DA is numerical (intensities can for instance be

Free Choice Profiling

1. Write down 3-5 attributes that best describe each sample

1.	2.	3.	4.	5.
_Oak_____	_____	_____	_____	_____
_Dried fruit____	_____	_____	_____	_____
_Caramel____	_____	_____	_____	_____
_Grapefruit____	_____	_____	_____	_____
_____	_____	_____	_____	_____

6.	7.	8.	9.
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Flash Profiling

2. Using the descriptors you generated, rank the samples from most intense to least intense

_Oak_____	8 1 6 7 4 3 9 2 5
_Dried fruit____	1 8 5 4 7 3 6 9 2
_____	

FIGURE 2. Free choice profiling and Flash profiling as rapid methods for wine sensory profiling.

rated as a number out of a total of 100), as well as descriptive, and can be easily combined with chemical measurements on the same set of wines. This would be desirable, for example, when correlations between chemical and sensory data are investigated. DA is the method of choice when detailed sensory information of the products is required, when a comprehensive list of all the attributes of a single product is required, or when one wants to quantify specific taste/aroma differences between a few products.

General wine aroma CATA list

AROMATIC DESCRIPTORS LIST

FRUITY

WHITE FRUITS
- ☐ Quince
- ☐ Pear
- ☐ Yellow Apple
- ☐ Green Apple
- ☐ Oxidized Apple

YELLOW FRUITS
- ☐ Apricot
- ☐ Peach
- ☐ Melon

CITRUS
- ☐ Grapefruit
- ☐ Lemon
- ☐ Orange

RED FRUITS
- ☐ Cherry
- ☐ Raspberry
- ☐ Redcurrant
- ☐ Strawberry

BLACK FRUITS
- ☐ Blackberry
- ☐ Blackcurrant
- ☐ Blueberry

DRIED FRUITS
- ☐ Dried Peach
- ☐ Dried Apricot
- ☐ Dried Pear
- ☐ Dried Apple
- ☐ Dried Fig
- ☐ Date
- ☐ Prune
- ☐ Raisin

NUTS
- ☐ Almond
- ☐ Hazelnut
- ☐ Walnut

TROPICAL FRUITS
- ☐ Pineapple
- ☐ Banana
- ☐ Guava
- ☐ Passion Fruit
- ☐ Litchi
- ☐ Mango
- ☐ Gooseberry
- ☐ Coconut

SWEET ASSOCIATED
- ☐ Ripe Fruit
- ☐ Marmelade
- ☐ Honey
- ☐ Fruit Jam
- ☐ Glazed / Crystallized Fruit
- ☐ Artificial / Chemical Fruit
- ☐ Muscat
- ☐ Cider

VEGETATIVE / GREEN

VEGETABLES
- ☐ Artichoke
- ☐ Asparagus
- ☐ Cabbage
- ☐ Green Beans
- ☐ Green Pepper
- ☐ Green Olive

FRESH
- ☐ Eucalyptus
- ☐ Herbaceous
- ☐ Tomato leaf
- ☐ Celery
- ☐ Green / Cut Grass
- ☐ Lemon Grass
- ☐ Mint

DRIED
- ☐ Hay / Dried Grass
- ☐ Tobacco

SPICY
- ☐ Bay Leaf / Laurel
- ☐ Thyme
- ☐ Juniper
- ☐ Nutmeg
- ☐ Cinnamon
- ☐ Ginger
- ☐ Clove
- ☐ Anise / Fennel
- ☐ Liquorice
- ☐ Curry
- ☐ Black Pepper
- ☐ White Pepper

FLORAL
- ☐ Camomile
- ☐ Linden Tree Flower
- ☐ Honeysuckle
- ☐ Orange Blossom
- ☐ Jasmine
- ☐ Rose
- ☐ Violet
- ☐ Lilac
- ☐ Geranium

TOASTED / WOOD

TOASTED
- ☐ Caramel / Burnt Sugar
- ☐ Toffee
- ☐ Vanilla
- ☐ Chocolate
- ☐ Roasted Coffee
- ☐ Toated Bread

WOODY
- ☐ Planky
- ☐ Oaky
- ☐ Burnt / Smoked Wood

ANIMAL
- ☐ Cat urine
- ☐ Horsy / Sweaty
- ☐ Leather
- ☐ Meat Stock
- ☐ Musk / Civet
- ☐ Smoked Meat
- ☐ Wet dog

FOREST FLOOR
- ☐ Humus / Earthy
- ☐ Mouldy
- ☐ Mushroom

OTHER
- ☐ Alcohol
- ☐ Butter / Lactic
- ☐ Chalky
- ☐ Iodine / Salty
- ☐ Mineral / Flinty
- ☐ Rubber
- ☐ Solvent / Chemical
- ☐ Sulphur
- ☐ Stuffy / Fusty / Dusty
- ☐ Tar
- ☐ Wet mop
- ☐ Yeast

FIGURE 3. General wine aroma Check-all-that-apply (CATA) list.
(ACKNOWLEDGEMENT: DOMINIQUE VALENTIN, AGROSUP, DIJON, FRANCE).

Sorting

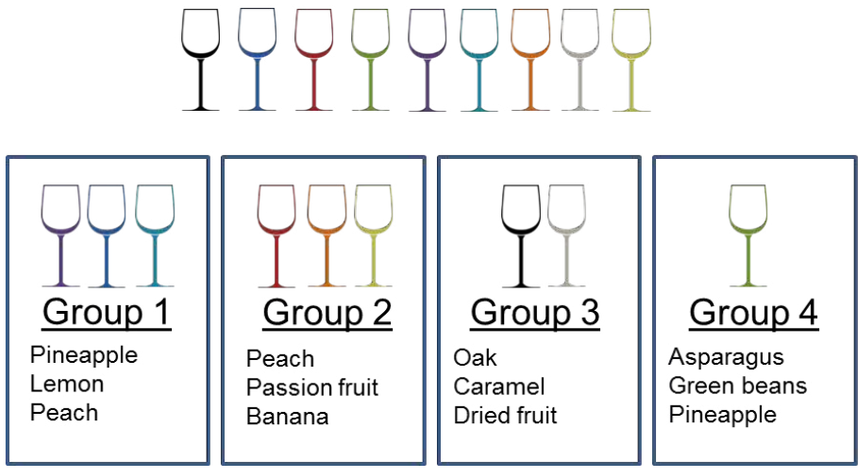

FIGURE 4. Sorting method for wine sensory evaluation. Wines are grouped-based on sensory similarities and differences and placed accordingly on a sheet of white paper.

Although DA is the most well-known and widely used method for sensory analysis, it is a lengthy and costly process. Training of a panel can take between 10 and 30 hours depending on how complex the wine is, and how many samples you wish to profile.

RAPID SENSORY ANALYSIS METHODS

It is also more than likely that one would wish to profile a considerable number of wines, as quickly and as accurately as possible. To fulfil this need for faster/less expensive sensory analysis, came the development of alternative more rapid sensory analysis methods. These methods still use panellists, but can be completed in a short period (in some instances, one hour is sufficient). Several of the rapid methods used in the food industry today were not initially developed for wine and the sensory facility of the IWBT-DWW and IGWS, SU, started a Winetech-funded research project a few years ago to test the efficiency of the rapid methods when applied to wine. Where necessary, methods were adjusted to improve their performance so that the industry can use them. Throughout this series of papers, we use the results of our research to make recommendations regarding applications to wine.

As can be seen in Figure 1, the rapid methods can be divided into two broad groups, namely:

- Verbal-based methods (descriptions); and
- Similarity-based methods (grouping).

Within these two groups they can be further split into:

- Flash profiling (FP);
- Check-all-that-apply (CATA);
- Sorting; and
- Projective mapping (PM).

Within the last four mentioned, there are a number of variations that differ in their degree of difficulty and complexity.

FREE CHOICE AND FLASH PROFILING (FP)

With Free choice profiling (Figure 2), panellists use their own words to describe a wine sample, as the name suggests. There is no training and no list of descriptors is given. The follow on from that is called Flash profiling (Figure 2), where panellists, using the list of descriptors they generated, then rank each wine sample in terms of how strongly they perceive each descriptor in each wine. FP is a good method to use when one needs to rapidly identify a product set's most important attributes. Free choice and Flash profiling are good methods to use to quickly identify the most important descriptors in a set of wines.

CHECK-ALL-THAT-APPLY (CATA)

With the Check-all-that-apply method the panellist is given the samples, as well as a list of descriptors or phrases (Figure 3). The panellist is then required to smell/taste the sample and choose descriptors that they feel most accurately describe the wine. For this method, if consumers are used, words or phrases such as 'like it', 'I would buy it' and 'I don't like it' can be included. From this CATA method, a variation was recently added to the portfolio of wine sensory methods and referred to as the attribute frequency of citation method. This modification requires that panellists are trained on the usage of the descriptors on the list, with the aim of improving consensus regarding the meaning of words and simplifying data analysis.

When setting up a CATA list, the aroma lists available for each cultivar are good starting points, and other attributes can be added at the panel leaders' discretion. One of the most important factors to keep in mind when setting up a CATA list is that the order which the terms appear in, can affect the results. Terms at the top of the list are used more often than those at the bottom. To combat this problem, the following recommendations can be kept in mind:

- The order of the terms on the list should be changed for each assessor;
- Grouping of terms and thereby shortening of the list; and
- Making use of multiple shorter lists.

CATA lists can also be used in combination with other sensory tests like Sorting or Mapping. Figure 3 is an example of the full general wine aroma CATA list originally developed by Dominique Valentin (AgroSup, Dijon, France) and adapted by the sensory laboratory at IWBT-DVO and IGWS, SU, to suit the South African wines and requirements of industry testing.

SORTING

Sorting is a similarity-based test (Figure 4), where samples are grouped according to similarities or differences. Each panellist sorts their set of samples into groups, on a sheet of paper for instance, in a way that makes sense to him/her, and then provides a list of attributes to describe each of their groups. All samples are presented simultaneously, and this technique can be used to evaluate aroma, taste or visual appearance. There are two simple rules, which must be followed; firstly the panellist must make more than one group and secondly, he/she cannot put each sample in its own group. Sorting is fast (normally it can be completed within one hour) and can be done by trained or inexperienced panellists, because it does not require consensus from panellists and there is no quantitative rating. Sorting can also be done with a large number of samples, ideally between nine and 20.

PROJECTIVE MAPPING (PM) AND NAPPING®

For Projective mapping (Figure 5), also referred to as Napping®, samples are sorted/mapped on a large sheet of paper (usually size A2) in two dimensions. The name Napping comes from when it was originally done on a tablecloth (*nappe* in French) instead of paper. Panellists are instructed to arrange each sample on the page according to their perceived sensory similarities and differences. The more similar the samples, the closer together they would be placed, and the more different, the further apart the samples would be placed. The positions of the glasses are then marked on the paper and using the bottom left hand corner of the page as the origin, X and Y co-ordinates are obtained for each sample. The distances (in cm) between the glasses represent their similarities and differences. Panellists also write three to five descriptors for each sample or cluster of samples. This method is fast and can be completed within one hour. It is easy for either ordinary consumers or trained panellists to complete.

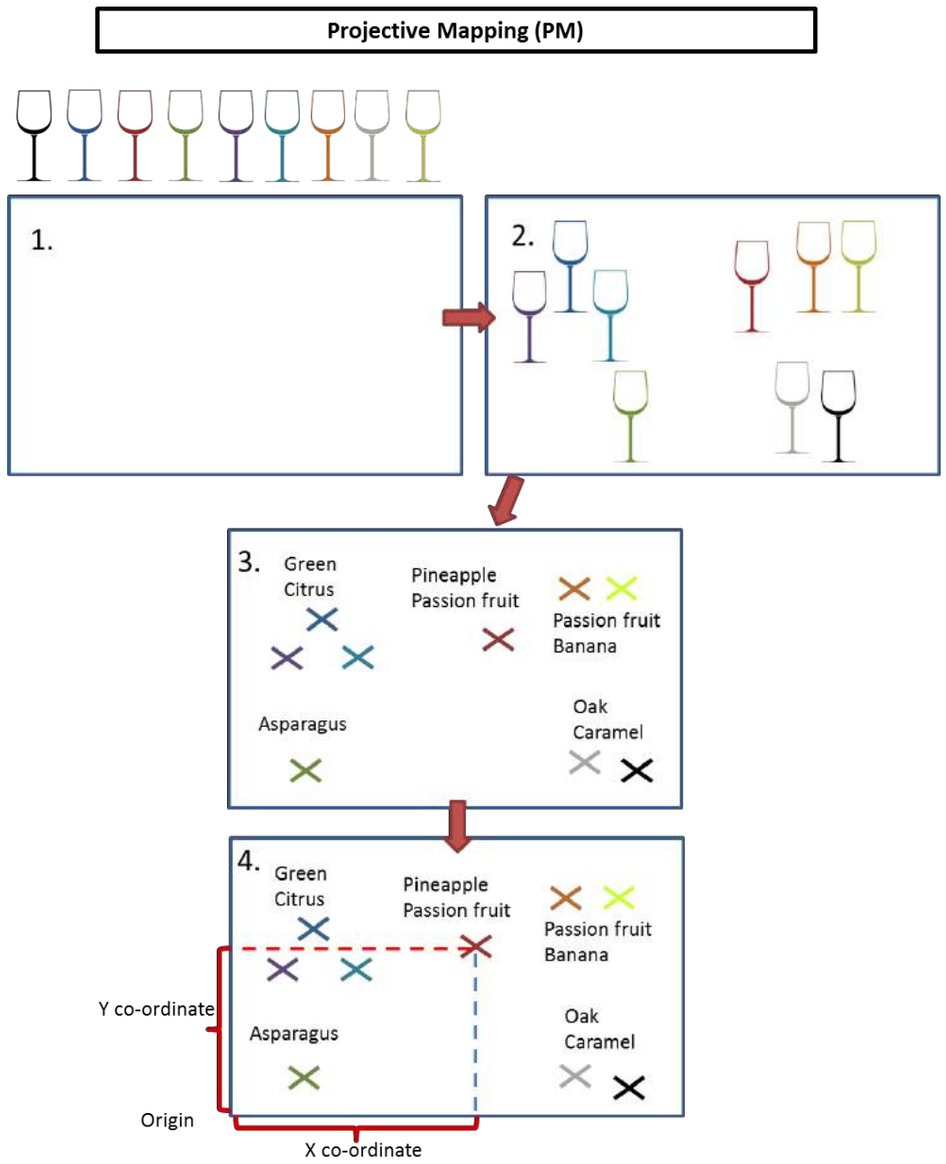

FIGURE 5. Projective mapping. Each rectangle represents a sheet of white paper. Markings show the positions of the glasses positioned on the sheet by the panellists after tasting of the wines.

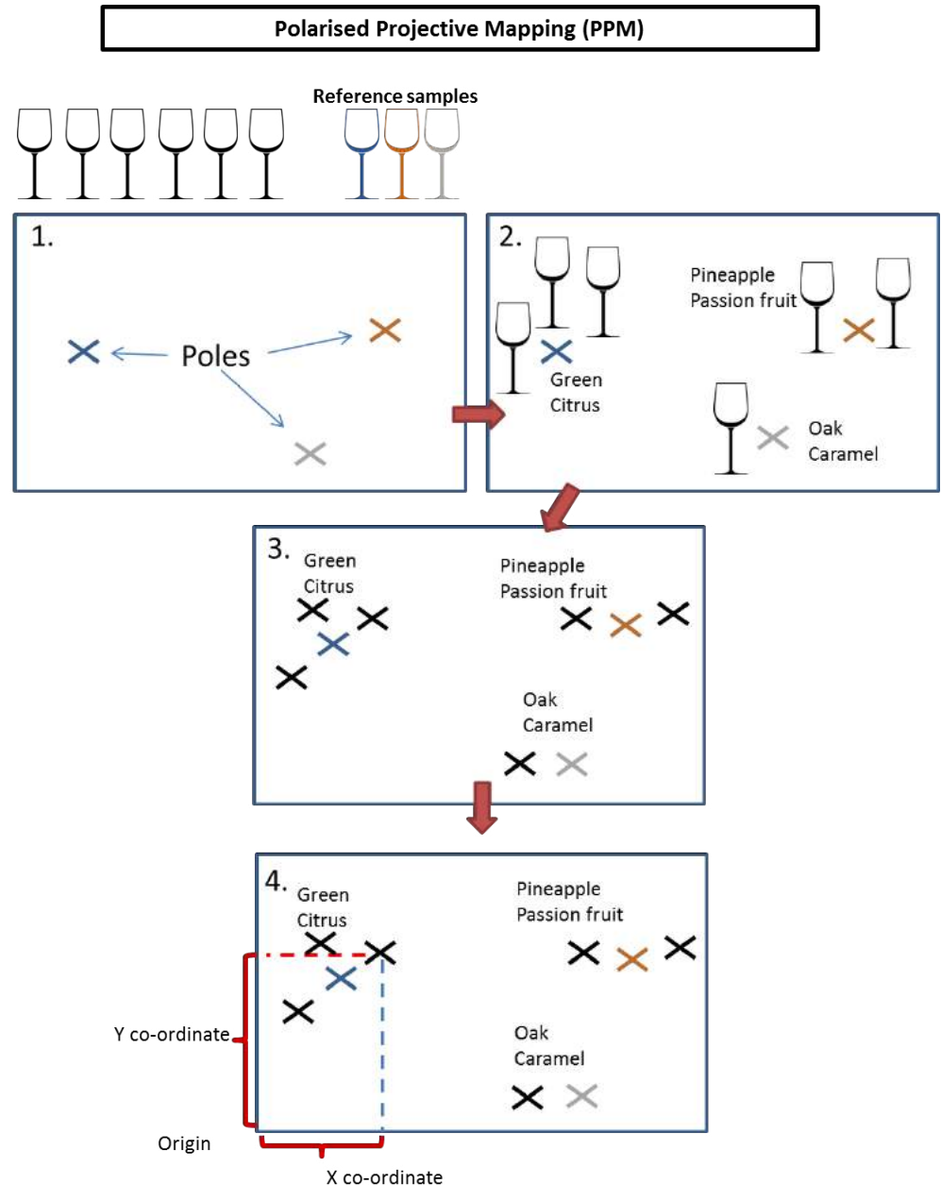

FIGURE 6. Polarised projective mapping. Each rectangle represents a sheet of white paper. Markings show the positions of the glasses positioned on the sheet by the panellists after tasting the wines.

POLARISED PROJECTIVE MAPPING (PPM)

Polarised projective mapping (Figure 6) is a similar process to Mapping, there is just one difference. Generally three wine samples are defined as poles. These poles are used as a reference and the other samples are arranged (mapped) in relation to them. A preliminary PM can be done to identify potential poles/ reference samples, or alternatively the poles can be chosen by the panel leader.

WHICH METHOD IS THE BEST?

DA is the method of choice when:

- Detailed sensory information of a product is required;
- When a list of all the characteristics observed by the panel, including their intensities, for each wine sample is required; and

- When specific taste or aroma differences between a couple of products needs to be quantified. Software programmes like *Compusense* are available and are convenient for capturing DA testing data. Although DA is the most commonly used method for sensory evaluation, it is a long and costly process.

The rapid methods do however also have limitations, and the choice of method depends on the size of the panel, the timeframe and budget that is available. For the results to be statistically valid for the rapid methods, you need 30 individual answers (or 15 answers in duplicate). Data obtained from DA is numeric, as well as descriptive (whereas for the more rapid methods the data is mostly descriptive), and can therefore be combined with other numerical measurements from the same set of wine, e.g. consumer liking or chemical data. Mapping however, because of the co-ordinate data it produces, has the potential to be combined with other data. This however is still being worked on.

With Flash profiling, CATA and DA all the wine samples are profiled individually, therefore if wines are added or removed, this will make no difference to your results. In contrast, Sorting and PM is a holistic approach where all the samples are compared to one another in order to be grouped or arranged on the tasting sheet. Due to the fact that the wine samples are grouped in relation to each other, adding or removing a sample from the set could alter the results. With CATA and Sorting methods, you will generally only pick up large differences between wines, and be able to identify the most dominant characteristics. These methods are limited in their ability to differentiate between samples where differences are intensity-based.

Overall, the advantages of the rapid methods are that they require less panel training as the panel does not have to come to consensus on scaling of descriptors, as is the case with DA. The rapid methods are definitely faster for the panel to complete, but not for the panel leader/analyst and more research is currently being done to speed up the data capturing and subsequent data analysis stages.

The following articles in this series will discuss three rapid methods for sensory evaluation of wine, namely Sorting (Part 2), Projective mapping (Part 3) and CATA (Part 4). These articles will supply more detailed explanations and illustrations, as well as guidelines on how to choose the best method for your task in the cellar environment.

ACKNOWLEDGEMENTS

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Sensory evaluation of wine (Part 2)

Sorting – a fast and simple method to describe sensory differences and similarities between wines

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KEYWORDS: Sensory evaluation, Descriptive analysis, Sorting, Projective mapping, Napping, CATA.

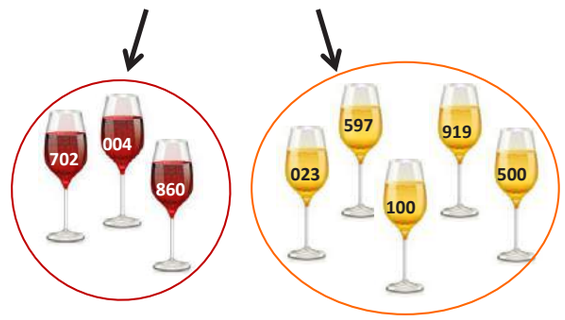
Step 1: Coded wine samples are provided to each taster and tasters sort the wines.

Group wines according to similarity

Provide 3-5 descriptors for each group

Taster 1
Group 1:
023, 597, 919, 500, 100
Citrus, peach, melon

Group 2:
004, 860, 702
Cherry, plum, spice, violet, oak

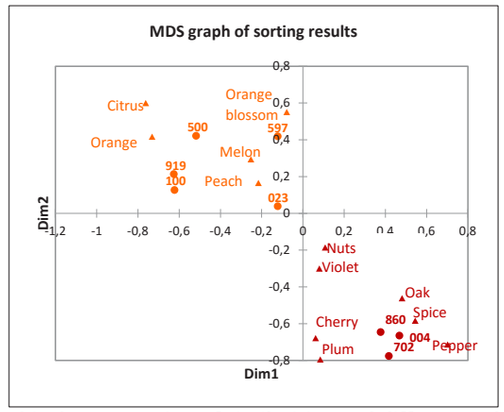


Step 2: Tasters describe each group.

Taster1	Citrus	Peach	Cherry	Plum
Wine 1 023	1	1	0	0
Wine 2 597	1	1	0	0
Wine 3 860	0	0	1	1
Wine 4 004	0	0	1	1

Taster 1	Wine 1 023	Wine 2 597	Wine 3 860	Wine 4 004
Wine 1 023	1	1	0	0
Wine 2 597	1	1	0	0
Wine 3 860	0	0	1	1
Wine 4 004	0	0	1	1

Step 3: Table with all tasters' data is constructed and analysed.



Step 4: Graphical presentation of similarities and differences between wines.

FIGURE 1. Work flow for wine sensory evaluation with the sorting task.

This article is the second in a series of four articles on methods for the sensory evaluation of wine. The articles are based on current sensory research at the Institute for Wine Biotechnology, Department of Viticulture and Oenology (IWBT-DVO) and the Institute for Grape and Wine Sciences (IGWS), Stellenbosch University (SU). As part of our research outputs, we aimed to optimise some of the methods so that they are fit for industry use. The explanations and workflow are presented in a clearly illustrated graphical format, with the industry person responsible for sensory quality control, or leading a sensory panel, in mind. References to additional information are provided for those readers interested in more background.

Part 1 gave an overview of the main methods including Descriptive analysis (DA), which gives a full description of each wine in the test set, as well as alternative faster methods. Sorting is discussed in Part 2 (this article). Part 3 deals with Projective mapping (PM) and Napping® and Part 4 with the Check-all-that-apply (CATA) method. A summary where practical considerations are compared for all the methods discussed in this series can be found in Part 4. This information can be useful for those who have to use these methods for industry applications.

BACKGROUND: ORIGIN OF SORTING AS SENSORY EVALUATION METHOD

Sorting, according to the psychologists, is a natural and intuitive task that people use daily to organise their thoughts, in order to understand their environment. Psychologists Hulin and Katz used the technique already in 1935 to group and classify facial expressions. Sensory scientists began to use the method in the 1990s to study people's perceptions of smell. Harry Lawless and co-authors (1995) were the first scientists who used the method in 1995 to group food products based on sensory characteristics. Since then, Sorting became one of the most popular alternative rapid methods for sensory evaluation of food, including wine and beer.

THE SORTING TASK: STEPWISE

Before discussing the steps involved in the sorting task, it is important to keep the two simple questions that this procedure is trying to answer, in mind:

1. Which products are similar and which differ in their sensory characteristics?
2. Which sensory characteristics are responsible for the observed differences and similarities between the wines?

The answers to these two questions are elicited during the sorting task, in the following practical steps:

STEP 1: Sorting of the wines in the sample set (which products are similar and which differ in their sensory characteristics?)

At the start of the sorting task, tasters receive all the wines simultaneously and are asked to group them according to similarity. Wines with similar sensory characteristics must be placed in the same group and wines with different characteristics, in different groups. Tasters must form more than one group. They may not put all the wines in a single group and each wine may not be placed in its own separate group.

There are several variations of the method. Two of these commonly used and providing good results for wine evaluation, are firstly free or uninformed sorting and secondly, informed or directed sorting. During a free sorting task, tasters can create any number of groups and use any of their own criteria to make groups. Informed or directed sorting applies when instructions are provided to the tasters on how to group wines. Typically, information pertaining to the number of groups in which the wines must be sorted, or the criteria to use for sorting the wines, can be provided. Colour, aroma, taste and mouth-feel can be assessed simultaneously or separately during different tasting sessions. It is often asked of tasters to use only aroma or taste during a sorting task.

A sorting task can be concluded at this stage, but often it is important to know which sensory characteristics are responsible for the perceived differences and similarities amongst the wines in the test set.

STEP 2: Description of the main sensory characteristics of each group (which sensory characteristics are responsible for the differences and similarities?)

During the second part of the sorting task, tasters are asked to describe the sensory characteristics of the groups they formed, a procedure that is referred to as labelled sorting. It can be a very time consuming process to do statistical analysis on the data when tasters all use their own terms. A pre-determined list of sensory descriptions can be provided to the tasters and they can select the terms used to describe the groups best. This will simplify the task for the tasters, as well as ensure that they all use the same terms for a particular characteristic. In this way, the statistical analysis is kept relatively straightforward. Aroma wheels and sensory descriptions of similar wines from previous studies are examples of such lists.

STEP 3: Statistical analysis and graphic projection of results

There are different approaches that can be used to analyse sorting data. The most common approach is to calculate (by counting) how many times each two wines were grouped together by all the tasters. The statistical method Multidimensional scaling (MDS) is used to produce a graph where wines that were grouped together by many tasters, are displayed close to each other, and wines often grouped into separate groups by the tasters, appear further apart.

The panel leader would typically reduce the number of descriptions given by the tasters, by combining terms with the similar meaning. Terms used by only one or two tasters are left out. The number of times a particular term was used by all the tasters to describe a wine, is then counted. Correspondence analysis (CA), are typically used to analyse data related to the descriptions. MDS and CA techniques can easily be done in XLSTAT software. There is expertise at IWBT-DVO, SU to support and train people who are interested to do these analyses.

SETTING UP A PANEL FOR THE SORTING TASK

One of the advantages of the sorting task is that training of a panel is not required. Wine experts, as well as people with lesser wine knowledge can participate as tasters. Numerous studies have been done with non-expert consumers. It is important to choose the right panel, in order to get the information required about the wines. Experts will more often be used if in-depth sensory profiles of the products are required. It may also be useful to do the same sorting experiment with both experts and non-expert consumers. An investigation into the similarities, and differences between the criteria used by the two groups to sort the wines, can be valuable for product and brand development.

ADVANTAGES AND DISADVANTAGES OF THE SORTING METHOD

Sorting is one of the best methods for the sensory characterisation of products such as wine and beer, when the differences between the products are clear, and the smaller differences are not so important. Research has shown that the results obtained with classical sensory Descriptive analysis (DA) and Sorting, often lead to the same conclusions. In some cases it will be necessary to rather do DA on the test set, if it is important to identify the less prominent sensory qualities in wines and quantify their intensities. The results Sorting produce are usually repeatable, even with non-expert users as tasters. The data analysis step can however be time consuming and it is sometimes difficult to interpret the descriptions of the groups formed by the tasters, if a pre-determined list was not used. The number of wines that can be tasted is limited, because the tasters receive all the wines to be tested simultaneously.

THE SORTING METHOD APPLIED TO WINE: SOME PUBLISHED EXAMPLES

Free sorting of wines was used by Bester and co-authors in 2011 to show that wine experts and non-experts alike, find it difficult to distinguish between some South African Chenin blanc wine styles. This was particularly evident for the “fresh and fruity” and “rich and ripe” unwooded Chenin product categories. Mineral-like characteristics in wine, as well as the meaning that experts associate with the term, was researched by Ballester and co-authors in 2013 by using a sorting task. This research group also investigated the sorting of wines based on aroma in 2009. Sorting is becoming increasingly important in wine sensory evaluation, due to the fact that training of the panel is not required, and both experts, as well as ordinary consumers can be used as tasters.

The other articles in this series on wine sensory evaluation methods include Part 3 that deals with Projective mapping (PM) and Napping®, while Part 4 focusses on Check-all-that-apply (CATA). A comparison of the practical aspects and requirements of all the methods discussed in this series is also given in Part 4.

ACKNOWLEDGEMENTS

This research was made possible by Winetech funding, project IWBT W13/02: “Rapid descriptive sensory methods for wine evaluation – special focus on further optimisation of rapid methods and streamlining of workflow”. Winetech, the Institute for Grape and Wine Sciences (IGWS), Stellenbosch University, NRF and THRIP are acknowledged for financial support.

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Sensory evaluation of wine (Part 3)

Projective mapping and Napping®

APRIL 2016

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KEYWORDS: Sensory evaluation, Descriptive analysis, Sorting, Projective mapping, Napping, CATA.

This article is the third in a series of four that deals with useful wine sensory evaluation methods for the industry. The articles are based on current sensory research at the Institute for Wine Biotechnology, Department of Viticulture and Oenology (IWBT-DVO) and the Institute for Grape and Wine Sciences (IGWS), Stellenbosch University (SU). As part of our research outputs, we aimed to optimise some of the methods so that they are fit for industry use. The explanations and workflow are presented in a clearly illustrated graphical format, with the industry person responsible for sensory quality control, or leading a sensory panel, in mind. References to additional information are provided for those readers interested in more background.

Part 1 of the series gave an overview with Descriptive analysis (DA), as well as some alternative, faster methods covered. The Sorting method was discussed in Part 2, while Projective mapping (PM) and Napping® are discussed in Part 3 (this section). Part 4 deals with yet another rapid method, Check-all-that-apply (CATA), and some of its variations that are useful for wine profiling. The series is concluded in Part 4 by a summary where the methods are compared in terms of their practical requirements, amongst others, the number of panellists required, time required for the completion of the tasting by the panel and difficulty of the tasks.

BACKGROUND: MAPPING AND NAPPING®

With the mapping approach, sensory panel members are given a set of wines poured in glasses and they are asked to evaluate the wines based on either aroma or taste, or both modalities at the same time, depending on the aim and requirements of the tasting. After tasting, the wine glasses are placed by the panellists on a sheet of white paper (for instance) in such a way that samples that are perceived by the panellists to have similar sensory characteristics are close to each other, and those with dissimilar characteristics, further apart. The mapping concept was borrowed from the field of psychology (Valentin & co-authors, 2012). Risvik and co-workers adapted the strategy to sensory profiling of foodstuffs in 1994, referring to the method as Projective mapping (PM). Pagès and co-workers (2005) referred to the technique as Napping®, originating from “nappe”, the French word for tablecloth, where the tasters were asked to arrange the products on a tablecloth. When using the name Napping® for your sensory test, certain rules apply: A2 or A3 paper sheets should be used as the tablecloth and specific statistical techniques should be used to analyse the data. Both Napping® and PM are commonly used in the food and beverage industries, including the wine industry.

THE PM METHOD FOR SENSORY EVALUATION OF WINE

The workflow of PM is illustrated step by step in Figure 1. Panellists receive all the wines simultaneously (Figure 1, Step 1) and are asked to focus on the sensory similarities and differences between wines. The wines are positioned, according to similarities and differences on a flat surface, such as a tablecloth, white paper or computer screen (Figure 1, Step 2). Each panel member decides how similar or different the wines are according to his/her own discretion. Similar wines are placed close to each other and different wines far apart. The distances between wines indicate the degree of similarity; the bigger the sensory differences between wines, the further apart the wines will be placed from each other. Panellists can taste each wine as many times as they want. After deciding on the final positions of the wines on the paper, tasters make a small cross and write down the three digit code of the wine on the paper to indicate the position of the wine (Figure 1, Step 3).

HOW DO WE ANALYSE PM DATA AND INTERPRET THE GRAPHS?

The X and Y co-ordinates of the positions of all the wines on the paper are measured (Figure 1, Step 4). These co-ordinates from all the tasters are recorded in a table (Figure 1, Step 5). The number of times a term is used by all the tasters to describe a wine, is then counted. When many terms are used by the tasters, similar terms are combined in the data analysis stage, to reduce the number of terms displayed on the graphs, so that the most important information obtained with the PM sample descriptions is shown. The sum of all the tasters’ descriptive data is calculated after reducing the terms and added to the data table as extra columns (Figure 1, Step 5). The statistical method most frequently used to analyse PM data is Multiple Factor Analysis (MFA). This technique takes all the individual tasters’ placing configurations of wines on the paper into account. The graphical presentation shows the most prominent sensory characteristics of the different wines or groupings of wines (Figure 1, Step 6). Other statistical techniques that can be used to analyse PM data include DISTATIS, INDSCAL, as well as General Procrustus Analysis (GPA). The graphs produced by these methods are similar to MFA graphs. There is expertise available at IWBT-DVO, Stellenbosch University to help readers interested in using these techniques.

MAIN SIMILARITIES AND DIFFERENCES BETWEEN THE PM AND SORTING METHODS

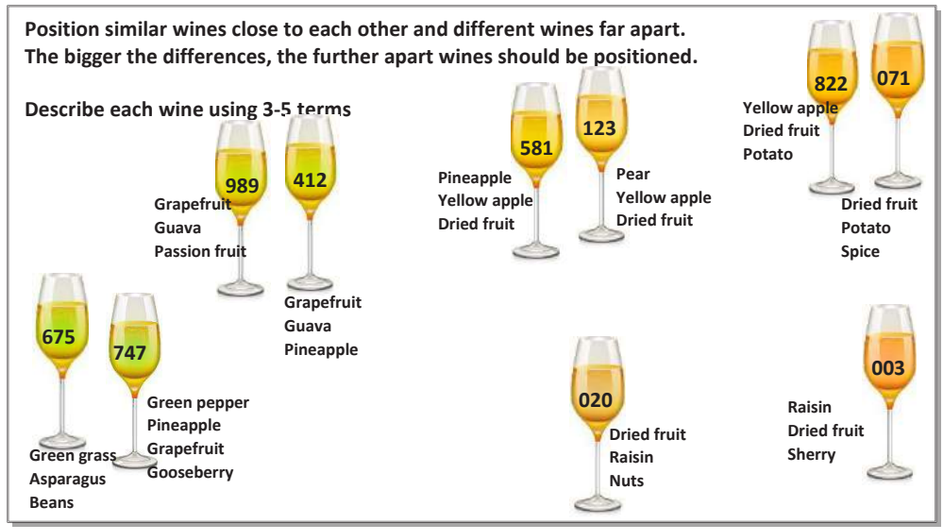
During Sorting (refer to Part 2 of the series on wine sensory evaluation), the taster has to decide whether the differences between wines are big enough for the wines to be placed in different groups. With PM, the taster does not have to make this decision. PM is particularly suitable for a set of wines where the sensory properties change gradually and no distinct groupings of wines are perceived. Pagès and co-authors suggested a method referred to as Sorted Napping® in 2010. This method is a combination of Sorting and Napping®. The tasters group the products, while at the same time position the products on the “tablecloth” or paper. Groups are indicated by circling products that belong together. The distances between the groups give an indication of the differences and similarities between the groups. The further apart groups appear from each other, the larger the differences between groups. The closer groups are to each other, the more similar they are. The differences between products within the same group can also be indicated with distances on the paper in the same manner.

An important difference between Sorting and PM is that with PM each wine is usually described separately, while with Sorting the samples belonging to the same group share the same descriptors as the group. Again, as with Sorting, the PM panel leader can decide whether a predetermined list of terms should be used for description, otherwise tasters can use their own terms.

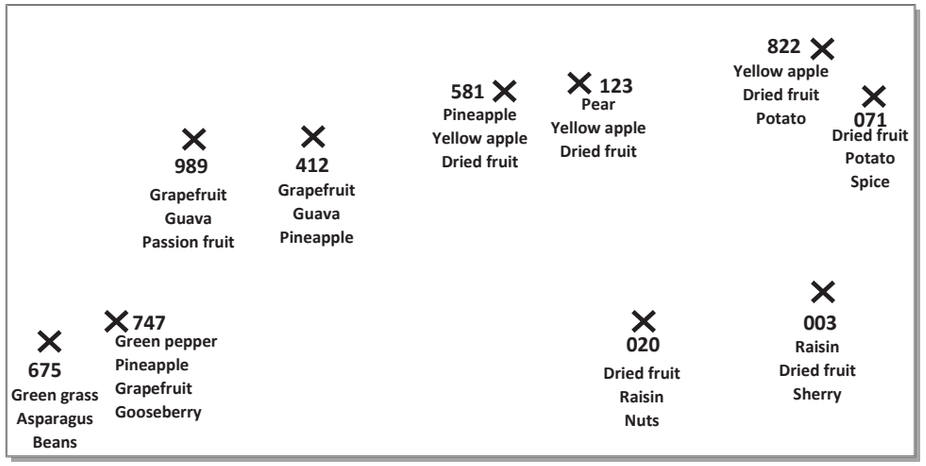
CHOOSING A PM PANEL

A wide range of tasters can be used for PM, including wine experts and ordinary consumers. Although the training of a panel is not required for PM/Napping®, researchers have obtained good results with trained panels. Such an example is

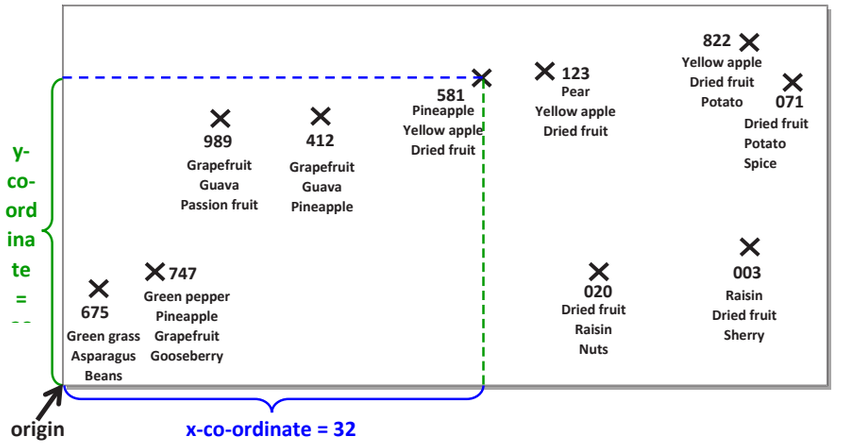
Step 1: Coded wines are presented to each taster.



Step 2: Tasters position wines on a plane and describe each wine.



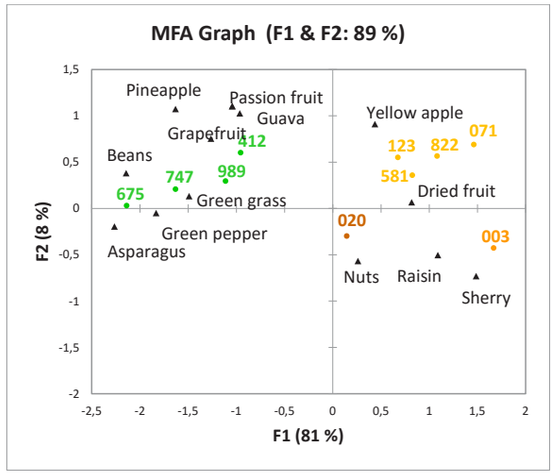
Step 3: Positions of wines are marked by the taster.



Step 4: Co-ordinates of each wine are measured by the panel leader.

	Taster1		Taster2		...	Sensory attributes – sum of all the tasters' data			
Wine	X1	Y1	X2	Y2	...	Pineapple	Yellow apple	Dried fruit	Grapefruit
581	**32**	**28**	45	12		**23**	**16**	**24**	2

Step 5: Table of all the tasters' data compiled and statistically analysed.



Step 6: Graphical presentation of the most important sensory similarities and differences between wines as described by PM.

FIGURE 1. Workflow for using Projective mapping (PM) for sensory evaluation of wine.

the sensory method development done by Louw and co-authors (2013) for brandy profiling, a study which showed that panel training improved the results. Several studies on wine compared results obtained by non-expert consumers to that of expert consumers. When selecting a panel, the information required from the tasting should always be kept in mind. Trained panels and experts consistently provide more in-depth and technical descriptions of the wines, and can also describe smaller differences between wines more accurately, than non-expert consumers.

HELPFUL TIPS TO OVERCOME DIFFICULTIES RELATED TO PM

PM is a useful technique commonly used as sensory technique for food profiling. It can be used for the evaluation of colour, aroma, taste, as well as mouth-feel. The quality of the data is better and the task is easier for tasters, when only one of these modalities is evaluated in a tasting session. It is therefore sometimes better to arrange separate sessions for aroma and taste, when it is necessary to take both aspects into account during profiling. This variation of the method is called partial mapping or Napping®.

When people who do not regularly participate in wine tasting are used as sensory panellists, it is essential to ensure that they understand the instructions. A "practice session" before the formal sensory evaluation, where tasters practise the mapping exercise, can improve the quality of the data and ease the task for the tasters. The number of wines that can be evaluated with most rapid sensory evaluation methods are limited, because tasters are presented with all the wines at the same time.

Polarised projective mapping is a variation of PM where predetermined wines are used as reference points (also called poles), in order to merge datasets obtained from various tastings. The reference wines are tasted at all tastings and their positions on the plane or paper are fixed and the same for all sessions.

The last article in this series, Part 4, will discuss Check-all-that-apply (CATA). A comparison of the practical aspects and requirements of all the methods discussed in this series, DA and the rapid methods, is also given, so that the industry end-user can choose the best method for his/her sensory tasks.

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Sensory evaluation of wine (Part 4)

Check-all-that-apply (CATA) – profiling of wine with multiple choice questions

MAY 2016

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KEYWORDS: Sensory evaluation, Descriptive analysis, Sorting, Projective mapping, Napping, CATA.

This article is the last in a series of four on methods for the sensory evaluation of wine. The articles are based on current sensory research at the Institute for Wine Biotechnology, Department of Viticulture and Oenology (IWBT-DVO) and the Institute for Grape and Wine Sciences (IGWS), Stellenbosch University (SU). As part of our research outputs, we aimed to optimise some of the methods so that they are fit for industry use. The explanations and workflow are presented in a clearly illustrated graphical format, with the industry person responsible for sensory quality control, or leading a sensory panel, in mind. References to additional information are provided for those readers interested in more background.

Part 1 gave an overview of methods used for sensory evaluation of wine. Descriptive analysis (DA) was included, as well as newer faster methods. Sorting was discussed in Part 2, while Projective mapping (PM) and Napping® were discussed in Part 3. Part 4 (this section) will deal with Check-all-that-apply (CATA) questions and some of its variations frequently used for wine profiling. This is an extensively researched method, and only the most important aspects and variations that make the method suitable for industry use are discussed.

BACKGROUND: THE ORIGIN AND USE OF MULTIPLE CHOICE QUESTIONS FOR FOOD AND WINE PROFILING

The multiple choice based method referred to as “pick-any” was used in the 1960s for market research on different brands. Sensory scientists began to use the method under the name Check-all-that-apply (CATA) to analyse consumer preferences for certain products. CATA is currently also used for profiling of wine and beer by product experts and producers.

COMPILING A CATA PANEL

CATA is mostly used for profiling and preference testing of food products by using consumers as tasters. In the wine industry, it is common to use experts and trained panellists, a procedure that is not so common for the food industry. Here, we will firstly discuss the workflow of CATA for consumer preference testing, followed by the workflow of CATA when used to compile sensory profiles by experts and trained panellists.

USE OF CATA TO UNDERSTAND CONSUMER PREFERENCES

One of the differences between CATA (Figure 1) and methods such as Sorting and Projective mapping, is the way in which wines are presented to tasters. During Sorting and Projective mapping all the wines are served simultaneously, while one wine at a time is served during a CATA tasting. It is therefore important that every taster receives the wines in a different order.

Tasters get a list of words or phrases to describe the characteristics of a wine. All of the words or phrases that apply are marked by the panellists. The words or phrases can include sensory terms, for example, aroma, taste and mouth-feel descriptions, as well as social or emotional aspects that describe their product preferences and liking. “I like it”, “delicious” and “I hate it” are examples of phrases related to the emotional aspects of product preferences and liking. Sensory descriptions can be combined with phrases about product liking in the same CATA list. It is also possible to use a separate question before or after CATA to evaluate liking. Varela

Step 1: Coded wines are presented one at a time to tasters.

Step 2: Tasters mark the terms that apply.

Judge 1	Tropical	Guava	Litchi	Green Pepper
Wine 1	**1**	**1**	**0**	**1**
Wine 2	1	0	1	0
Wine 3	1	0	0	1
Wine 4	0	0	0	1

Sum across Judges	Tropical	Guava	Litchi	Green Pepper
Wine 1	**21**	**15**	**0**	**18**
Wine 2	11	0	8	0
Wine 3	15	8	0	7
Wine 4	5	0	0	22

Step 3: The number of times that an attribute is used to describe a wine is counted.

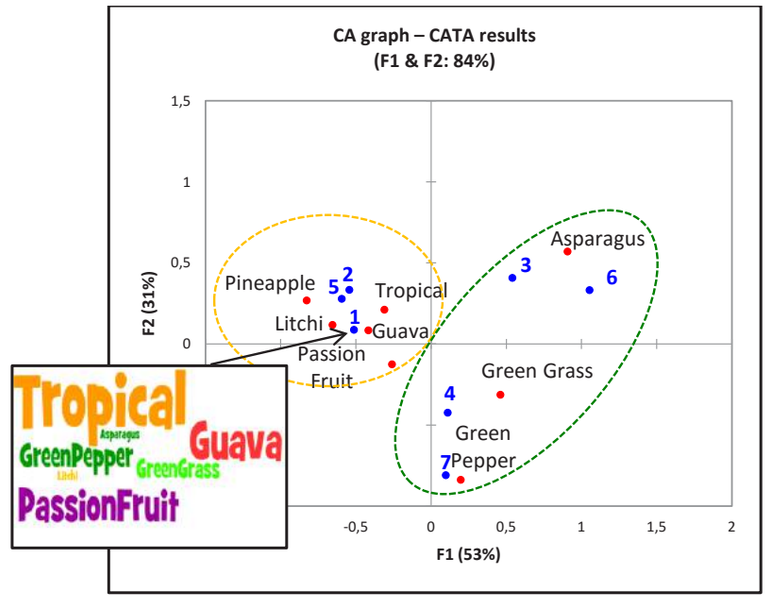

FIGURE 1. CATA workflow for sensory profiling of wine.

and co-authors (2012) suggested that the liking question is asked before the CATA procedure is done, if it is not included as part of the CATA list.

The order of the terms on CATA lists is important. Research has shown that consumers tend to not pay an equal amount of attention to all of the terms. Words at the top end of the list are often given preference and used more often. The order of the terms can be changed for each taster if the list is very short. It can even be changed for different wines tasted by the same taster. The person who sets up these lists and determines the order of the terms, should remember that wine is complex and difficult to taste. Changing the order of terms should not be confusing or frustrating for tasters. If the CATA list is very long, it makes sense to group similar terms, for example group fruity aroma or woody aroma terms together, etcetera. Different lists can even be used for different modalities; colour, aroma, taste and mouth-feel.

THE USE OF CATA TO PROFILE WINE USING EXPERTS AND TRAINED PANELLISTS

Pick-K-attributes is a variation of CATA and is commonly used for sensory profiling of wine, with wine experts and trained panellists as tasters. This variation differs from the standard CATA method, in the sense that tasters are asked to check the most prominent/dominant terms only, instead of marking all the terms that apply. Since CATA does not measure the intensities of the sensory characteristics, it is usually a better idea to use only the most prominent sensory characteristics for profiling, rather than bringing all characteristics into account. This however, depends on the purpose of the sensory tests. In some cases CATA will rather be used for profiling, as a more complex description with more terms that can be obtained than with Pick-K-attributes.

When wine experts are used as the panel during a Pick-K-attributes profiling test, the assumption is made that all the terms are interpreted and understood in the same manner by all the panel members, which is usually the case. However, when a complex set of wines with small differences between them is evaluated by using a long list of terms, the degree of consensus among wine experts without sensory training, is not always good enough. A trained panel can be used for scenarios like this. This variation of the CATA method is not fast and if the panel is paid, it can become a costly exercise. Campo and co-authors (2010) found that the description of the products obtained using this method was indeed excellent. Results compared well to classical Descriptive sensory analysis (DA) results also obtained during the study on the same set of products. More information on DA was given in Part 1 of this series.

STATISTICAL ANALYSIS OF CATA DATA

The number of times a description is used for a wine by all the tasters is counted and a table constructed of all these counts (referred to as a frequency matrix). The most common statistical method used to analyse the data is Correspondence analysis (CA). Other methods, for example Multiple factor analysis (MFA) can also be used. The end result is a graph representing all the wines and descriptions used (Figure 1). Wines are positioned close to the terms or phrases that were often used by tasters to describe them. Wines for which the same descriptions were used often will also appear close to each other on the graph. All the wines in a data set are thus represented on a graph.

Graphical representations and descriptions of each individual wine are sometimes useful and word clouds are commonly used to visualise frequency data. Words used by more tasters to describe a wine will appear larger (a larger font) than words used by fewer tasters (Figure 1). These simple tools can emphasise the information captured by CATA and make it easy to understand for the reader.

POSSIBLE PITFALLS: KEEP THE FOLLOWING IN MIND WITH CATA-TYPE METHODS

Since wine can be a challenging matrix to taste, especially for consumers, it can be tiring when CATA lists are unnecessarily long. CATA lists can be shortened by focus group tastings prior to the formal CATA experiment.

The reality that CATA-type methods do not measure intensities of sensory attributes, should be kept in mind. This is especially important when the purpose of the tasting is to compare different wines. When the same terms are found at high frequencies for two wines, it does not necessarily mean that the intensity of those sensory properties is high in both wines. It only indicates that both wines have those properties and that it is prominent/intense enough for most of the tasters' perceptions. When intensity differences between wines are an important aspect that needs to be tested, CATA with intensity rating (Rate-all-that-apply, RATA) or DA should be used.

BENEFITS AND APPLICATIONS OF CATA IN THE WINE INDUSTRY

CATA-type methods are mostly used in the wine industry for profiling, although it is sometimes used for consumer preference (liking) test. Campo and co-authors (2010) pointed out an important benefit that the Pick-K-attributes approach has over the DA method. During DA analysis, the panel members are limited to use a small number of sensory descriptors to describe a set of wines. This is a disadvantage when the data set contains various wines with vastly different sensory properties. In such a case, it is not always possible to describe all the wines with a maximum of 15 to 20 terms (normally used in DA). The Pick-K-attributes list can be much longer than 20 terms, indeed, for some reported tastings, more than 100 terms were used for profiling using a trained panel. Finally, CATA-type methods are quick and simple for both the tasting panel and the analyst who must do the data analysis.

HOW DO DA AND THE ALTERNATIVE, RAPID METHODS COMPARE?

The different sensory evaluation techniques discussed in the articles in this series is compared briefly in Table 1, to provide readers with practical guidelines on choosing the appropriate method. These guidelines are supported by scientific research done at the IWBT-DVO, SU.

Results obtained with the rapid methods compare well to DA results when product experts or trained panellists are used as tasters. The average time tasters need to finish the tasting, as well as the difficulty of the methods varied. CATA variant Pick-K-attributes was perceived as the easiest method and also took the least time to finish. Mapping was rated by the tasters as the most difficult and time consuming

TABLE 1. Comparison of practical implications of using DA and selected rapid sensory methods for the profiling of wine.

	DA	Pick-*K*	Rate-*K*	Sorting	Mapping
Number of sensory sessions	11	1-2	1-2	1-2	1-2
Lab time for sensory task	1 month	1 day	1 day	1 day	1 day
Training time	>20 hours	None	None	None	None
Time spent on sensory task by judge (average)	±2 hours	<30 min	<30 min	<45 min	<1 hour
Difficulty of sensory task (average out of 10)	6	3	5	4	8

method, and it was the only rapid method that was rated more difficult than DA. The time needed for sensory evaluation and the difficulty of the sensory task are both important considerations. However, the nature of the set of wines to be tasted, the information required from the tasting and expectations about the outcome should be the first priority when choosing a sensory evaluation method.

Pick-K-attributes can provide useful results when the characteristics of each sample are needed and little information is available or needed with regard to similarities between the wines. Sorting is a good alternative to DA if some of the wines in the set are similar, or if the purpose is to specifically investigate the similarities and differences. Mapping could be particularly useful to investigate product similarities when wines present a continuum of characteristics that change gradually, rather than presenting as discrete groups. It is also a useful method to investigate the magnitude of differences between groups of wines or wines within the same group.

The references provided here provide more background. You are also welcome to contact us for more information or assistance with the setup of a rapid sensory evaluation session.

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- Statistical analysis and interpretation of data.

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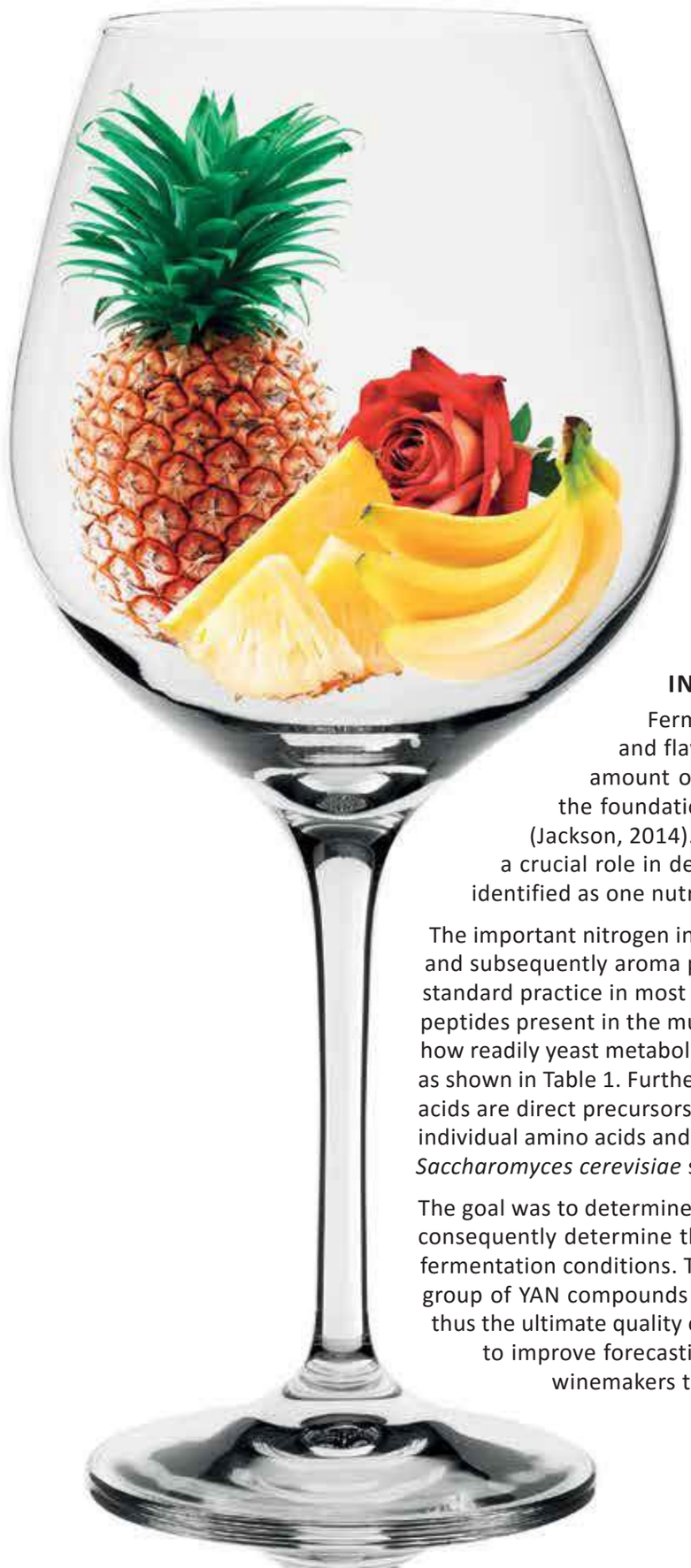

The complex relationship between wine aroma and amino acid utilisation by yeast

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KEYWORDS: Wine aroma, amino acid, yeast, nitrogen.

INTRODUCTION

Fermentation-derived aroma compounds are one of the defining features of wine quality. Primary aroma and flavour compounds such as acetic acid, ethanol and glycerol are largely dependent on the quality and amount of carbon in the starter must and on fermentation conditions. Nonetheless, the jury is still out on the foundation of some distinctive flavours and aromas usually associated with particular varieties and locales (Jackson, 2014). Scientists and winemakers have long suspected that yeast nutrition, along with other factors, plays a crucial role in determining the chemical profile of wine aroma. Nitrogen concentration in the grape must has been identified as one nutritional factor crucial in aroma biosynthesis during winemaking.

The important nitrogen in grape must, referred to as yeast assimilable nitrogen (YAN), determines yeast growth, development and subsequently aroma production. As a result, addition of diammonium phosphate (DAP) as a nitrogen supplement is now standard practice in most cellars. Yeast can meet its nitrogen needs by breaking down proteins, and by using amino acids and peptides present in the must. Amino acids are a significant source of YAN, but are not all used at the same rate. Depending on how readily yeast metabolise the amino acids, they can be classified as preferred, intermediate, non-preferred and non-utilised as shown in Table 1. Furthermore, their use by yeast will result in the production of various aroma compounds, and many amino acids are direct precursors of specific aromatic features. This report is a summary of a study that investigated the influence of individual amino acids and amino acid classes on the aroma profiles of synthetic grape must (SGM) fermented by two industrial *Saccharomyces cerevisiae* strains.

The goal was to determine the effect of individual amino acids as the sole source of YAN on the growth and aroma profile, and consequently determine the predictability of aroma using various nitrogen concentrations and sources under standard wine fermentation conditions. This is essential for the further optimisation of yeast nutrition, because amino acids form the largest group of YAN compounds (Bell & Henschke, 2005) and their metabolism is directly related to many flavour compounds, and thus the ultimate quality of wine (Styger *et al.*, 2011). As such, it is worthwhile to assess the amino acid and aroma relationship to improve forecasting of wine aroma by using grape must characteristics. Knowledge of such relationships will enable winemakers to make more informed decisions on how to proceed with the fermentation process.

GROWTH KINETICS

Two *Saccharomyces cerevisiae* strains were used in this study to ferment synthetic grape must. The effect of individual amino acids shown in Table 1 on growth was assessed for the two yeast strains using yeast nitrogen base media (YNB) containing 10% (w/v) glucose at a pH of 3.8. Only one of the amino acids was added to give a YAN concentration of 150 mg L^{-1} and YNB with only ammonium used as the control. Optical density (representing biomass production), duration of lag phase were used as indicators of growth. The slope of the exponential growth phase was used to calculate the growth rate.

FERMENTATION KINETICS

The defined grape must was used to determine the aroma production pattern of the two yeasts when presented with single amino acids as nitrogen sources. Just like with the growth experiment, amino acids were added to the media to contribute a YAN of 150 mg L^{-1}, but the media were fermented to dryness, i.e. until sugar levels were less than 4g L^{-1}. At the end of fermentation, final biomass was quantified to determine the effects of different amino acids on biomass production. Productivity showed notable variations between different amino acids. For both strains, the preferred amino acids, glutamine, glutamate, alanine

TABLE 1. Amino acid classification based on the efficiency of utilisation by wine yeast (Ljungdahl & Daignan-Fornier, 2012). The classes are important in predicting the wine aroma when single amino acids are used as sole sources of nitrogen.

Preferred	Intermediate	Non-preferred	Non-utilised
Asparagine	Proline	Leucine*	Histidine
Glutamine	Valine*	Isoleucine*	Lysine
Serine	Phenylalanine*	Methionine	Cysteine
Aspartic acid		Tyrosine	
Alanine		Threonine	
Arginine		Tryptophan	
Glutamic acid			

* Amino acids with asterisk are also grouped as the branched chains and aromatic amino acids.

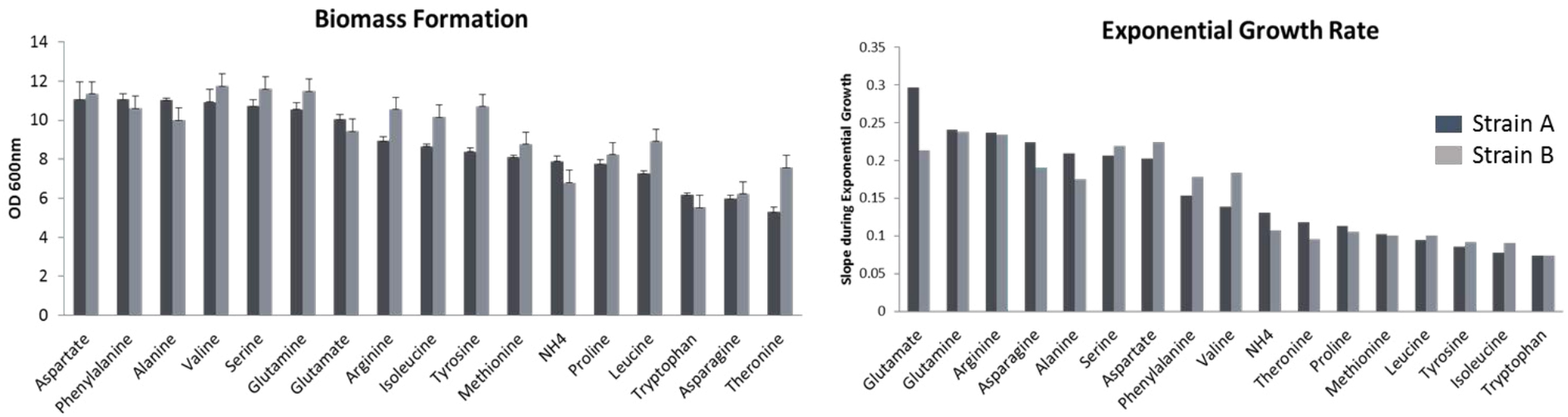

FIGURE 1. A comparison of mean biomass accumulation (A) and growth rate (B) of Strain A and Strain B cultured in media with only one amino acid or ammonium as the sole source of nitrogen.

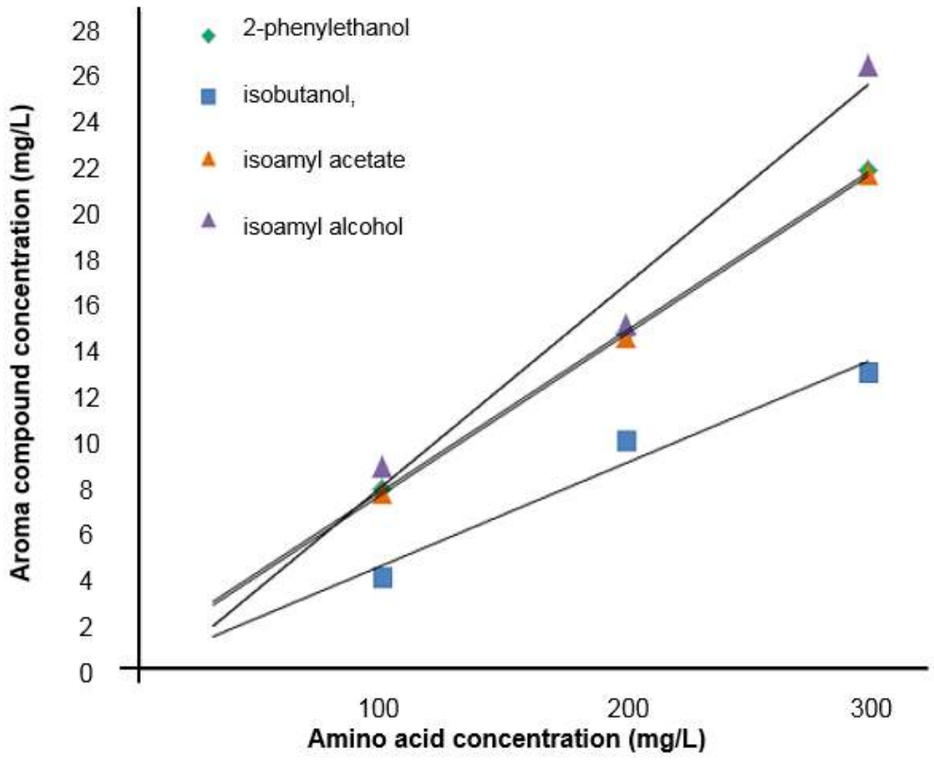

FIGURE 2. Relationship between BCAAs and related volatile metabolite produced by VIN13 during fermentation.

aspartate, arginine and serine exhibited high growth rates which were however not directly correlated to biomass accumulation. For instance, phenylalanine had a final biomass of 2.87 g L^{-1}, but had a comparatively low growth rate of 0.15 as shown in Figure 1. Nevertheless, the preferred amino acids were efficiently utilised in comparison to the other classes and had higher growth rates. The preferred amino acids also led to the shortest time to complete fermentation.

The amino acid consumption pattern is broadly consistent for both strains, especially for the preferred amino acids, with the exception of arginine. There was no significant difference in the final biomass accumulation between the strains as is shown in Figure 1 except for fermentation driven by arginine, leucine, isoleucine, tyrosine and threonine which had strain B producing more cells at the end of fermentation. From this it can deduced that strain B is a better fermenter than strain A given that they had similar media to begin with.

These data highlight the fact that total YAN only has limited potential to predict the fermentation performance of yeast. Indeed, two musts with the same YAN, but very different amino acid composition, and in particular a different ratio of preferred vs. non-preferred amino acids, will lead to different fermentation dynamics.

AROMA PRODUCTION

Aroma production was determined by using gas chromatography coupled with a flame ionisation detector. The aroma compounds were extracted using ethyl acetate and a total of 32 compounds were analysed. The volatile compound production was more driven by the amino acids used as nitrogen source than by the yeast strain. With the exception of acetic acid and ethyl acetate, aroma compounds were positively correlated to their related amino acid. For instance the two related amino acids, leucine and isoleucine resulted in aroma profiles dominated by isoamyl alcohol (banana), isoamyl acetate (fruity) and isovaleric acid (cheese), compounds whose formation is directly linked to leucine and isoleucine metabolism. A similar trend is observed for phenylalanine and its corresponding volatiles; 2-phenylethanol (floral) and 2-phenylethyl acetate (rose), and valine which increased the synthesis of isobutanol (solvent) and isobutyric acid (rancid). Acetic acid (vinegar) and ethyl acetate (sweet, nail polish remover) appeared positively correlated across for all amino acids and both strains showed no pronounced difference.

Further experiments were conducted with only strain A to investigate the effect of increasing branched chain and aromatic amino acid (BCAA) concentration of the quantity of the resultant aroma metabolites. In these studies SGM contained 100 mg N L^{-1}, 200 mg N L^{-1} and 300 mg N L^{-1} YAN provided by individual amino acids. The total YAN was always 300 mg N L^{-1} and in cases where amino acids were used at lower rates, the YAN was supplemented with ammonium.

Taking 2-phenylethanol, isobutanol, isoamyl acetate (banana) and isoamyl alcohol (banana) to represent volatiles from metabolism of phenylalanine, valine, leucine and isoleucine respectively, Figure 3 shows the strong and positive relationship (R^2 is greater than 0.99 for all compounds) between the amino acids and resultant volatiles. Similar relationships were also observed for the other related compound and lead to the development of the theory that if all the BCAAs are added simultaneously to the SGM, all compounds associated with the amino acids included will be positively altered.

To validate the predictability of the linear relationship between BCAAs and aroma compound, another experiment was designed to test the model. Four classes of amino acids (preferred, BCAAs, non-utilised and non-preferred) were used as nitrogen source at a concentration of 200 mg N L^{-1} and the rate of volatile compound production was compared with the predicted values for BCAAs (Figure 3). Of all the volatiles analysed, only isobutanol and 2-phenylethanol had high predictability. For all other compounds (isobutyric acid, propionoic acid, butanol, 2-phenylethyl acetate, decanoic acid, and valeric acid) there were no similarities between the observed and the predicted concentration.

Thus, predictability of aroma production is possible when individual amino acids are used for the provision of YAN. On the contrary, the predictability is reduced the more complex the nitrogen source. The reason lies with the nature of the metabolic networks involved in aroma production, since many of the amino acids share metabolic enzymes and precursors. In a complex mix, the competition between substrates, the variable preferences of enzymes and the many indirect drivers such as the need to maintain a healthy redox balance result in a more

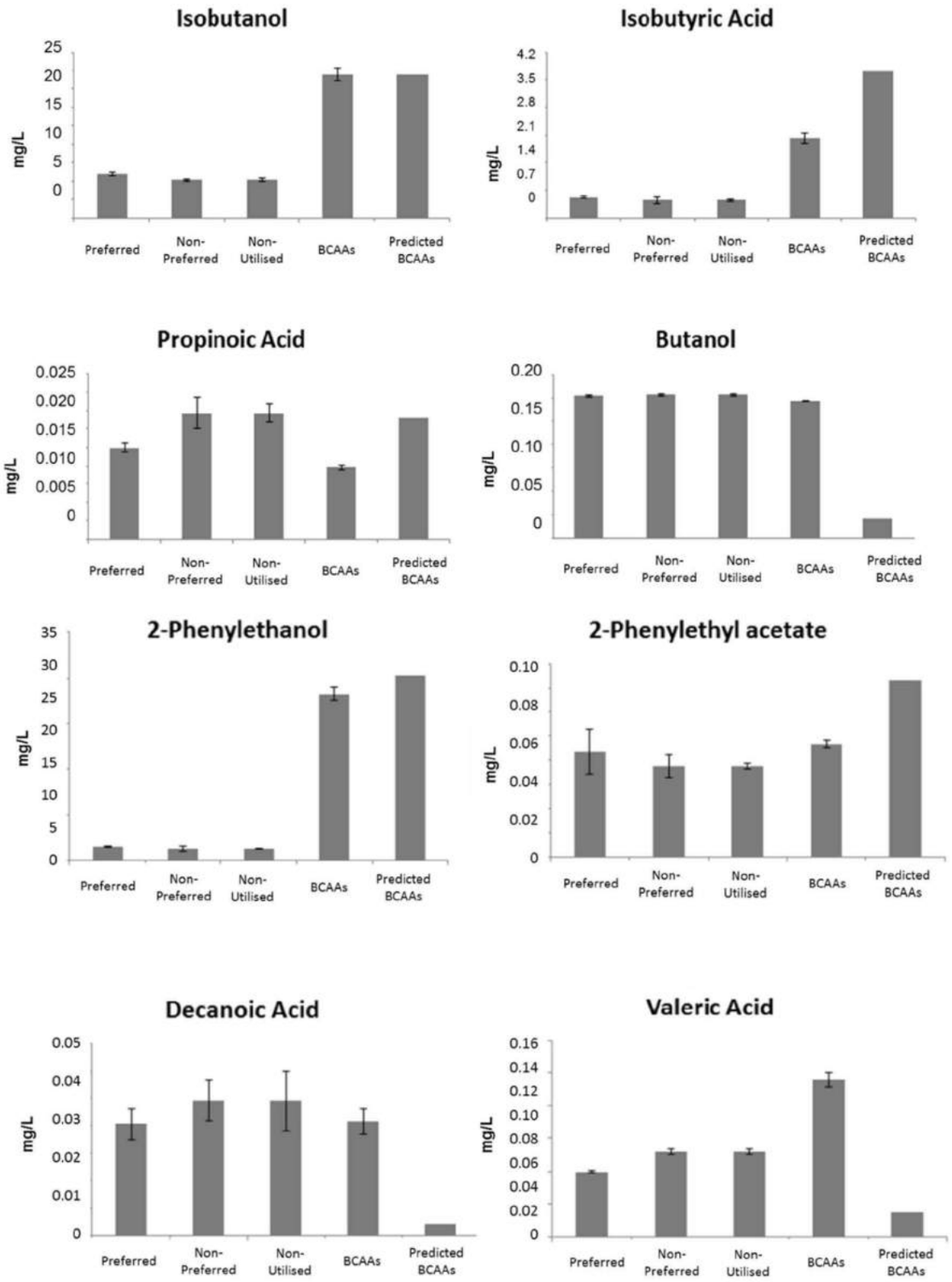

FIGURE 3. Effect of amino acids classes (YAN = 200 mg/ℓ) on the accumulation of major volatile compounds and the predictability of BCAAs-linked aroma compounds.

"chaotic" output. However, this study provides baseline data from which to extend further analysis to link amino acid nutrition to yeast aroma production. A better understanding of this relationship is indeed necessary to optimise the use of nitrogen addition to grape must.

CONCLUSION

Generally, yeast utilisation of amino acid classes is similar across the two strains used in this experiment. The type of nitrogen source played a major role in fermentation kinetic and aroma production, and it would clearly be in the interest of winemakers to be able to monitor levels and types of amino acids in the must before fermentation in order to adjust nitrogen not only with regard to total YAN, but also with regard to potential aromatic impact. The BCAAs are particularly important as they have direct bearing on the production of many major aroma compounds that can either add character or render the wine unpalatable. This study provides a good baseline investigation from which to further explore the effect of more complex nitrogen treatments. Since cultivar and regional differences have been shown to also lead to differences in amino acid present in grape must, this study also contributes to the body of knowledge attempting to explain the basis of varietal and regional wine characteristics.

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Oxygen permeating through oak barrels – new results

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Oxygen plays a critical role in the chemical and sensory composition of red wine. Too much oxygen exposure can lead to excessive levels of acetaldehyde being formed in red wine with it becoming oxidised. However, it is well known that the introduction of small amounts of oxygen leads to red wines with a higher colour density, more stable colour, as well as the polymerisation of anthocyanin and tannin molecules. All these reactions lead to red wine maturing when aged in oak barrels. It has been established in the past that red wine maturing in new oak barrels undergo these changes in their phenolic and colour composition quicker than when the same wine is matured in stainless steel tanks or older oak barrels. This is thought to be due to the introduction of small amounts of oxygen into the wine via the staves of the barrel. Other sources of oxygen into red wines when maturing in oak barrels can be due to the addition of topping-up wine, as well as through the bung opening.

Research that was published on this topic in the early 2000s indicated that the amount of oxygen entering through a barrel depends on the age of the barrel, the grain of the wood, as well as the type of bung stopper used. According to these results, levels of oxygen entering a new barrel ranges from 28-45 mg/ℓ/year. High levels of oxygen also penetrate if a silicone bung is used. In older barrels these levels decrease to about 10 mg/ℓ/year. The usage of oak wood staves with a tighter grain was also found to increase levels of oxygen entering the barrels. These researchers indicated that 16% of the oxygen enters through the wood itself, 21% through the bung and 63% enters between the staves. However, the measurement of oxygen entering barrels has always been a challenge due to limited equipment sensitivity and the inability to measure oxygen diffusion in real time in a barrel. This led to these experiments being done on staves instead of barrels, as well as measurements only being done a few times. Theoretical oxygen transfer rates were

then calculated. However, recently published data has challenged these figures and will be discussed in more detail.

Recent advances in measuring oxygen in a non-destructive way has led the way for more real-time measurements. This technology uses a principle where oxygen concentrations can be measured inside a bottle, tank or barrel without opening these containers. Eight of these oxygen sensors were placed at locations inside four new fine grain French, five new, fine grain American oak and four new medium grain American oak barrels which were filled with model wine. This model wine consisted of only 15% ethanol at a pH of 3.5 of which the oxygen was previously removed. This was done in order to measure the oxygen transfer more accurately, as no anti-oxidants were present in this model wine which could have reacted with the oxygen as would happen in real wine.

The researchers found oxygen levels to be higher closer to the bung hole, with levels decreasing as the distance from the bung hole increases (closer to the bottom of the barrel). Levels were sometimes up to 2x higher in the area closer to the bung hole. This illustrates the importance of obtaining representative samples from barrels when oxygen studies are conducted.

The sources of this oxygen going from the barrels to the model wine were initially due to oxygen inside the staves, between the joints between the staves and permeating through the staves. For the three types of barrels, the rate of oxygen transfer was the quickest in the first four months. The fine-grained American oak barrels for instance supplied the model wine with 7% of the total yearly oxygen additions within one week, 23% after one month and 50% after four months. The same trends were also observed for medium-grained American and the French barrels. The transfer of oxygen into wine from a new barrel is thus not linear. If one thus for instance matures wine A in a new barrel for four months and then replaces it with wine B for eight months then both these wines would only obtain about 50% of the total oxygen supplied from the barrel in a year. However, what was interesting was that American oak barrels yielded more oxygen to the wines than the French barrels. The calculated average oxygen additions for the medium-grained American barrels were 11.3 mg/ℓ, with that of the tight-grained American barrels being 11.6 mg/ℓ. The French oak barrels' calculated average value was only 8.2 mg/ℓ. The higher amounts of oxygen entering through American oak barrels probably have to do with the physical structure of the wood. Tyloses block the rays in American oak, but in French oak the occurrence of tyloses is much lower. Wood from France thus has to be split in order for these rays to run parallel with the staves to prevent leakage. American oak can however, be sawed due to the higher occurrence of tyloses which leads to more rays in the staves not being parallel with the wine. The tyloses, although preventing wine leakage, are not impermeable to gasses and more oxygen thus comes into contact with wine in American oak barrels, compared to French oak barrels.

These results shows much lower levels of oxygen permeating into the model wine than previously thought (around 8-11 mg/ℓ/year, versus around 30-45 mg/ℓ/year).

Micro-oxygenation is a process where small controlled amounts of oxygen is added to wine in tanks to simulate the amount of oxygen coming into contact with wine through a barrel. In this process up to 4 mg/ℓ/month can be added to the wine (around 48 mg/ℓ/year), but these dosages thus need some re-thinking considering the above-mentioned results.

These researchers also found more oxygen to permeate into the model wine in the first few hours of the trails, but this effect was short lived and at the end of the one-year trail the calculated oxygen transfer between the tight and medium grain American oak barrel was the same. These results thus disproves the theory that the grain plays a large role in the amount of oxygen entering a barrel. Past research only measured oxygen transfer into wine over a relatively short period of time and also found that tight grain leads to more oxygen permeating through the wood, but the recent results showed that oxygen permeation should be followed over a prolonged period of time.

The oxygen transfer rate is also to a large extent governed by the humidity of the wood. Researchers have calculated that it takes about 82 days after filling with wine for the water saturation front to occur in staves in new barrels. Oxygen travels much slower through a liquid than air and after this period the rate of oxygen diffusion will decrease and thus reaches its minimum. Toasting and bending of rough staves also reduce the oxygen transfer rate with up to 4.5-fold once they have been assembled into a barrel.

Using these results researchers were able to construct barrels with a low oxygen transfer rate and a high oxygen transfer rate. This rate was twice the amount in the high oxygen transfer rate barrel, compared to the low oxygen transfer rate barrel. The classification of staves according to how much oxygen they can transfer seems like a future option for coopers to deliver a barrel to winemakers with certain characteristics, of which one could include its oxygen transfer rate.

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PHOTO: P MYBURGH.

Bioremediation – a method for reducing chemical oxygen demand and turbidity in winery wastewater

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KEYWORDS: Aeration, bioreactor, chemical oxygen demand, turbidity, winery wastewater.

INTRODUCTION

Winery wastewaters vary in quantity and quality during the annual cycle of winery activity. The disposal of winery effluent into rivers, streams and onto soil in South Africa is regulated by the National Water Act No. 36 of 1998. To meet the standards set, most winery wastewaters must undergo some form of pre-treatment before disposal. One method involves directing the effluent through a constructed wetland in which organic wastes are trapped by plants and decomposed by microbes. These microbial breakdown processes require copious amounts of oxygen. This oxygen may be taken either from the soil, in the case of land application, or water, where effluent is discharged directly into water bodies. Under oxygen deficient (anaerobic) conditions, decomposition is slow and noxious odours are produced. Anaerobic conditions are likely to arise where the rate of inflow is high and the organic contents of the wastewater are too concentrated. Constructed wetlands cease to be effective where the chemical oxygen demand (COD) of the wastewater exceeds 15 000 mg/ℓ (Mulidzi, 2005). COD is a measure of the amount of oxygen that is required to biologically oxidise a given quantity of residue in the wastewater. The higher the COD, the greater the risk to fish and other organisms in water, and the greater the likelihood that anaerobic, root inhibiting conditions will develop in soils. Clearly, high-COD effluents must undergo some form of pre-treatment before they enter a constructed wetland. A potentially viable method of pre-treatment involves passing the wastewater through containers filled with inert materials having very large surface areas. These materials form substrates on which the organisms that oxidise organic material become established, forming permanent coatings (biofilms). Such a device is known as a bioreactor.

This article reports on the effects on COD and turbidity of passing winery wastewaters of varying composition through aerated and non-aerated bioreactors.

MATERIALS AND METHODS

The bioreactor consisted of four, covered, 1 000 ℓ plastic tanks linked by a plastic pipe running from the base of one tank to the top of the next downstream tank (Figure 1A). Each tank was slightly lower than its predecessor to facilitate gravity flow, and was filled to 75% of its capacity with inert plastic spheroids moulded to create large internal surface areas (Bioballs™) (Figure 1B & 1C). Commencing in May 2006, the tanks were filled through the opening in the top of Tank 1, from which effluent flowed into the successively lower tanks. The time period over

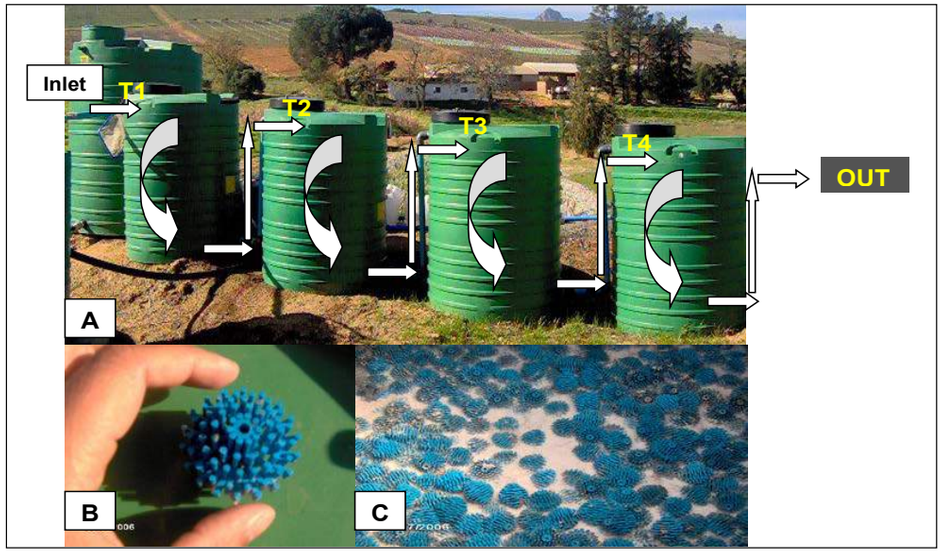

FIGURE 1. Bioreactor, consisting of four 1 000 ℓ tanks. Arrows indicate the movement of wastewater. B. An individual Bioball™. C. Bioballs™ on the surface of the wastewater in one of the tanks.

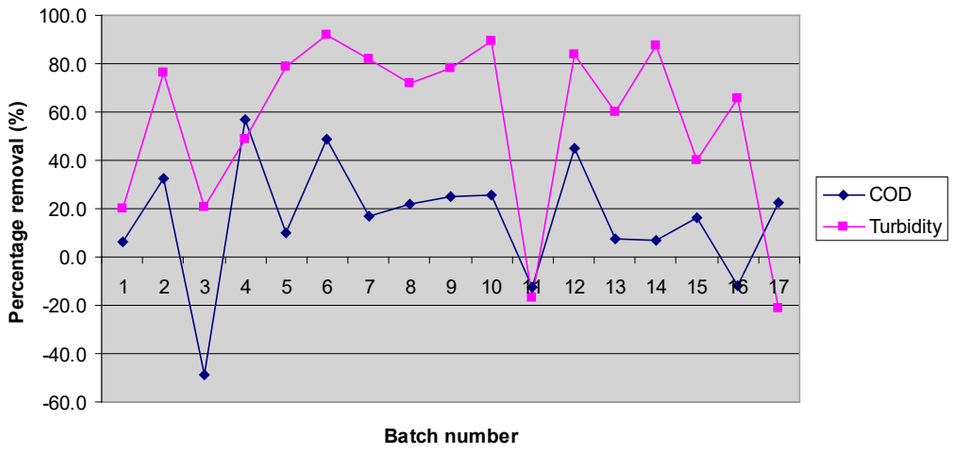

FIGURE 2. Reduction in COD and turbidity in 17 consecutive batches of winery wastewater where the rate of wastewater inflow into the bioreactor was adjusted to give a theoretical retention time of 12 days.

which effluent remained in the reactor was regulated by adjusting the amount of effluent added to the top of Tank 1. The newly added wastewater was assumed to displace the effluent that already occupied it into the following tank, finally exiting from the outlet at the bottom of Tank 4. Since some mixing was inevitable, the time period for a hypothetical single drop of wastewater to pass through the reactor was regarded as the theoretical residence time (TRT). The wastewater used in this trial was obtained from a commercial winery in several bulk consignments (batches). These varied in composition. Additions of effluent were carried out at intervals and volumes sufficient to result in TRTs of four, eight and 12 days (820 ℓ/day for four days, 410 ℓ/day for eight days, and 270 ℓ/day for 12 days, respectively). Several batches of effluent were used for each TRT treatment. The effluents were tested to determine their COD and turbidity (a measure of clarity, or optical translucency) before entering Tank 1, and again at the outflow of Tank 4, one day after the final effluent addition of the treatment. Two bioreactors operated in parallel. One of these received no aeration. Ambient air was pumped into the tanks of the second reactor for periods of six, 15, 60 and 120 minutes per day.

RESULTS AND DISCUSSION

EFFECTS OF WASTEWATER QUALITY AND RETENTION TIME

The effect of TRT was inconsistent (Table 1). Outflow COD was appreciably lower after eight than after four days TRT (49.3% and 8.3% reduction, respectively). Turbidity followed a similar pattern (82.6% and 49.3% decrease, respectively). For most batches within a given TRT treatment outflow COD and turbidity were similar. These values did not improve appreciably between the first batch of the trial (4-day TRT, reduction in COD, 6.5%; turbidity reduction, 51.5%) and seventh batch (COD reduction 7.8%; turbidity reduction 39.2%), implying that efficiency did not improve over successive batches. After 12 days TRT the decreases in COD and turbidity were lower than after eight days TRT. This anomaly stemmed from the fact that the effectiveness of the bioreactor increased as the COD and turbidity of the inflowing wastewater increased, i.e. with worsening wastewater quality (Table 2). The appreciably lower CODs observed after four compared with eight days TRT may therefore have been at least partly due to the fact that the average inflow CODs of the wastewaters used in the 4-day TRT trial runs (1 327 mg/ℓ) were lower than in the 8-day trial runs (19 974 mg/ℓ). It is also probable that the biofilms were less well established, and less effective during the 4-day trial runs, which were the first trial runs after the bioreactor was commissioned, than during the subsequent 8-day runs. A second factor which contributed to the poor performance in the 12-day, compared with the 8-day TRT was that, after some batches, both COD and turbidity were worse after, than before, treatment in the bioreactor (denoted by negative values in Table 2 and shown for batches 3 and 11 in Figure 2). This occurred most frequently where the influent quality was reasonably high (low CODs, i.e. <6 000 mg/ℓ, as in the 4-day trial runs and in batches 3, 5, 11 and 16 in the 12-day TRT trial runs), and where the bioreactor was refilled many times during a single trial run, as in the 12-day TRT treatments). These anomalies arose, because aggregates of organic material from the wastewater, and fragments of biofilm, periodically broke free from the substrate, probably when fresh wastewater was added to Tank 1. These aggregates were then flushed through the bioreactor in the wastewater flow. Most of these aggregates could probably have been removed if appropriate sieves had been fitted on the outflow side of each tank. The sieves would have needed to be cleaned frequently.

EFFECTS OF AERATION ON COD

Averaged over a range of TRTs and wastewater compositions, COD reduction was not consistently affected where aeration times were less than two hours per day (Figure 3). However, where aeration was carried out for two hours per day, COD removal averaged 78%, compared with 66% in the non-aerated reactor.

TABLE 1. Effect of theoretical retention time (TRT) on average reduction in COD and turbidity of winery wastewater flowing through a 4-tank reactor containing an inert plastic substrate having a large surface area.

TRT (days)	Batches tested	COD decrease (%)	Turbidity decrease (%)
4	7	9.0	48.0
8	4	51.0	86.8
12	17	15.7	56.3

TABLE 2. Effect of influent wastewater quality (COD) on average reduction in COD and turbidity of winery wastewater flowing through a 4-tank reactor containing an inert plastic substrate having a large surface area. Residence time: 12 days.

COD at inflow (mg/ℓ)	Batches tested	COD decrease (%)	Turbidity decrease (%)
>9 000	5	41.5	75.8
6 000-9 000	8	15.5	53.8
<6 000	4	-15.8	36.9

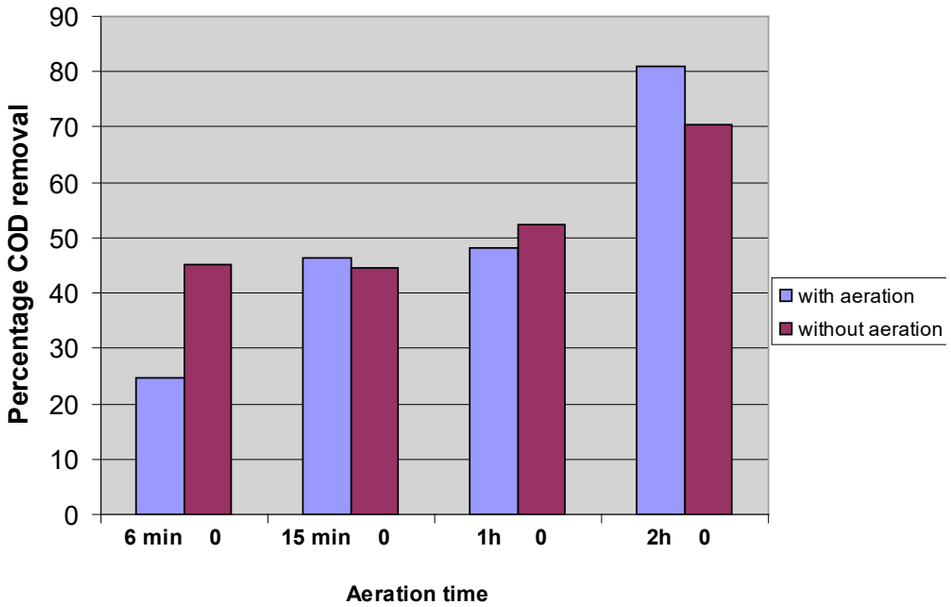

FIGURE 3. Removal (reduction) of COD from wastewater in aerated and non-aerated bioreactors following aeration for six, 15, 60 and 120 minutes per day. Values are averages of multiple batches of wastewater and different retention times.

CONCLUSIONS AND RECOMMENDATIONS

Over the range of CODs tested (4 606 to 10 437 mg/ℓ), passage through a 4-tank bioreactor brought about a c. 50% reduction in COD, and a c. 80% reduction in turbidity after a retention time of eight days. Aerating the reactor for periods of two hours per day promoted bioreactor efficiency. These results suggest that bioreactors may be a practical way to reduce COD in winery wastewaters before they are discharged into wetlands, particularly if prolonged aeration is provided. The primary disadvantage of bioreactors is that aggregates of organic material periodically break free from the substrate and may exit the bioreactor. If adequate filtration were provided to remove these aggregates from the bioreactor it is likely that greater efficiencies would be achieved at retention times in excess of eight days, possibly permitting a continuous flow system to be designed and effectively operated.

SUMMARY

The ability of a bioreactor to reduce the chemical oxygen demand (COD) and turbidity of winery wastewater was tested at theoretical retention times of four, eight and 12 days. At these intervals COD reduction averaged 9%, 51% and 16%, respectively. Associated decreases in turbidity were 48%, 87% and 56%. Effectiveness decreased with decreasing inflow wastewater quality and with frequency of refilling. Filtration is needed to remove planktonic fragments of organic material.

ACKNOWLEDGEMENTS

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Bioremediation of winery wastewater – effects of aeration on microbial diversity

PHOTO: P MYBURGH.

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KEYWORDS: Aeration, bacteria, biofilm, bioreactor, fungi, plankton.

INTRODUCTION

In a previous trial Du Plessis *et al.* (2016) showed that bioreactors reduce chemical oxygen demand (COD) and improve clarity in turbid, high-COD winery wastewaters. Aeration for 120 minutes per day improved bioreactor efficiency. The effect of bioreactor aeration on microbial biodiversity is, however, not known. Bioreactors of the type used by Du Plessis *et al.* (2016) rely mainly on bacteria and fungi to break down waste products in the wastewater. Bacteria break down winery wastes at moderately high pH levels, whereas fungi metabolise wastes under acid conditions. Bacteria and fungi both form mucilaginous films (biofilms) on solid surfaces. To maximise surface area, bioreactor tanks contain inert plastic spheroids (Bioballs™) moulded to create very large, wastewater-accessible internal surface areas. Bacteria and fungi also occur in free-floating (planktonic) form. The bacteria and fungi in the biofilms and plankton must be able to process winery wastewaters over wide pH, composition and concentration ranges. The greater the diversity of the microbial population, the more resilient the system will be. In closed systems, such as bioreactors, microbiological activity may be limited by shortage of oxygen.

The present trial, which was carried out in the same bioreactor used by Du Plessis *et al.* (2016), aimed to investigate the effects of aeration on the diversity of the bacterial and fungal populations in the plankton and biofilms.

MATERIALS AND METHODS

Two adjacent bioreactors were used, each consisting of four 1 000 ℓ capacity plastic tanks, filled to 75% of their capacity with Bioballs™ (Figure 1). The tanks in each reactor were installed at progressively lower heights and linked with PVC pipes which carried wastewater from the base of each upstream tank to the top of the next downstream tank. Wastewater was introduced into the top of Tank 1 and discharged through an outlet at the base of Tank 4. This outlet was extended up to the level of the top of Tank 4 to prevent the system from draining, except when wastewater was displaced through the tanks by additions of wastewater to Tank 1. Wastewater was periodically obtained, in bulk, from the holding dam of a nearby winery. After filling for the first time, 270 ℓ of wastewater was pumped into the top of Tank 1 each day for a period of eight months. Allowing for the percentage of the usable tank volume that was occupied by the Bioballs™ this application rate was equivalent to a theoretical retention time of approximately 12 days, or 3 days per tank. One of the bioreactors was aerated for 120 minutes each day. During this time interval ambient air was pumped into the base of each tank through a diffuser. The tanks in the second bioreactor received no aeration. At 12-day intervals, commencing 12 days after filling, samples of wastewater were drawn from the top of each tank, and 10 randomly-selected Bioballs™ were collected. The Bioballs™ were rinsed with water to remove residual planktonic microorganisms, then agitated in an ultrasonic bath to remove the biofilms. After centrifuging to concentrate the separate plankton and biofilm samples, the genomic DNA was extracted, purified, and separated on an agarose gel. DNA amplification was carried out using the polymerase chain reaction. Community diversity was determined by automated ribosomal intergenic spacer analysis, and expressed in terms of the Shannon-Weaver index. In this index, values increase with increasing microbial diversity.

RESULTS AND DISCUSSION

Microbial diversity increased from Tank 1 to Tank 2 in all categories, except for the aerated planktonic bacteria. This result was probably anomalous (Figure 2). Planktonic and biofilm bacterial diversities, both aerated and non-aerated, tended to be progressively higher in Tank 3 and 4 than in Tank 2. Conditions in Tank 3 and 4 were thus more conducive to increased bacterial diversity than those in Tank 1 and 2, probably because conditions were more stable, and water quality higher, due to previous processing in Tank 1 and 2.

Fungal diversities in the biofilms were lower than the planktonic bacterial diversities in all four tanks, and did not differ greatly between Tank 2, 3 and 4.

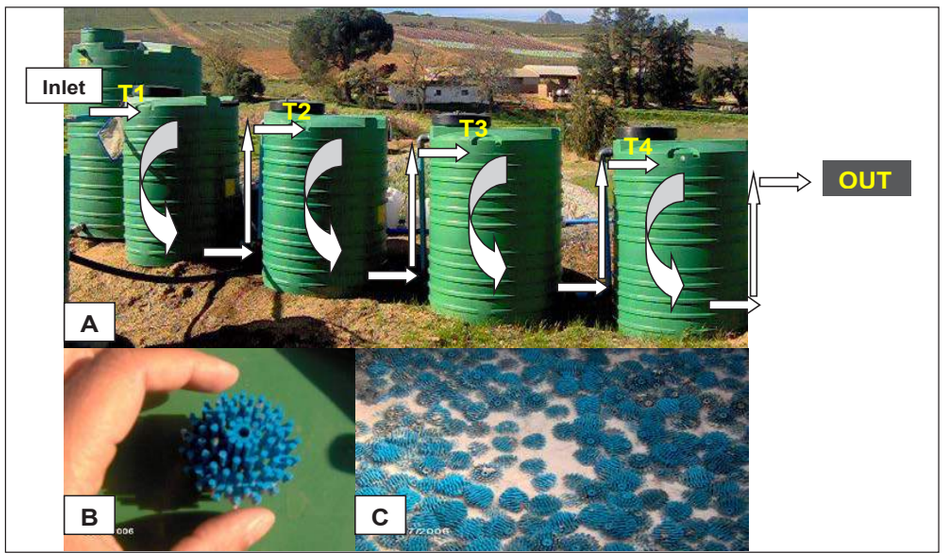

FIGURE 1. A: Bioreactor, consisting of four 1 000 ℓ tanks. Arrows indicate the movement of wastewater. B: An individual Bioball™. C: Bioballs™ exposed on the surface of the wastewater.

Planktonic fungal diversities were nevertheless lower in Tank 3 and 4 than in Tank 2. This was most notably the case for the planktonic fungi in Tank 4 of the aerated bioreactor.

Averaged over Tank 3 and 4, the effects of aeration on diversity were near zero for the biofilm bacteria and fungi, slightly positive (6.3%) for the planktonic bacteria, and negative (9.5%) for the planktonic fungi. This latter figure is misleading since, as shown in Figure 1, planktonic fungal diversities in aerated Tank 1, 2 and 3 were higher than in the non-aerated tanks.

The generally low diversity indexes in Tank 1 were attributed to turbulence during filling, and to changes in acidity and in the availability of easily decomposable nitrogen and carbon-bearing substrates as the raw wastewater introduced into Tank 1 was progressively acted upon by microorganisms in the subsequent tanks.

These findings indicate that although diversity tended to increase from tank to tank (except for planktonic fungi in which diversity declined), the positive effects of aeration, where present, were small. Conversely, aeration exacerbated the observed decline in planktonic fungal diversity.

CONCLUSIONS AND RECOMMENDATIONS

Under the prevailing trial conditions, aeration, which was shown to increase bioreactor effectiveness in reducing COD and improving turbidity in a previous trial, had a small positive effect on planktonic bacterial diversity, no effect on bacterial and fungal diversity in biofilms, and suppressed planktonic fungal diversity, compared with the non-aerated bioreactor. However, this suppression was only observed in Tank 4. From a practical viewpoint, any disadvantage that may stem from an aeration-induced decline in fungal diversity is likely to be outweighed by the greater COD reducing, and turbidity improving, capabilities of aerated, compared with non-aerated bioreactors.

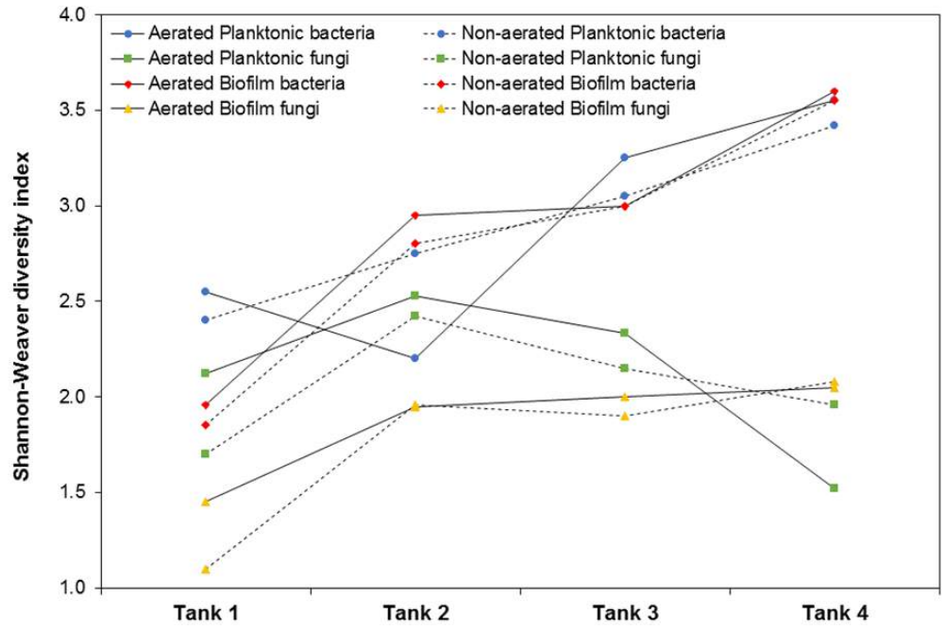

FIGURE 2. Effect of aeration and position of tank in bioreactor on Shannon-Weaver community diversity index for planktonic and bioflim bacteria and fungi in parallel aerated and non-aerated bioreactors. Data acquired at 12-day intervals over an eight month period. Values are treatment averages.

SUMMARY

The effects of microbiological processing of winery wastewater on bacterial and fungal diversity in plankton and biofilms were determined in aerated and non-aerated bioreactors. Aeration had little effect on bacterial and fungal diversity in biofilms, but slightly suppressed planktonic fungal biodiversity.

ACKNOWLEDGEMENTS

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For more information contact Reckson Mulidzi at mulidzir@arc.agric.za.

Genetic test takes cholesterol and alcohol consumption into account

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KEYWORDS: Genetic test, cholesterol, alcohol, heart attack, Alzheimer's disease, CVD.

Biochemical testing combined with genetic screening is increasingly used by doctors to inform clinical and therapeutic decision making in the risk management of chronic and degenerative diseases. It is well known that increased cholesterol levels are associated with heart attacks and Alzheimer's disease. It has recently been demonstrated that certain genetic markers can be responsible for these increased cholesterol levels when they are activated by obesity or environmental influences such as an unhealthy diet. For instance, the cholesterol-raising apolipoprotein E (APOE) polymorphism (ε-4 allele) provides a link between cardiovascular disease (CVD) and Alzheimer's disease. This genetic alteration is present in about 25% of the general population. People with the APOE ε-4 allele do not experience the same protection from CVD related to alcohol consumption than those without this genetic variation. Knowledge of this genetic marker enable doctors to warn their patients to limit their alcohol consumption to reduce the risk of CVD and Alzheimer's disease.

INTRODUCTION

Modern Western medicine made spectacular advances in the past millennium in the treatment of acute, life-threatening diseases presenting as medical or surgical emergencies. Most acute infections can be treated with modern antibiotics, and serious injuries and other surgical emergencies such as acute appendicitis, can effectively be treated with life-saving operations due to safe anaesthetics and sterile operating procedures.

Unfortunately, the same is not true for the modern epidemic of the chronic and degenerative diseases due to unhealthy lifestyles and adverse environmental conditions. Despite the advances made in the discovery of new medicines and lifesaving operations, modern medicine does not have all the answers for these lifestyle-related conditions caused mainly by the destructive lifestyle of modern man. The pharmaceutical industry developed medicines for the management of diseases such as diabetes, CVD, arthritis, osteoporosis and cancer. Patients also insisted on medicines to cure all their ailments without taking responsibility for their own health. Unfortunately, most of these medications relieve the symptoms only, and do not cure these diseases except if the

real cause is addressed where possible. Some of these medications such as the anti-inflammatory medicines and cholesterol-lowering agents may cause serious and life-threatening side effects in some people due to differences in their genetic make-up. The Medicines Control Councils were forced to recall some of these common approved prescription medicines due to unforeseen fatalities and serious side effects.

Medical scientists realised that more should be done to prevent these lifestyle-related diseases before it became manifest, rather than to rely on medicines only to treat these conditions. These chronic and degenerative diseases develop over a period of several years, and doctors identified various factors contributing to the development of these diseases. Epidemiological data identified several associations of these chronic diseases with factors such as smoking and air pollution, obesity, inactivity, unhealthy diet and alcohol abuse, as well as ultraviolet irradiation. Great advances were made in the development of biochemical tests to identify early manifestations of diabetes and heart diseases. Abnormalities in glucose- and cholesterol metabolism can be detected years before a heart attack or the manifestation of diabetes mellitus. This enabled doctors to introduce the necessary lifestyle modifications such as diet interventions, exercise and weight loss programmes, as well as certain supplements to restore the homeostasis in the body without only relying on medications.

The unravelling of the human genome in 2003 brought a new dimension in the diagnostic and therapeutic armamentarium of the modern doctor. Major and minor genetic abnormalities were detected to identify the cause of specific diseases in families and individuals. Certain genetic abnormalities could be linked to specific diseases, and it was also established that certain abnormal biochemical results are also linked to a genetic profile. Clinical features such as overweight and high blood pressure could also herald the risk to develop serious diseases. The combination of related clinical-, biochemical- and genetic-information became a valuable decision-making tool in the management of these chronic and degenerative diseases. In this research project we demonstrated that genetic testing should not be done in isolation, but must be combined with a lifestyle questionnaire, including the family history and careful analysis of the diet and alcohol consumption.

The determination of cholesterol levels is of particular importance in South Africa, where the prevalence of familial hypercholesterolaemia (FH) is increased 5 to 10 times, compared to most other populations in the world due to a founder effect. This led to the development of a genetic test by our laboratory for FH to identify these patients with a high risk of cardiovascular disease. These patients need urgent treatment with cholesterol-lowering agents to prevent a premature heart attack, despite their APOE status.

It is well established that abnormal cholesterol levels are also associated with cardiovascular disease and Alzheimer's disease in people that do not suffer from FH. We demonstrated that certain minor genetic markers could cause elevated cholesterol levels when they are activated by overweight, inactivity and an atherogenic diet. The cholesterol-raising apolipoprotein E (APOE) polymorphism (ε- 4 allele) provides a link between cardiovascular disease (CVD) and Alzheimer's disease. This genetic alteration is present in about 25% of the general population. Our previous research demonstrated that moderate intake of alcohol protects people from CVD. However, people with the APOE ε- 4 allele do not experience the same protection from CVD than those without this genetic variation. Knowledge of this genetic marker enable doctors to warn their patients to limit their alcohol consumption to reduce the risk of CVD and Alzheimer's disease.

WHAT DOES THIS RESEARCH MEAN FOR THE WINE-LOVING COMMUNITY?

The good news is still valid; moderate and responsible wine consumption provides some protection against the development of cardiovascular disease. This is supported by various epidemiological and experimental observations worldwide, despite the very negative comments made in the UK recently about the adverse effects of alcohol. Of course we assume that the moderate and regular consumption of wine is associated with a healthy lifestyle including a balanced diet, weight management, no smoking, and regular exercise.

However, genetic testing enabled us to detect people with elevated cholesterol suffering of FH. Our laboratory identified that most people with FH goes undetected, because of the unavailability of genetic testing procedures. They are at risk to develop cardiovascular disease, and need urgent treatment with cholesterol-lowering agents to prevent this catastrophe, irrespective of APOE status. In contrast, people without FH, but with the cholesterol-elevating APOE polymorphism (ε-4 allele), respond better to lifestyle modification such as diet, weight loss, and exercise than on cholesterol-lowering agents such as the statins. This has important implications, because it has recently been demonstrated that statins have various negative side effects in some patients such as muscle pains due to muscle breakdown. The use of statins is also implicated in the development of cerebrovascular attacks in these patients. People in this genetic subgroup should therefore not be treated with these potential toxic medications such as the statins. Alcohol abuse should also be avoided in this subgroup of patients, because they do not derive the same cardioprotective benefits than people with a normal genetic profile.

CONCLUSION

Chronic and degenerative diseases as the result of a destructive lifestyle derive more benefit from lifestyle interventions rather than medications. In fact, some of the current prescription drugs for these diseases do have serious side effects, and efforts should be made to detect biochemical and genetic markers to guide us in our search for more appropriate therapy.

However, certain inherited diseases such as FH, needs urgent identification and aggressive therapy. Our research also indicated that many people with FH remain undetected, because the genetic profile is unknown. Patients with abnormally high cholesterol and a family history of CVD, need to undergo genetic testing.

Moderate and regular alcohol intake, and more specific red wine, in conjunction with a balanced diet and a responsible lifestyle, provides health benefits to the majority of the population. Personal and family history, in conjunction with biochemical blood analysis and certain genetic testing, provides a scientific platform for doctors to diagnose and treat these conditions more appropriately.

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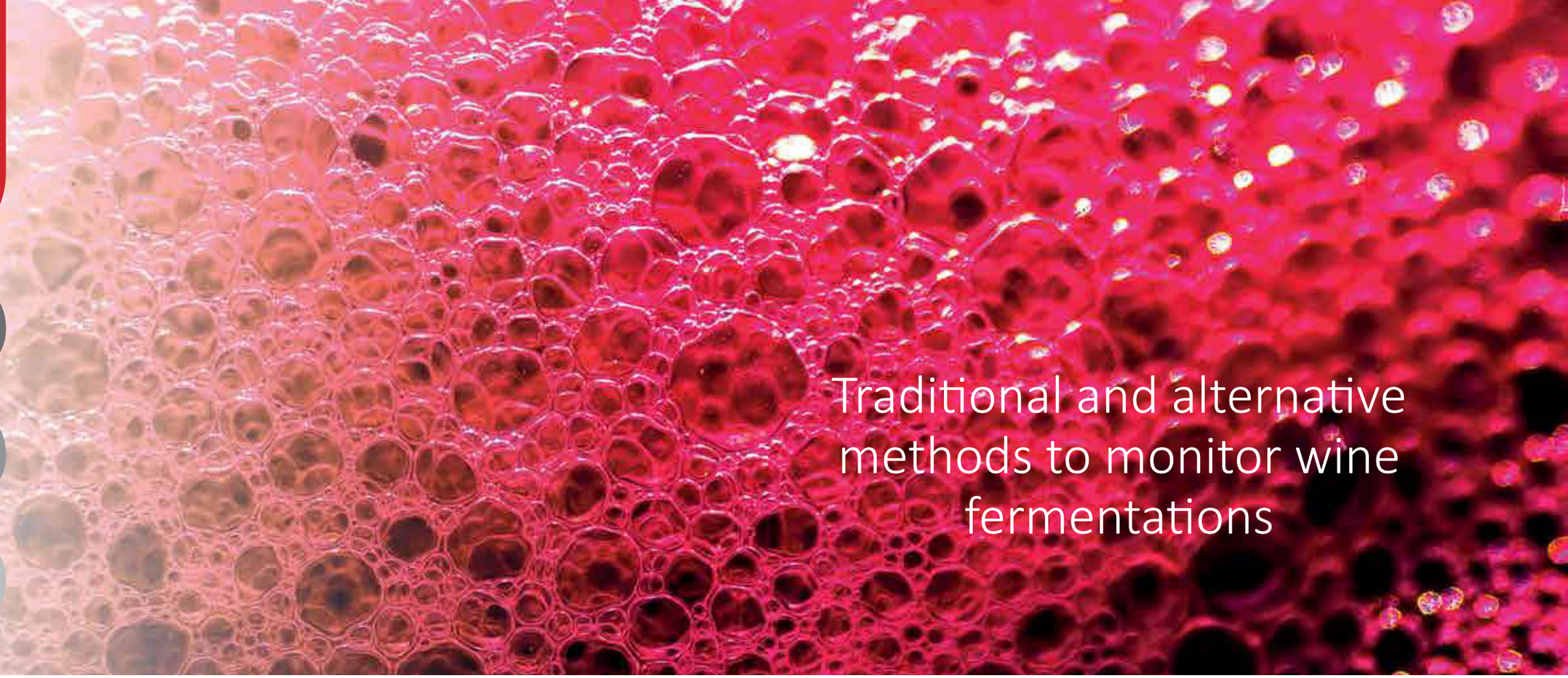

Traditional and alternative methods to monitor wine fermentations

NOVEMBER 2016

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KEYWORDS: Fermentation, infrared spectroscopy.

The purpose of this article is to illustrate the potential contribution of infrared spectroscopy to the monitoring of wine fermentations.

In the wine context, the term “fermentation monitoring” usually refers to the process whereby samples are removed from fermentation tanks or barrels and taken to the laboratory for chemical analyses. Concentrations of important compounds or parameters are measured and the information is subsequently interpreted to obtain an indication of the progression of the fermentation process, or the quality of the end product.

In the wine industry, so-called wet chemical methods are largely used to monitor the progression of fermentations. Although these practices are well-established, there is a tendency world-wide to also make use of available alternative strategies that are able to generate the same information in more cost- and time-effective ways.

Infrared spectroscopy (IRS) is widely considered to be one of the technologies which will potentially impact most on the ways in which fermentations are monitored in future. Reasons for this contention are the unmatched low unit cost and huge amounts of physical-chemical information in the fermenting matrix that are captured by infrared measurements. Furthermore, the technology is well-established in the food and pharmaceutical industries.

Results and conclusions presented here are based on research funded by Winetech and conducted in recent years at the Institute for Wine Biotechnology, Department of Viticulture and Oenology, Stellenbosch University, in conjunction with SA wine cellars.

TRADITIONAL METHODS OF MONITORING ALCOHOLIC FERMENTATION AND MALOLACTIC FERMENTATION

The conversion of grape juice to wine during fermentation may be considered a biotechnological process that is largely catalysed by the metabolic activities of microbes. The two most important fermentation processes, namely alcoholic fermentation (AF) and malolactic fermentation (MLF), are characterised by the production and consumption of metabolites by yeasts and malolactic bacteria, resulting in complex, ongoing and often very rapid changes in the chemical composition of the fermenting juice or wine.

The effective management of the progression of AF and MLF requires regular chemical analyses of the most important compounds and parameters, such as reducing sugars (RS), glucose, fructose, alcohol, pH, volatile acid (VA), titratable acid (TA) and malic acid. For these tests, wine laboratories use established and accurate methods. Some of these methods have disadvantages, such as lengthy, time-consuming procedures and consequently a long time delay before the result is known. Furthermore, the unit costs of certain analyses are high and a considerable amount of chemical waste is generated.

It is common knowledge that the progression and duration of AF and MLF are unpredictable. Apart from the juice composition, the population dynamics of the microbes (wine yeasts and bacteria) also play an important and often unpredictable role. Factors such as the initial sugar concentration of the juice, availability of nitrogen for yeast metabolism, temperature fluctuations during fermentation and specific inoculation practices may also impact on the progression.

Furthermore, variation may also be caused by sluggish or stuck fermentations. All these factors together result in huge variations, as well as a measure of unpredictability regarding the progression of AF and MLF. This is very clearly illustrated in Figure 1 that shows the results of an experiment in which one batch of Shiraz juice was divided into equal smaller volumes, each fermented separately under the same controlled cellar conditions with a co-inoculation strategy. The yeast *Saccharomyces cerevisiae* NT 202 was used, together with one of four different MLF cultures of *Oenococcus oeni* (S5 and Lalvin VP41) and *Lactobacillus plantarum* (V22 and 56).

Samples were removed from the fermentation containers on a daily basis and analysed for the malic acid concentration and the IRS spectrum. Results (Figure 1) show that the duration of degradation of malic acid took place at different tempos and durations. In certain instances the entire fermentation process was completed within 18 days, while others took more than 30 days. These are well-known phenomena, but illustrate the challenges faced by winemakers to manage cellar space when large numbers of fermentations take place simultaneously.

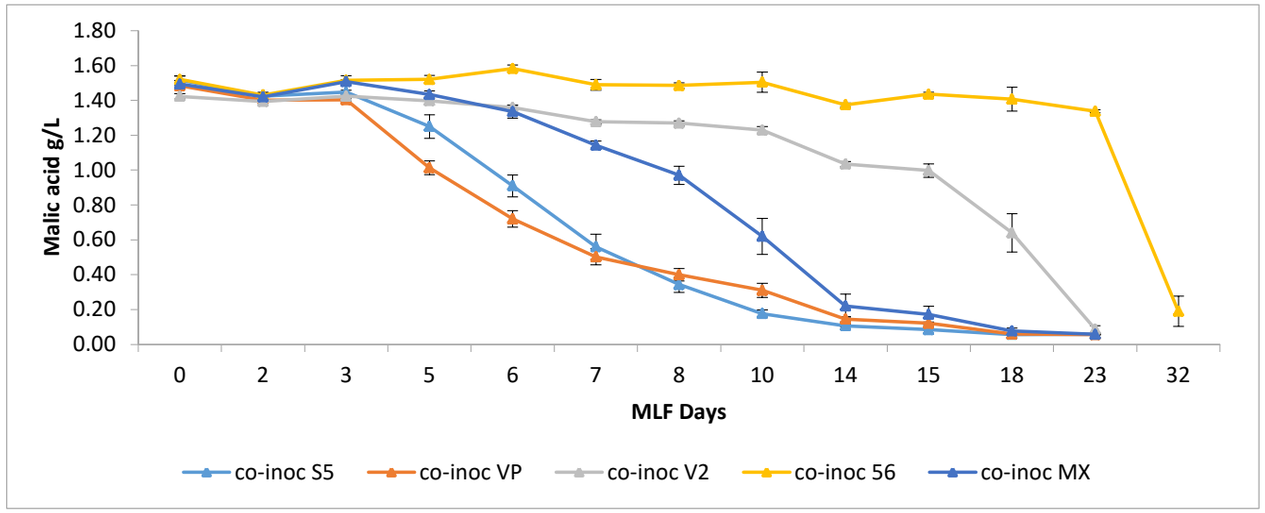


FIGURE 1. The progression of malolactic fermentation with different co-inoculation strategies. *S. cerevisiae* NT 202 was used with one of four different combinations of MLF cultures: *O. oeni* S5 and Lalvin VP41; *L. plantarum* V22 and 56.

QUANTITATIVE MONITORING OF FERMENTATIONS WITH INFRARED SPECTROSCOPY

Quantitative monitoring implies that the concentrations of the chemical components in samples removed from the fermentation are calculated at regular intervals. As mentioned previously, this can be achieved with laboratory analyses, but modern wine industries are increasingly looking at quicker methods to follow the progression of AF and MLF, especially with a view to automatisation and improved management of fermentations. A considerable amount of developmental work with infrared spectroscopy has been conducted in the SA wine industry through research in collaboration with cellars and laboratories.

Infrared spectroscopy refers to the measurement of the absorbance of infrared light by chemical components. Fermentation samples are measured in seconds and a so-called “fingerprint” of the fermentation at the exact moment of measurement is displayed as a simple line spectrum (Figure 2A). The industry uses both near (800-2 500 nm) and mid-infrared light (2 500-5 000 nm).

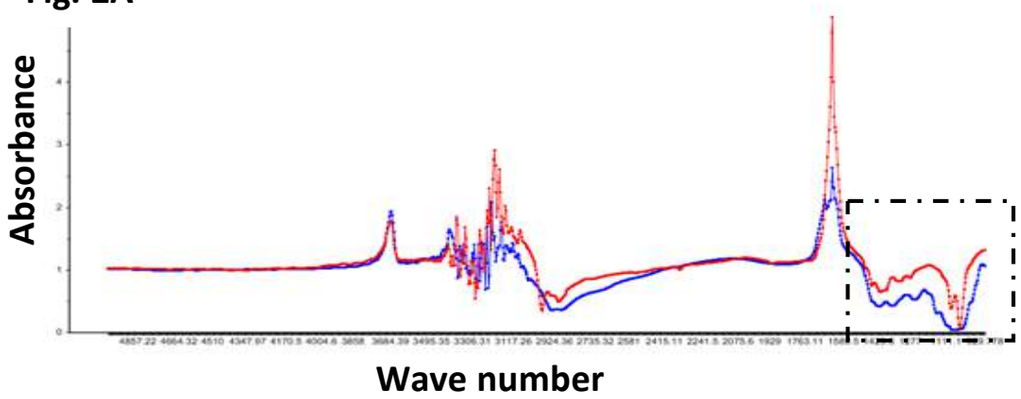
The spectra contain the information about the chemical composition of the fermentation, which is loaded onto the IR spectrometer by means of a mathematical calculation in the form of a so-called “calibration model” to analyse future samples. Figure 2B shows a graph of the calibration model for malic acid developed by the research.

The indirect measurements are plotted against the measured values and graphically represented to evaluate the prediction error (Figure 2B). Established statistical indicators such as RMSEP and RPD give a very good indication of the accuracy of the predicted values. During the calibration phase, reference values should also be measured, but this is no longer necessary once the model has been established.

In instances where the accuracy of the quantitative values does not comply with the requirements that have been set, the information may still be used to monitor the progression of the fermentation, to the point where chemical methods are used to obtain a precise value. In this way the IRS technology may play a role in limiting the number of tests that are conducted using expensive analytical methods.

In the course of the research a wide range of accurate calibrations were developed for both AF and MLF, for quality parameters such as glucose, fructose, RS, alcohol, VA, TA, pH, yeast-assimilable nitrogen (YAN), malic acid and lactic acid, which were loaded on infrared spectrometers in laboratories. The technology is

Fig. 2A



Calibration

Fig. 2B

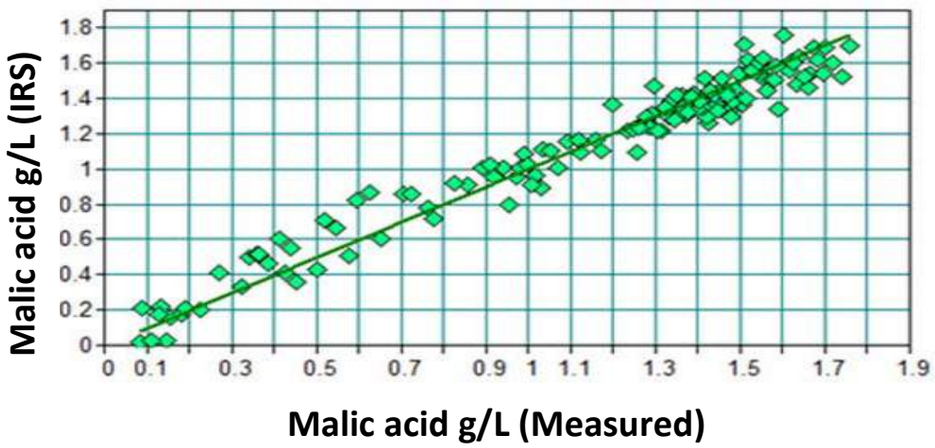


FIGURE 2A. Mid-infrared spectra of alcoholic fermentation show how the spectra differ at the beginning (blue line) and the end (red line) of the process, especially in the area indicated with the dotted line.

FIGURE 2B. The chemical information in the spectra may be processed in a calibration model and used to indirectly predict the concentration of chemical components, in this case malic acid, rather than using a direct laboratory analysis.

not only limited to the laboratories; IR detectors can also be mounted inline and online to the fermentation tanks. The ideal strategy would be to have real time readings displayed graphically and the metabolic condition of the process determined, especially to identify problematic fermentations. This has enormous logistical benefits in view of the fact that problems may be addressed quickly through informed decisions and applicable follow-up actions. In this context one should consider infrared spectroscopy as a solution that forms part of a process, rather than a specific technology that functions in isolation.

Although the quantitative use of infrared spectroscopy, rather than laboratory measurements, is very handy to predict concentrations of chemical components, the calibration process may be time-consuming and expertise is required. The next section on qualitative monitoring, discusses the strategy developed by the research to eliminate the calibration step and simply use the infrared spectra to determine the progression of AF or MLF.

QUALITATIVE MONITORING OF FERMENTATIONS WITH INFRARED SPECTROSCOPY

Given the initial developmental work that has to be done with infrared spectroscopy to establish the calibrations for quantitative measurements, the research also looked at methods to eliminate this step. A very simple test, known as the conformity test, was developed. It is based on comparing the absorbance per wavelength between spectra. The standard deviation (SD) at each data point (measurement at a specific wavelength) gives an indication of how similar or different the spectra are. Large SD values indicate that the spectra are very different, while small SD values show that the spectra are very similar. The analyst also has the option of comparing only spectra in specific areas with each other, for example the areas where malic acid is absorbed, rather than the entire spectrum.

Small SD between spectra can also be interpreted that the samples from which the spectra were taken, are chemically very similar. Figure 3 illustrates the process as applied to AF: Each marker on the graph represents an IR spectrum which was taken daily from the fermentations in the course of progression. The green reference spectra refer to samples taken at the end of AF and analysed for RS to ensure that the fermentation is completely dry. The blue markers indicate the spectra measured at different stages during the AF.

From Figure 3 it is clear that the distance between the blue markers and green reference spectra, gradually becomes smaller at each subsequent stage of the fermentation progression. At stage 7 the blue markers lie on the same horizontal line as the green markers (small SD between the blue and green spectra) and it is therefore possible to deduct that the fermentation is completely dry.

To illustrate the explanation a graph was drawn in the right insert on Figure 3, showing the RS values measured in the laboratory, so that the

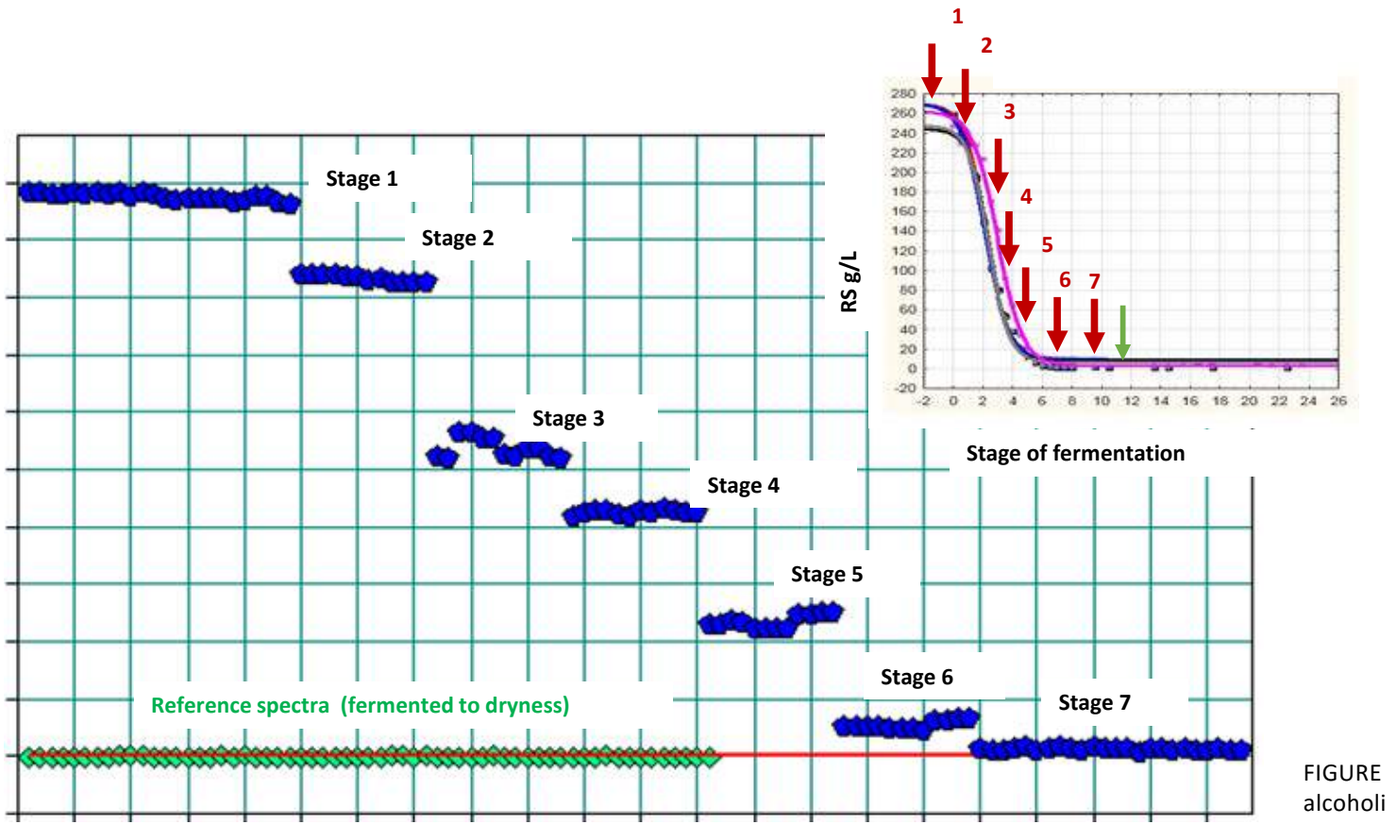


FIGURE 3. Conformity test to monitor the progression of alcoholic fermentation by using mid-infrared spectra.

relationship between the reduced RS concentrations and smaller SD of the two sets of spectra can be clearly seen.

With this qualitative approach, it is therefore only necessary to examine the spectra and chemical analyses are quite unnecessary. The reference spectra may also represent malic acid concentration, which makes the method suitable for MLF. Several other applications of the conformity test are currently being investigated.

SUMMARY

The research conducted in this industry funded project laid a very valuable foundation for the monitoring of AF and MLF. Several quantitative calibration models were created by making use of infrared spectra and relevant reference values, and are currently being used not only in the research, but also in the wine laboratories of South African cellars. Qualitative models, using spectra only and no reference values, were also developed to eliminate the time-consuming calibration process.

Although it is clear that infrared spectroscopy can make a huge contribution to the monitoring of wine fermentations, the technology is not yet being used to its full potential in the industry. The input of wine laboratories and cellars has been considerable to optimise the technology for other users and make it as accurate as possible.

In a subsequent phase of the research the intention is to mount the IRS detectors online on fermentation tanks and expand the calibration models to measure colour components in juice and wine. This will bring the industry another step closer to complete automatisation of the monitoring of wine fermentations.

This article originates from research funded by Winetech and the final report of project IWBT B 08/11, “Bioprocess monitoring of fermentations with infrared spectroscopy”, can be downloaded from http://www.sawislibrary.co.za/dbtextimages/NieuwoudtH4.pdf.

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Testing for allergenic residues in wine – a storm in a teacup?

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KEYWORDS: Allergen, residue, fining, filtration, ELISA.

A study was commissioned by Winetech to determine whether it is necessary for wineries to have every batch of wine tested for potential residues if allergenic proteins were used as processing aids during the winemaking process. Such a practice will not just be time consuming, but also very costly. We are happy to report, based on this study and similar studies done in Australia and Europe, that the testing of every batch is not required if good winemaking practices in terms of the use of fining agents, as well as specific filtration guidelines, are followed. Producers who fine their wines, but intend to sell them unfiltered will have to label the allergenic agent used or alternatively test each batch, since residues above the OIV/EU legal limit of 0.25 mg/ℓ may remain.

WHAT THE LAW REQUIRES

EU Commission Regulation (EC) No. 607/2009, that deals with the labelling requirements of allergens in wine (when the ingredients used during the

production of wines are still present in the final product), has been amended by EU regulation 579/2012 on 29 June 2012. This regulation determines that wine exported to, or produced in the EU, must be labelled for allergens (terms concerning sulphites, milk and milk-based products and eggs and egg-based products) under the following conditions:

- All wine from the harvests of 2011 and wine of the 2012 harvest, labelled before 30 June 2012, will be exempt from mandatory egg/milk allergen labelling.
- Wine of the 2012 harvest, labelled after 30 June 2012, will not be exempt.

THE SOUTH AFRICAN STUDY

The determination of allergenic fining agent protein residues in wines were done using an ELISA method. Sampling was done with the assistance of the Sawis inspectors. Wine samples were requested from wine cellars who wished to participate in the project. These samples must have been treated with either casein (milk powder), egg white proteins (ovalbumin) or lysozyme. The winemaker was requested to declare the amount of allergen or fining agent that was added, the type of fining/filtering techniques used and the size of the filters, e.g. 0.6 µm filter.

Study 1

Samples were drawn at various intervals during the winemaking process: before fining, after fining, after bulk filtration, before bottling and after bottling. The sample set was red wines of the 2013 and 2014 harvests and therefore only comprised of wine processed with albumin or lysozyme.

Study 2

In addition to the voluntary sampling, a questionnaire was introduced on Wine Online for a period of one month for all certified wine submissions. Samples were collected from certified wines that were treated with allergens, which were then submitted to the laboratory for analyses. All the wines in the sample set were final bottled and labelled red and white wines, ready to be sold on the market or exported. These samples were analysed for the presence of casein, egg white and lysozyme and the results quantified for concentrations above 0.25 mg/ℓ.

The results

In Study 1 a total of 79 samples were collected for analysis. The sampling procedures were statistically sound. All wines were treated with either ovalbumin (egg white) or lysozyme. Only four producers however used lysozyme. Ten g/hℓ of lysozyme was added to a relatively small volume and even though the wines were not filtered, no residual lysozyme could be detected in the wine samples. A larger sample set will however have to be studied before a conclusive observation can be made in the case of this allergen.

The average dosage of albumin used by producers was between 2 and 3 g/hℓ of wine. All wines were fined between three and 11 days after production. All wines that were analysed directly after fining, but before filtration, showed the presence of allergens at different concentrations, except for one wine. However, all wines that were positive for the presence of allergens, showed that no residual allergen could be detected after the wine was filtered. The size of the filters that were used for filtration was between 0.2 and 0.65 µm. Only in one instance was residual albumin detected after filtration. The wine was then filtered with a filter size of 0.45 µm and cold stabilised before bottling. The wine was re-analysed after bottling and no residual albumin could be detected.

In Study 2 a total of 43 samples were analysed. All wines in the sample set were final bottled and labelled samples that were ready to be sold in the marketplace or exported. Wines were fined using the following type of proteins: ovalbumin (egg white), casein and gelatine. Gelatine is not considered an allergen. Only one sample was treated with lysozyme and it tested negative for the presence of residual lysozyme. The size of the filters that were used for filtration before bottling was between 0.45 and 0.65 µm. All bottled samples (both red and white wine) tested negative for the presence of residual albumin or casein.

CONCLUSIONS FROM THE SA STUDY

In general wines that tested positive for the presence of allergens after fining, tested negative for the presence of allergens after filtration, with the exception of one wine. This suggests that filtration, using the correct filter size (between 0.2 and 0.65 µm), could indeed remove residual allergens from the wine (if the dosages in this study are applied) and therefore negate the need to label or test for allergens and therefore save costs.

All certified final bottled and labelled wine in the sample set tested negative for the presence of residual albumen or casein. The levels of residual allergens were all found to be below the 0.25 mg/ℓ detection level, above which allergen labelling becomes mandatory.

If a higher dosage of allergen was added to a smaller volume of wine, then the initial filtration after fining can be followed up by a second filtration just before cold stabilisation and bottling to ensure that all residual allergens are removed.

No correlation could be found between the initial dosage of fining agent added, the volume of wine and the quantified residual allergen remaining in the wine after fining. Literature suggests that different fining agents react differently with different wines and even with the same wine.

RESULTS FROM INTERNATIONAL STUDIES

A 2015 publication by Elena Penas and co-workers reviews the allergen residue studies done over the past few years. Here are the results from some of the studies mentioned in the review:

- No detectable amounts of egg white with ELISA in four experimental wines treated with egg white at 4 g/hℓ; egg white detected with ELISA at a level of 0,2 mg/ℓ when a dosage of 20 g/hℓ was used.
- No detectable amounts of casein in four experimental wines fined with 6 and 30 g/hℓ potassium-caseinate; treatment with bentonite and crossflow filtration after casein treatment leaves no detectable casein residues.
- No ovalbumin detected with ELISA above 1 mg/ℓ in 40 commercially available Australian wines fined with egg white proteins.

- No casein detected with ELISA in 153 commercially available Australian wines.
- The use of bentonite or sheet filtration, followed by sterile filtration can eliminate egg allergens.

The authors conclude that most of the studies they reviewed showed that most wines are free (below 0.25 mg/ℓ) from allergens after bottling. However in some cases egg white proteins were detected after bottling and egg white residues are also more frequent than milk protein residues.

The removal of allergenic additives or fining agents can be optimised following specific guidelines set by the OIV. These guidelines can easily be followed by winemakers and labelling can be avoided.

For more results on international studies refer to the above-mentioned open access review.

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This article originates from research funded by Winetech and the final report of project Allergen 1-2013, “Analyses of allergens in wine to establish the impact of filtration and fining of wine”, can be downloaded from http://www.sawislibrary.co.za/dbtextimages/JonkerW.pdf.

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3
In the Vineyard

When and how to fertilise

FEBRUARY 2016

BRAHAM OBERHOLZER

KEYWORDS: Soil, fertiliser, irrigation, manure, water.

It is very important to follow the correct fertilisation programme. Both your grapevines and your wallet will benefit.

Provided nutritional deficiencies were adjusted according to prescribed recommendations at the time of soil preparation, grapevines should mainly have to receive nothing but a nitrogen supplement in the course of their lifetime. Grapevines have a good root system which enables them to take up most of the nutrients they require from the soil. The best times for application are when the roots of the grapevine grow actively, which of course depends on soil temperatures. For grapevines the times of root growth are four weeks after bud burst, after set and immediately after the harvest.

FERTILISATION OF YOUNG GRAPEVINES

Young grapevines differ from production grapevines in that their roots grow actively throughout summer. Consequently they are given fertiliser throughout the entire season. Small amounts of fertiliser applications are given on a monthly basis. The fertiliser is either applied via the irrigation system or placed by hand alongside the grapevines. If fertiliser or manure is applied manually, it should not be heaped against the trunk. If this happens, the fertiliser will burn the trunk and the grapevine will die. The fertiliser and manure should therefore be placed a small distance from the young grapevines' trunks and preferably also under the drippers of a drip irrigation system.

It is important not to apply the fertiliser required by the grapevines all at once, rather divide it into applications that can be spread out across the growing season.

Fertiliser may be applied in three different ways, namely:

- By hand;
- Using tractors and fertiliser sprayers; or
- As fertigation.

Where fertiliser is applied by hand, it is mostly strewn on the berm/vine row. Even application of fertiliser is an art, especially with highly concentrated products such as urea, where only a small amount is applied per hectare. If excessive amounts of

Use a cooldrink bottle cap to measure fertiliser that is applied by hand.

A cooldrink bottle cap of LAN weighs between 3 and 5 g.

Fertiliser may also be applied by tractors and fertiliser spreaders.

Water soluble fertiliser applied via the irrigation system.

urea are strewn in one spot, the roots may burn. To ensure accuracy, the amount of fertiliser to be given per grapevine must be calculated carefully. It should be weighed so that the people involved in the application have a good notion of the amount to be given to each grapevine. A small tin or cap is mostly used to measure the right amount for each grapevine.

EXAMPLE

Young grapevines' requirement for the first season amounts to a total of 90 kg nitrogen (N) which is given in three applications, namely after bud burst, after set and after the harvest. The most commonly used source of nitrogen fertiliser is limestone ammonium nitrate (LAN) with a nitrogen content of 28%. Therefore 100 kg LAN (two bags of 50 kg each) contains 28 kg N and almost complies with the requirement of 30 kg N per application. Suppose there are 3 000 grapevines per hectare (100 x 100 m), then 100 kg LAN per 3 000 grapevines will amount to ±33,3 g LAN per grapevine. Three applications will deliver 100 g LAN per grapevine.

The fertiliser for each block must be meticulously calculated and weighed. This is to ensure that each block receives the recommended dosage.

If tractors are used to apply fertiliser and/or manure, the spreader must first be calibrated. This means the spreader must be set to apply the correct amount of fertiliser at a time. Also ensure that the spreader bin is clean and that there is no clogging. It is important to note that different blocks require different fertilisation. This means that the calibration of the strewer must be adjusted depending on the product and the amount to be applied.

Manure or lime spreader may be used to apply manure and/or enriched organic mixes. Such spreaders must also be calibrated. It is always possible that the manure may contain stones which will damage or break the spreader. To prevent this a sieve may be applied to the top of the spreader.

Liquid fertiliser is usually delivered in bulk by tankers directly into the fertiliser tank on the farm. From here it is distributed via the irrigation water to the blocks that require fertilisation. For an accurate application the irrigation system should be in good condition and no sprayer or drippers should be clogged.

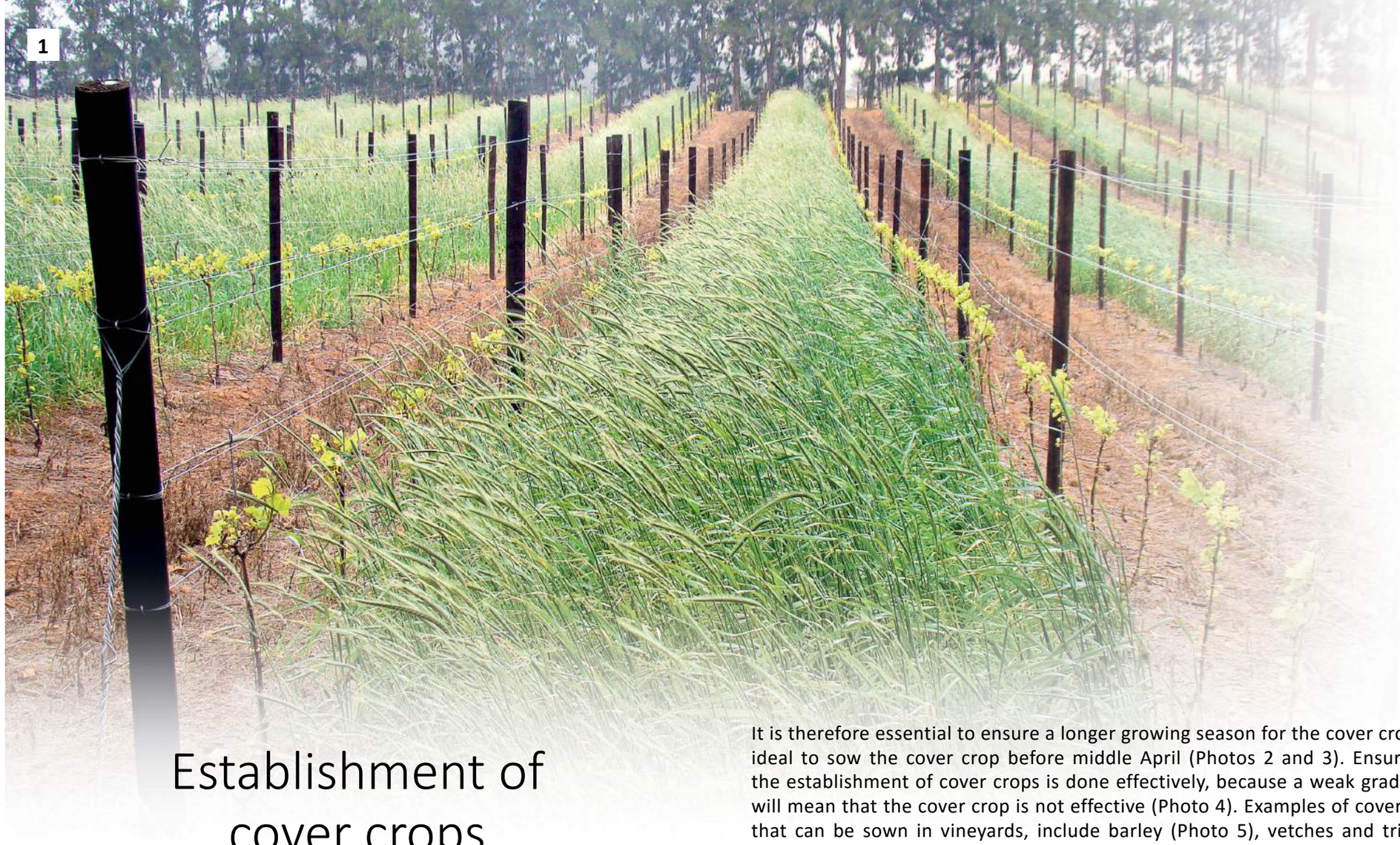
Water soluble fertiliser is high quality concentrated products that are easily dissolved in water and applied by the irrigation system in the vineyards. When water soluble products are mixed, it is essential to adhere to the recommended dosages. If too much fertiliser is added to the tank, everything will not dissolve and the irrigation lines may become clogged. There should be water in the tank before adding the fertiliser. If not, the fertiliser at the bottom of the tank may form a hard clump which will dissolve with difficulty.

If too much fertiliser is applied, the portion not used by the grapevines may be leached out of the soil. Such leached nutrients end up in soil water and rivers and cause pollution. The sandier the soil, the easier it will be for nutrients to leach out.

Too much fertiliser, nitrogen especially, can also cause excessive nutrient uptake by grapevines, which will then grow profusely. Profuse growth means that a lot of topping is required and leaves will have to be broken out. The grapevine also bears less grapes because it becomes infertile and the quality of the grapes is moreover inferior. In addition profuse canopies are conducive to diseases and rot.

It is therefore important to apply the right nutritional elements in the right quantities. Not only does this ensure that the requirements of the grapevine are met, it also prevents money being wasted on too much or unnecessary fertiliser.

For more information contact Braham Oberholzer at boberholzer@nulandis.com.



Establishment of cover crops

APRIL 2016

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KEYWORDS: Establishment, cover crops, vineyards.

The effective establishment of cover crops is essential. A cover crop that is not of good grade or not giving good density will not be effective for weed control which leads to weeds dominating the cover crop.

There is much value in a good grade cover crop (Photo 1). The most important benefits of cover crops, are moisture retention by means of dead plant residues, weed control, increased water infiltration, improvement of soil structure, limitation of run-off and evaporation, and a certain degree of nutrient distribution.

It is therefore essential to ensure a longer growing season for the cover crop and ideal to sow the cover crop before middle April (Photos 2 and 3). Ensure that the establishment of cover crops is done effectively, because a weak grade crop will mean that the cover crop is not effective (Photo 4). Examples of cover crops that can be sown in vineyards, include barley (Photo 5), vetches and triticale, sometimes mixed (Photo 6) and rye grass, to name a few.

Basic principles to keep in mind when establishing cover crops:

- Use good quality seeds.
- Sow early to extend the growing season of the cover crop and ensure maximum production of dry material.
- Start preparing the seed bed at the end of March.
- Use a cover crop that will flatten by itself once it has been sprayed with a herbicide (clovers, vetches and barley) or drag it flat (Photo 7).
- Leave the dead plant residues on the surface.
- Spray ridges with a herbicide in the middle of winter over a one metre wide strip in the vine rows.
- Spray the cover crops with a herbicide just before bud break.
- Know the cultivation requirements of the cover crop, in other words which soil type is more suitable for a specific cover crop.

Cover crops have a stabilising effect on soil structure, because:

- Dead plant material is an energy source to soil microbes which form structure stabilising bonds from it.

(PHOTOS: FRANCOIS VILJOEN)

– Cover crops contribute to the organic material content of the soil and promote formation of new structural units.
– Cover crops protect the soil against the impact of water and raindrops.

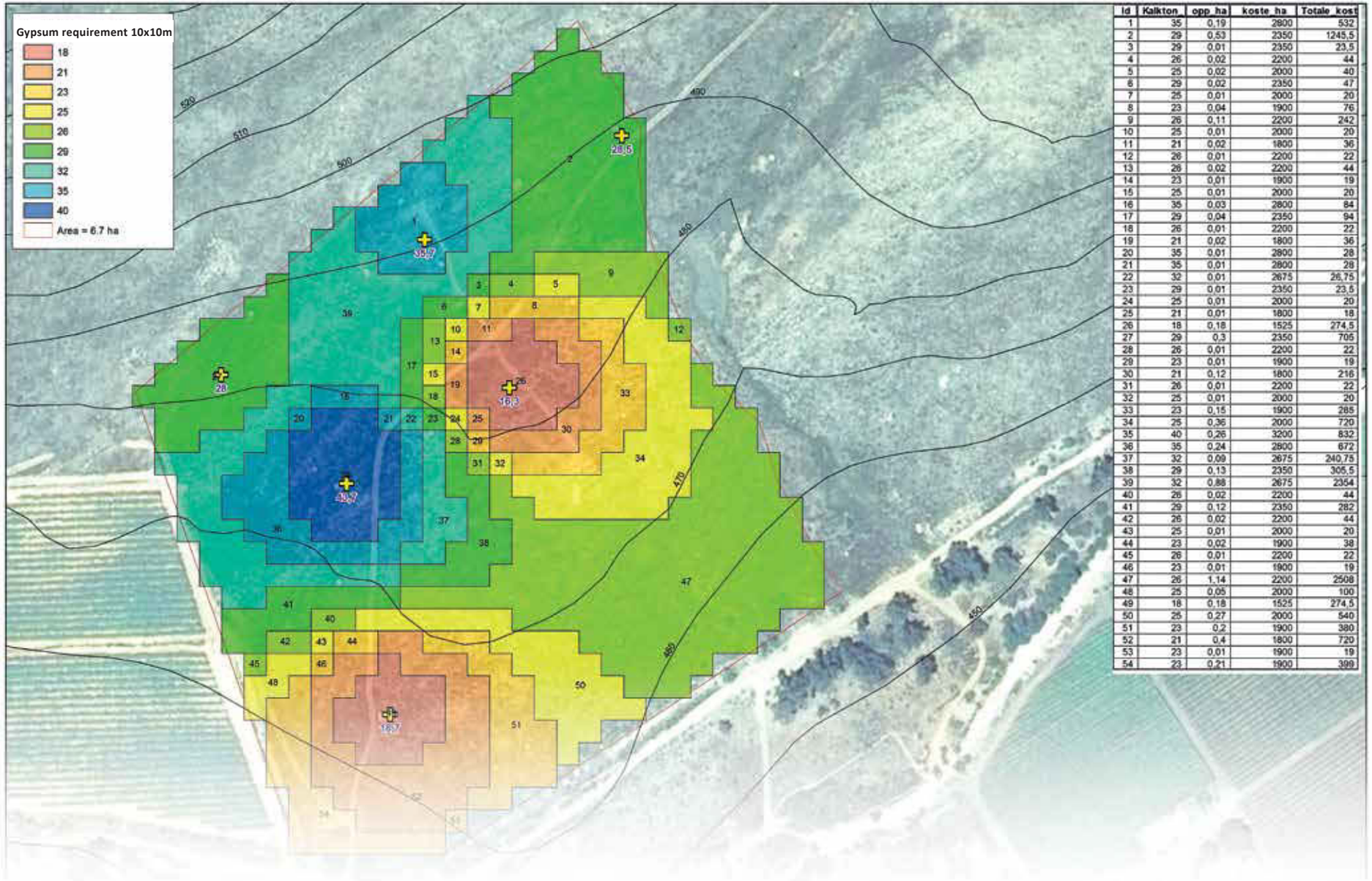
There are various ways to handle cover crops before the new growing season:

– Working them into the soil with a ghrop or disc plough so that they may serve as a soil fertiliser (Photo 8).
– Spraying them with a chemical herbicide at the beginning of the growing season and flattening or cutting them so that plant residues remain on the surface to obtain maximum moisture retention (Photo 9).
– Destroying them mechanically with a disc plough, slasher or rotovator without working them into the soil.
– Keeping them short using a slasher.
– Leaving cover crops or natural weeds to grow in the vineyards and die naturally.
– Ideally cover crops seed before budding, then die naturally and germinate and grow the following winter.

Problems that may be encountered with cover crops:

– The carpet of dead plant residues from the previous year is destroyed when the seedbed is prepared to sow the following year's cover crop (a cover crop that re-establishes itself is ideal).
– A cover crop cannot be established successfully using drip irrigation, because moisture distribution to the middle of the row is insufficient.
– In winter cover crops offer snails a place to hide while serving as a source of nutrition.
– When switching from clean cultivation to minimum cultivation, there may be a slight decline in yield, but this may be rectified with additional fertilising (70 kg N/ha per annum).
– If cover crops are sown at an early stage, germination problems may be experienced in high trellising systems (gables and factory roof) as a result of overshadowing.
– Problematic grasses or weeds will not be suppressed by cover crops.
– Certain cover crops exude toxic substances which may impede the growth of grapevine roots.
– The large quantity of dry material creates a fire hazard in the dry summer months.

For more information contact Kahné Ross at kachne@vinpro.co.za.



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52	21	0,4	1800	720
53	23	0,01	1900	19
54	23	0,21	1900	399

Precision application of ameliorants

JUNE 2016

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KEYWORDS: GIS, ameliorants, soil, GPS.

By using GIS technology, ameliorants may be applied more precisely within a block, which may achieve considerable benefits for precision viticulture and entail cost savings.

The application of ameliorants (fertilisers) is traditionally managed at block level. Different soil samples from a block are combined, whereafter the average chemical composition of the specific block is obtained. Depending on the chemical demand and surface, the spreader is calibrated at a fixed application rate for the entire block. In this way the block is fertilised evenly, without taking into account chemical variation within the block.

This method is only effective if the block's physical and chemical soil composition is homogenous, which is seldom the case, especially in the Western Cape where the soil can differ physically and chemically over very short distances, even within a block.

A selective spreader.

The correct application of ameliorants is a science which is currently not commonly applied in the wine industry. Automated spreaders are able to vary the application rate using GPS technology and a calculated application template. This template is built within a GIS system once a soil scientist has taken soil samples using a GPS apparatus. The placement of soil samples can be based on soil maps or infrared aerial images that indicate the natural variation within the block.

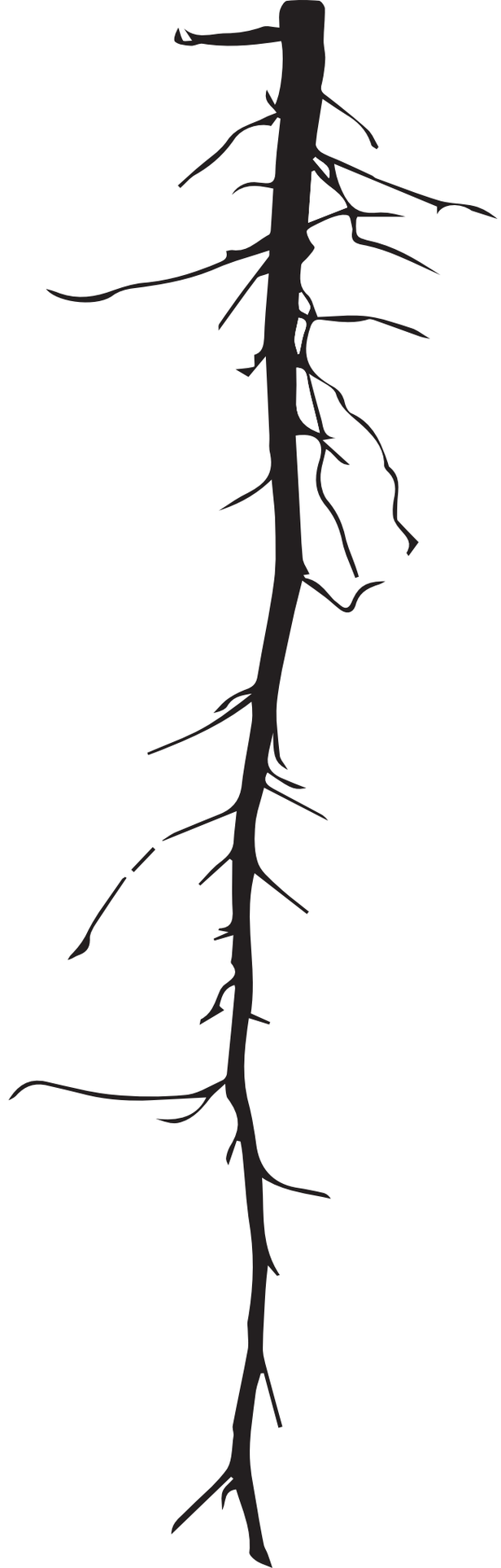
The fertilisation recommendation is calculated per point and the values interpolated to draw up a fertilisation template. The ameliorant template is used to calculate the total amount of fertiliser to be applied. Subsequently the fertilisation template is sent to the application contractor, who loads it onto his GPS. While the contractor drives up and down with the spreader, the GPS is continuously communicating with the variable applicator to adjust the application according to the template.

Large amounts of ameliorants can be saved since only the correct amounts are applied according to the specific requirements.

The end product is a tract of land where the chemical variation is now much more homogeneous and excessive, as well as insufficient fertilisation is eliminated. In this way yields can be considerably increased, seeing that it is easier to achieve the optimal nutritional status of the soil.

Selective application companies such as Agri-Balance may be contacted to do the spreading.

For more information contact Heinrich Schloms at heinrich@vinpro.co.za.



Planting of grapevines

JUNE 2016

JACQUES FERREIRA
Plant Improvement South Africa, Paarl
KEYWORDS: Plant, grapevines, graft, nursery.

Jacques Ferreira provides guidelines for the planting of grapevines.

PLANNING

- Plan ahead and get advice from your consultant/ viticulturist to plant the most suitable graft combination of a chosen variety for a specific soil type.
- Make an attempt to place your order for grafted vines at least 18 months before the grapevines have to be planted, and insist on certified vines.
- Certified vines are only available from nurseries registered with the DPA (table and raisin grapes) and VIA (wine grapes) and are indicated with a blue or candidate certification label (Fig. 1).
- Follow up on your order every three months and confirm that the correct quantities of each graft combination have been grafted and can be delivered.
- Visit the nursery towards the end of the growing season to take a look at the overall condition of the nursery.

COLLECTION OF GRAPEVINES

- Nursery vines should preferably be in a totally dormant condition when collected from the nursery.
- Nursery vines should be handled as little as possible, stored as briefly as possible, and planted as soon as possible.
- Make prior arrangements with the nursery for the collection of the vines.

NB: Some nurseries practice warm water treatment (WWT, namely 50°C for 45 minutes) on grapevines. In such instances the nursery is obliged to inform you about this so that the vines may be collected and planted as soon as possible. WWT stimulates bud burst in vines and bare roots increase the risk of drying out.

- Make sure that:
  - Each bundle of vines has been labelled properly.
  - Information regarding cultivar, clone, nursery certificate and status is correct (Fig. 2).
  - The number of vines tallies with your order.
- During transport the nursery vines should be kept moist and the soil should preferably not be washed from the roots.
- Take precautions to prevent the vines from drying out by:
  - Covering them with a tarpaulin if they are transported in an open truck.
  - Avoiding transportation of the vines on hot days.

ARRIVAL ON THE FARM

- Upon receipt ensure that the nursery vines comply with the minimum physical requirements for grafted vines, as prescribed in terms of the SA Plant

FIGURE 1. A certification label.

FIGURE 2. Certification labels should be controlled.

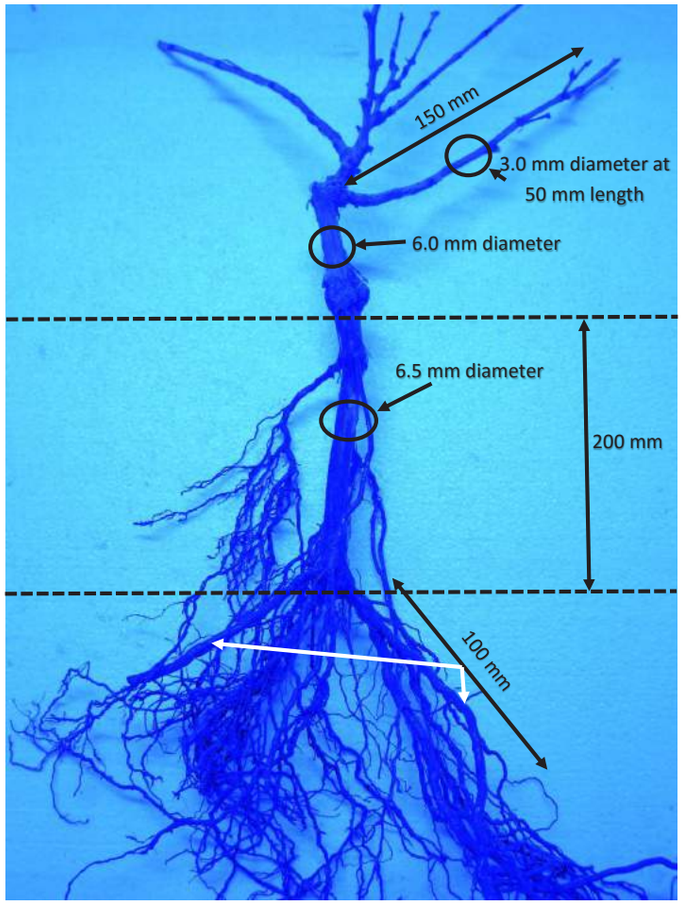
Certification Scheme for Wine or Table Grapes (Fig. 3). If the vines do not comply, follow up immediately with the nursery.

- For control purposes it is recommended to keep photos of each graft combination vines and the certification labels on record. This will improve traceability in the event of possible disputes and complaints.

STORAGE BEFORE PLANTING

- Nursery vines that underwent WWT must be planted out as soon as possible, because the treatment stimulates bud burst in the vines and the bare roots increase the risk of drying out.
- Nursery vines that are not planted out immediately, can be stored in airtight plastic bags in a cold room for a few months.
  - Maintain temperatures of 1 to 4°C with relative humidity (RH) 70 to 80% and take the necessary precautions against fungal infections.
  - Nursery vines must be left steeped in water for at least 24 hours before planting with the water surface level above the graft joint, but below the buds to prevent them from rotting.
  - If the vines have been in a cold room for more than six months, they should be left to stand in water for at least four days.
  - An increase in temperature after cold storage stimulates bud burst in vines and they should therefore be planted out as soon as possible.
- Nursery vines that are left in the soil should preferably be placed in shaded sandy soil.

Physical requirements for grafted table and wine grape vines



- Diameter of scion shoot = 3.0 mm at 50 mm length in the case of all varieties.
- Permitted tolerance: 3% of all the vines in consignment.

FIGURE 3. Physical requirements for grafted table and wine grape vines.

  - Sand must be washed between the vines with water and the graft joints should also be covered with sand.
  - Vines should be kept moist at all times.
  - Sites should be well-drained – avoid drowned or saline soil.

DURING PLANTING

- Plant the vines before the buds start swelling and before bud burst.
- Do not plant vines in soils that are too wet or too dry. No free water should occur in the root zone, because the roots will die back due to oxygen deficit and rotting.
- Do not take a large amount of vines from the soil where they have been laid in or from the cold room, in view of the fact that drying out is a big risk. Only take

out enough vines to be planted comfortably in one day. As far as possible vines should not be placed back into the sandy soil or cold room.

- Vines that come from a site where they have been laid in, should be steeped in water for at least six hours before planting – follow the method above.
- Pruning back of shoots before or during planting entails a significant risk of drying out and pathogen infections and is not recommended. If this is nevertheless done, apply wound sealant.
- If vines are trimmed back to one shoot or four buds, a mound of soil should cover the graft joint to prevent the vines from drying out.
- Vines should be kept in moist bags on the ground and not be allowed to dry out at any stage.
- Plant holes should be large and deep so that the vines may be planted without having to trim the roots back too much. A large part of the vine's reserves are in the roots and therefore they should not be trimmed shorter than 20 cm.
- If the plant holes have smooth walls, these should be loosened with a fork to improve root distribution.
- Plant vines so that the roots all point downwards and with the graft joint approximately 50 mm above the soil surface on even soils and 100 mm above the soil surface on uneven soils.
- Fill the plant hole with fine topsoil and trample down the soil.
- Fill the plant hole with water or irrigate to bring the roots properly into contact with the soil, but in certain soils care should be taken to avoid excess water.
- Fill the plant hole with topsoil.
- No fertiliser, manure, lime, phosphate or gypsum must be applied to the plant hole. All supplementary or remedial fertilisation should be done before or during soil preparation.

ALTERNATIVE PLANTING METHODS

- If one shoot remains, trim it back to four buds if bud burst occurs in the top bud.
- If vines are planted early in the season, they should first be pruned back when bud burst occurs in the top buds.
- If shoots are trimmed back, wound sealant should be applied.
- If vines are planted late in the season, plant as is and do not trim back at all.
- Vines that have undergone warm water treatment should be planted as is and only trimmed back if the top bud bursts.

POST-PLANTING

- In cases where plastic is laid and cut back earlier, the soil should not be too wet or too dry. If planting on plastic, special precautions should be taken against drying out with a small mound above the soil covering the graft joint.
- To prevent drying out of the graft joint and root, the vines should be irrigated judiciously.
- In the case of drip irrigation, ensure that the root zone of the young vines is moistened effectively.

AFTERCARE

- When bud burst starts, precautions should be taken against weevils and other pest infestations and disease infections.
- In areas where strong winds prevail during the growing season, ringbark often occurs on sandy soil at the surface of the soil.
- Mealybugs that feed on the bark just below the soil surface may also cause ringbarking in young vines.
- Young planted vines should be protected against feeding damage by antelopes. Do not use fresh (white) straw, because this may lead to sunburn damage.
- Weed control should be undertaken regularly to avoid competition with the young vines.
- Replacement vines should preferably be planted in plant bags so as to achieve better success with replacement.
- If vines have to be replaced at a later stage, make the plant holes double the size of the original holes and still follow all the above-mentioned planting guidelines.

POSSIBLE PROBLEMS AFTER PLANTING

- After planting a young vineyard, any problems must be detected as soon as possible.
- Report problems directly to the nursery, at an early stage, otherwise follow the VIA/DPA complaints procedure.
- If the problem cannot be ascribed to the quality of the nursery vines, advice should be sought from a viticulturist.

For more information contact Jacques Ferreira at jacques@plantsa.co.za or visit www.plantsa.co.za.

PHOTO 1. An example of a grapevine with Eutypa dieback.

Wound protection in vineyards

AUGUST 2016

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KEYWORDS: Wound, Eutypa dieback, sealant, Trichoderma.

During winter pruning plenty of pruning wounds are inflicted on grapevines as a result of manual and/or mechanical pruning. This also includes large wounds where arms are pruned back for renewal. When grapevines are sawed off, the wounds are even larger. Such wounds allow ingress to all the important trunk pathogens which cause premature decline in grapevines by growing in the xylem vessels thereby impeding sap flow. One of the best known and most common trunk diseases is Eutypa dieback.

CONTROL AIDS

PREVENTION OF INFECTION/WOUND PROTECTION

- Pruning wounds can be painted with a wound sealant immediately after pruning.
- Pruning wounds can be painted or sprayed with a biological control agent immediately after pruning. Several products that contain Trichoderma are available.
- Chemical products such as benomyl and flusilazole are effective for a short period after being painted or sprayed onto the pruning wounds, but lose their effectiveness quickly, especially when it rains.
- Do not prune when it rains, in view of the fact that spores which infect pruning wounds are released at such times.

PHOTO 2. A cross section of a cordon arm with typical symptoms of Eutypa dieback.

PHOTO 3. A large wound caused by arm renewal.

PHOTO 4. Grapevines that are sawn off have large wounds.

WOUND SEALANTS

- Apply with a brush, sponge or aerosol can to fresh wounds (bigger than a 20 cent coin) as soon as possible after pruning.
- After application sufficient time should be allowed for the product to dry thoroughly, otherwise it will be rinsed off by rain showers.
- Wash brushes and sponges thoroughly in cold water after each use.
- The person making the renewal cuts should keep a container with wound sealant and a brush/sponge on hand.
- Several products are available for these purposes.

BIOLOGICAL CONTROL

TRICHODERMA

- Trichoderma species are fungi that occur naturally in soil, rotting wood, compost, roots and in plants above the surface of the soil.
- Trichoderma can be selected as control agents for certain fungal pathogens. They produce toxins and/or enzymes that control the specific pathogen.
- Trichoderma are living organisms and available as powders and granules.
- Follow the storage instructions on the label and do not expose to direct sunlight. A new Trichoderma solution must be prepared each day.
- Use a brush or sponge to paint large wounds with a Trichoderma paste. Grapevines that have been pruned already, can be sprayed with a Trichoderma solution.
- Trichoderma colonise (grow) so quickly that they eliminate trunk pathogens on the pruning wounds.

ACKNOWLEDGEMENTS

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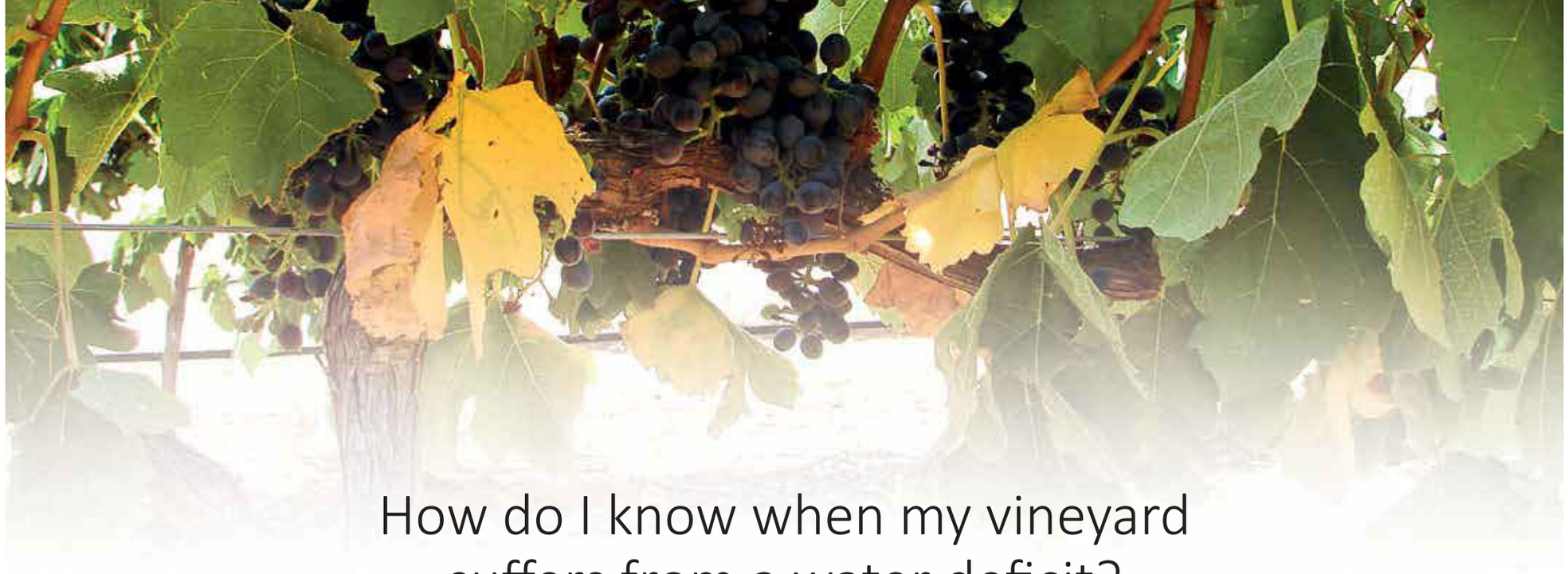
Dr. Brendon Neumann, Madumbi Sustainable Agriculture.

For more information contact Pierre Snyman at pierre@vinpro.co.za.



How do I know when my vineyard suffers from a water deficit?

OCTOBER 2016

LEON DIPPENAAR
VinPro, Breedekloof
KEYWORDS: Water deficit, soil moisture.

It is important to monitor water deficits and soil moisture in vineyards.

An actively growing vineyard which does not suffer from any water deficit (Photo 1) is characterised by the following traits: The growth tip is active and grows above the closest, young, unfurled leaves. The tendrils are active and the top tendril on the shoot extends above the growth tip. The leaves are perpendicular to the angle of the sun's incidence; they are glossy and dark green in colour.

Water deficits or moisture stress in a vineyard can be divided into four stages roughly; generally you do not want a vineyard to progress beyond stage three. Remember, these observations are mostly applicable to the pre-*véraison* period. After *véraison* it is better to rely on soil moisture measurements.

PHOTO 1. An actively growing vineyard.

THE FOUR STAGES OF WATER DEFICIT ARE: STAGE ONE

The growth tip is still growing actively and the closest unfurled leaves remain below the growth tip. The leaves are still at a perpendicular angle to the incidence of the sun, but the tendrils on the bottom half of the shoot are beginning to droop.

PHOTO 2. Stage one of water deficit.

STAGE TWO

At this stage the growth tip is less active and the closest unfurled leaves are beginning to fold around the growth tip. The tendrils that have not attached to anything are beginning to dry out from the bottom half of the shoot. The tendrils nearest to the growth tip are beginning to droop. A small percentage of the leaves start turning away from the sun and tend to have a dull green sheen.

PHOTO 3. Stage two of water deficit.

STAGE THREE

At this stage the growth tip is yellow to brown in colour and no longer growing actively. The nearest unfurled leaves envelop the growth tip entirely. The tendrils near the growth tip are drooping and detach fairly easily if touched. A bigger percentage of leaves start turning away from the sun and a small percentage of yellow leaves are present.

PHOTO 4. Stage three of water deficit.

STAGE FOUR

At this stage the growth tip is dead or detaches easily if touched. All tendrils that have not attached, dry out and fall off. The leaves on the bottom half of the shoot turn yellow and start falling off. The remaining leaves have a dull green colour, feel leathery to the touch and are turned away from direct sun. At this stage the shoots start showing signs of ripeness.

PHOTO 5. Stage four of water deficit.

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4 In the Cellar

The colour of wine is an important specification requirement.

Blending and mixing of wine (Part 1)

JANUARY 2016

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KEYWORDS: Blending, mixing.

Wine cellars can only be financially successful if they sell wine profitably and wine can only be sold if it complies with the requirements of the consumer. The consumer requirements of a product can be measured according to the specifications and the aim of wine blending is consequently to comply with the product specifications.

To blend wines successfully, analyses, legal, local export and foreign import, as well as quality management, consumer and taste requirements need to be complied with.

Analyses requirements include concentrations like alcohol, sugar, total acid (TA), volatile acidity (VA), sulphur dioxide, and pressure (in the case of perlé and sparkling wines) and are usually seen as basic wine analyses. These analyses will differ for different products and blends.

Apart from above-mentioned analyses requirements, local legal analyses requirements also need to be complied with. These requirements are usually published as regulations in the Government Gazette and its compliance is controlled by the South African Wine Industry Information and Systems (Sawis) and the Department of Agriculture. It usually includes minimum and maximum concentrations of alcohol, sugar, volatile acidity (VA), sulphur dioxide and pressure.

Local alcohol requirements (% per volume)
Natural wines (unfortified wines)
Like white, rosé, red, perlé and sparkling wines must have an alcohol concentration between 4.5 and 16.5.
Fortified wines (wines with added alcohol)
Like jerepigos, port and sherry must have an alcohol concentration between 15.0 and 22.0.

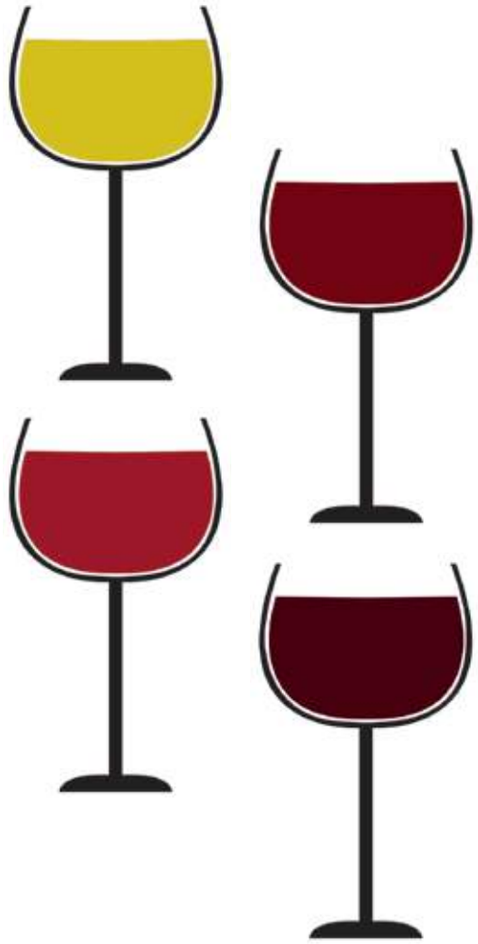
Local pressure requirements (kilopascal) (kPa) Most motor tyres have a pressure of 200 kPa
Perlé wine: 75 to 300.
Sparkling wines: More than 300.

Local sugar requirements Residual sugar (RS) in g/ℓ: Total acid (TA) in g/ℓ
Extra dry**:** 2.5 or less.
Dry: 5.0 or less or 9.0 or less, if TA is less than 2 lower than the RS.
Off dry: More than 5.0, but equal to or less than 12.0 or 18.0 or less, if the TA is less than 10 lower than the RS.
Semi-sweet: More than 5.0, but less than 30.0.
Late harvest: 20 or more.
Noble late harvest: More than 50.
Sweet natural: More than 20.

Local volatile acidity (VA) requirements (g/ℓ)
Noble late harvest and wine from sundried grapes: 1.8 or less.
Bulk wine for export: 0.8 or less.
Other wine: 1.2 or less.

Local total sulphur dioxide requirements (mg/ℓ or ppm)
Red wine with RS less than 5: 150 or less.
White wine with RS less than 5: 160 or less.
Natural wine with RS more than 5: 200 or less.
Noble late harvest and wine from sundried grapes: 300 or less.

If wine is sold with indications of the grape cultivar used for it, the origin of the used grape or the vintage grapes which were used must, in addition to the above analytical requirements, also comply with certain composition percentages. These composition requirements are prescribed by the Wine of Origin scheme.



Cultivar, origin and vintage requirements (%)
Cultivar: At least 85% if a single cultivar is indicated.
Origin (region, district, ward, estate or single vineyard): 100% of the indicated origin.
Vintage: At least 85% if a single vintage is indicated.

In order to export local wines, an export certificate of the Department of Agriculture is required and the countries exported to, also have certain requirements to be complied with.

Although all the above requirements can be complied with, the consumer will only buy the wine if it meets their expectations. Different factors like the wine type (white, red, perlé or sparkling), the age of the wine, wood character, sugar-acid ratio, sweetness degree and consistent quality, will determine whether the consumer likes the wine and will possibly buy it again.

The blending of wine does not only consist of the mixing of wines, but is a well-planned execution to ensure that above-mentioned requirements are met. It is consequently one of the most important tasks of a winemaker.

The colour of wine is an important specification requirement.

Blending and mixing of wine (Part 2)

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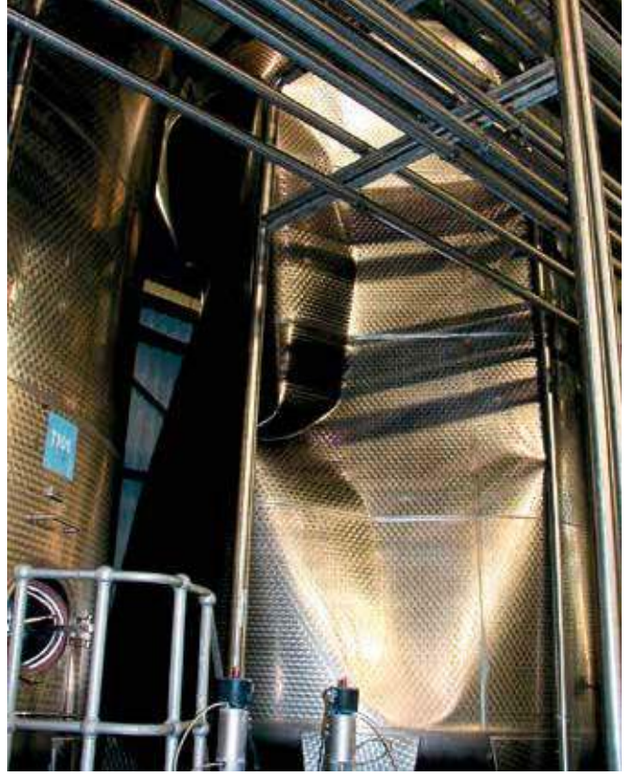
KEYWORDS: Blending, mixing, transfer.

The blending of wines is the mixing of different wines from different containers according to specifications (like alcohol and sugar concentrations) which were identified for different products. The processes of wine blending can be divided into different stages. Firstly, the quantities of the different wines must be calculated. The blend must then be mixed in small scale in the laboratory, where after it is evaluated and the necessary adjustments are made to the blend. Usually a fining (like gelatine or bentonite), which is also applied in small scale in the laboratory, is required. After the pilot blend has been approved, a work instruction will be compiled by the winemaker. The following particulars will be included in such work instruction:

- The number, wine description and quantity of wine that will be used from each tank.
- The number and capacity of the receiving tank(s) where the wines will be mixed, as well as the cleaning process of these tanks, before it is used.
- The sequence in which the different containers will be transferred to the receiving tank(s).
- The expected wine quantities in the receiving tank(s).
- Which pumps will be used for the transfer and whether the mixing will be done by pump overs or mixers.

Tank covers must be open before wine transfers.



An imploded tank.

- Which samples must be drawn after the mixing for tasting and analysis (for example alcohol content, volatile acid concentration, sulphur dioxide concentration, acid concentration, sugar concentration and pH).
- Adjustments after the analysis by adding for example acid and sulphur dioxide and mixing it properly with the wine.
- Additions of fining agents (for example bentonite and gelatine) and how these must be prepared and mixed with the wine.
- How (for example by rackings or filtration) the wine will be clarified after mixing, additions to be made, and to which tanks the clarified wines should be transferred.
- Which samples should be drawn again for tasting and the necessary analytical purposes.
- All these actions of the work instruction must also be documented and recorded.

After the work instruction has been received, preparations must be made to execute it and it is especially important to attend to the following:

- Ensure that the container numbers, wine detail and volumes of the wines to be mixed are correct.
- Taste the wines in the different tanks to ensure that they correspond with the details of the work instruction.
- Control whether the volume capacity of the receiving tanks is sufficient to accommodate the wine and control whether these tanks are empty and clean and no equipment like lights, valves or gaskets were left inside.
- Ensure that the tank covers of all tanks are open, before the pumping of the wine is commenced. If this is not done, the delivery tank can implode.
- The delivery tank and receiving tank(s) can be connected by fixed pipelines or wine hoses which were cleaned beforehand. Wine hoses are usually fixed to the racking valve of the supply tank and the bottom valve of the receiving tank. The pump must be as close as possible to the supply tank, where it is fixed with a flexible hose to the racking valve. The hose must be long enough to bend into the doorway, if the wine is racked from lees.
- If air contact needs to be avoided, the fixed pipelines, wine hoses and receiving tank(s) can be flushed with inert gasses like nitrogen, carbon dioxide (dry ice) or argon. This can be done by connecting the hoses to the pump, opening the bypass of the pump and flushing the line until inert gas has reached the end of it. The hose can then be connected to the bottom valve of the receiving tank to flush it also. It is preferable to put dry ice in both tanks while the wine is transferred.
- To ensure that no leakage occurs and valves are open, it is advised to attach all the hoses, open the bypass of the pump and then without starting the pump, open the valve of the supply tank. The flow of the wine can consequently be followed and potential problems can be identified and solved.
- If barrels need to be mixed, it is always better to evaluate the barrels individually, before they are mixed.

During the transfer of wines no leaks should occur and sound hoses and tight fittings must be used. Damaged parts of leaking hoses can host undesirable micro-organisms, which can spoil the wine. After the transfer action has been completed and the pump was switched off, all tank valves must be closed and the hoses loosened and drained. All equipment, containers and hoses must therefore be cleaned according to the cellar's work instructions.

Addition of exogenous tannins during winemaking

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KEYWORDS: Tannins, astringency.

Tannins are especially associated with their astringent taste. The perception of astringency is the result of the reaction between tannins and the proteins in saliva. Tannic red wines form a precipitate in the mouth, but due to the lack of tannins in white wines the precipitation will not occur. The tannins occurring in wine, can originate from the grapes or the stems, but they can also be added as exogenous tannins during winemaking.

Tannins are phenolic compounds that occur in many plants. The ability of tannins to react with proteins, is important during winemaking, because enzymatic reactions are inhibited by them and they can also contribute positively to the protein stability of wine. As result of the structural characteristics, they can also contribute to the oxidative and colour stability of wines. They also play an important role in the sensory characteristics of wine. A variety of tannin products has become available over recent years and it is important that winemakers are informed about the characteristics of the different products. Available tannins include hydrolysable tannins (ellagic and gallic basis) and condensed tannins (catechinic basis). Ellagic tannins originate from oak and chestnut wood, gallic tannins from oak wood wounds, tara and myrobalan exotic trees, while catechinic tannins originate from grapes and quebracho trees. The traditional tannins for winemaking (exogenous tannins) are extracted from the nutgalls of certain trees.

The most important effect of tannins added to wines, is the structuring of the mouth feel. Together with the alcohol and different fixed acids, they contribute to the balance of dry wines. If exogenous tannins are used, they must be used from the commencing of the alcoholic fermentation and during maturation. As result of the differences in the composition, different tannin products have different effects and winemakers must consider this before using a specific product. Recommendations regarding different exogenous tannins are summarised in Table 1.

TABLE 1. Recommendations regarding the use of different exogenous tannins during winemaking.

Problem	Desirable effect of tannin addition	Recommended tannin base	Timing and application
Spoiled grapes. Juice oxidation.	Minimise loss of natural polyphenols by reacting with O_2 and free radicals.	Gallic tannins.	Prior to alcoholic fermentation to react with oxygen.
		Split addition of ellagic or ellagic/gallic and catechinic mix. Initial addition at crushing and next at pressing of skins.	During winemaking tannin react with O_2. Mixed tannin will scavenge O_2 and enable binding of liberated anthocyanins.
		Catechinic tannin.	During pressing of skins or prior to maturation. React with proteins.
Unripe grapes lacking anthocyanins and potential of bitter and astringent tannins.	Preserve anthocyanins and soften tannins.	Combination of ellagic oak and catechinic tannins.	During winemaking ellagic tannin will add structure and bind green tannins. It will also initiate tannin-anthocyanin condensation.
		Gallic, ellagic or catechinic tannins.	Prior to MLF skin tannin will improve middle palate and soften harsh acidity. The addition of seed tannins will focus more on back palate structure.
Young wines with unstable colour and subtle tannins.	Stabilise colour before MLF and improve tannin potential.	Ellagic tannins.	Best tannins to bind free anthocyanins, so should be added before completion of MLF.
		Toasted ellagic or catechinic tannins.	Toasted ellagic or seed tannins for back palate structure. Skin tannins improve middle palate thus suitable if extended maceration is impossible.
Young wines with an astringent taste.	Balance the natural phenolic character of the wine.	Catechinic tannins.	Added during maturation or to improve the middle palate, unbalanced acidity and astringency.

The activity of different available tannins can differ considerably. Highly active tannins are used at a lower dosage and will have less impact on the sensory characteristics of wine, contrary to the effects of products with a lower activity. It is consequently important to execute laboratory evaluations before adding tannins to wine (Gore, 2015).

REFERENCE

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Microbial monitoring and sanitation practices in wine cellars

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KEYWORDS: Microbes, sanitation.

Proper sanitation practices in wine cellars prevent the development of undesirable micro-organisms. Although the alcohol concentration of wines limits the microbial spoilage thereof, in comparison with fruit juices and food, certain harmful micro-organisms can cause considerable sensory problems in wines. During the winemaking process favourable conditions must also be created for certain micro-organisms, without also favouring the undesirable micro-organisms.

Micro-organisms occur everywhere in a cellar and can mainly be divided into three groups, namely moulds, bacteria and yeasts. Sources of the different micro-organisms include the grapes, harvesting equipment, cellar equipment and tools, hoses, barrels, sampling equipment, corks and bottling lines. The mould *Botrytis cinerea* which occurs in rotten grapes, can cause premature oxidation in grape juice, but can also as noble rot contribute to the making of noble late harvest wines.

The most commonly known bacteria are acetic acid bacteria and lactic acid bacteria. Acetic acid bacteria like *Acetobacter* can cause high volatile acid concentrations. Lactic acid bacteria (LAB) include *Oenococcus*, *Pediococcus* and *Lactobacillus* and can convert malic acid to lactic acid during malolactic fermentation (MLF). *Lactobacillus* can however also cause a mousy flavour in wine, which cannot be removed easily. *Pediococcus* can cause ropiness in wine which will spoil it visually. Diacetyl, which has a buttery flavour, can in excess lead to a rancid character and unhealthy biogenic amines with off-flavours, can also be formed during MLF.

The most-used yeast is *Saccharomyces* species, although some winemakers prefer spontaneous fermentation to create unique characteristics in their wines. The results of the spontaneous yeasts are however unknown and can also deplete the nutrients in the juice, which can limit the fermentation of *Saccharomyces* yeasts. The wild yeasts can also contribute to the undesired formation of volatile acid, ethyl acetate and aldehydes and *Zygosaccharomyces*, *Picchia* and *Candida* yeasts have a resistance against SO_2. *Brettanomyces bruxellensis* is however the most important spoilage yeast and can form off-flavours like wet dog and band-aid. It

Unacceptable sanitation.

Acceptable sanitation.

requires few nutrients and can for example utilise the sugars occurring in toasted barrels. It is also difficult to prevent, because it can have resistance against low pH, high alcohol and SO_2.

The growth of micro-organisms is promoted by factors like available sugar, malic acid, nitrogen nutrients, pH's higher than 3.6, high temperatures and available oxygen. Their growth is however limited by SO_2, ethanol, pH's lower than 3.6 and low temperatures. In order to maintain sound cellar hygiene, it is advisable to keep the cellar temperature below 15°C and maintain the pH of wines below 3.6.

In order to manage the sanitation status of a cellar properly, it is essential to test for the presence of micro-organisms and monitor their status. It is recommended that the stage between alcoholic fermentation and malolactic fermentation (MLF) during maturation until the blending of wines and the stage prior to bottling, are seen as the three most important critical control points during winemaking. Tests must obviously also be executed if fermentation problems occur or off-flavours are identified. The monitoring and detection of micro-organisms are of cardinal importance to prevent spoilage. General techniques that are used to do this, are microscopic investigation, microbiological culture plating or the use of laboratory equipment, which uses PCR (polymerase chain reaction) for analyses. Luminometers are bioluminescence equipment used to detect the presence of ATP (adenosine triphosphate). ATP is energy molecules, which occur in all living organisms. The analysis of swab samples taken from cellar surfaces, is consequently a good indication of the micro flora population, although specific organisms cannot be identified. It is consequently a rapid method to monitor the efficiency of cleaning and sanitation practices.

Effective sanitation also includes the prevention of microbiological distribution. Seeing that micro-organisms are not locality bound and can occur anywhere, it can be distributed by cellar workers and wine flies. The maintaining of workers' hygiene and limitation of wine flies are consequently essential supporting actions of cellar sanitation. It is important to distinguish between cleaning and sanitation. Cleaning is the removal of soil and solid residue, while sanitation is the decreasing of micro-organisms.

The cleaning and sanitation procedures can consequently be divided in five steps:

- A pre-rinse action to loosen solid residue.
- The application of cleaning agents and a scrubbing exercise.
- The rinsing of the cleaning agent.
- The application of the sanitiser.
- The rinsing of the sanitiser (in some cases unnecessary).

The execution of effective cleaning and sanitation procedures will prevent the formation of biofilms. Biofilms in the cellar, which are layers of organic matter of slime or moulds on cellar or equipment surfaces, or mineral or tartrate precipitation on tanks and barrels, can be an important source of microbiological contamination.

Cleaning agents can be surface active and have either an acid base (for the cleaning of mineral precipitates) or an alkaline base (for the cleaning of tartrates). It can be applied together with foaming, soaking, or spraying at high pressure. Heat is the base of most sanitation procedures, either as hot water at 77 to 85°C or steam.

The surface that needs to be sanitised and the focus organism will determine which one is used. The chemical sanitisers used in cellars, changed considerably over recent years. Chlorine products were very popular, but due to the problems with trichloro-anisole (TCA) it is not used anymore. Iodine products, quaternary ammonium products, acid-ionic combinations (like peracetic acid), hydrogen peroxide, ozone and ultraviolet are more frequently used nowadays. No sanitising agent is perfect and different aspects must be considered before a specific product is chosen (see the *Winetech Tegnies* article on the sanitation of barrels: September 2014). It is also important that cleaning and sanitising products are rotated at least weekly, because micro-organisms can develop product resistance. It must also be used at the right concentration to ensure efficiency, but unnecessary high concentrations are a waste of money and safety and regulatory prescriptions can be exceeded.

The sanitation of barrels and bottling lines require specific attention and both are critical points to prevent contamination and later losses. *Brettanomyces* can penetrate barrels up to 8 mm and heavy toasted barrels are more susceptible to contamination by it. Even new barrels must also be treated before use to prevent contamination.

The following procedures can be used as guidelines for the sanitation of barrels:

- Barrels must be cleaned before sanitation practices are applied.
- The burning of sulphur wicks, containing 5 g sulphur, is effective and has a long term influence. It must however be burnt completely and barrels must be sealed effectively during storage afterwards.
- The steaming of barrels for 10 minutes or water at 82°C is effective to a depth of 8 mm.
- Treatment with 1 ppm ozonised water for 10 minutes decreases the contamination of barrels.
- Peracetic acid, also known as peroxi-acetic acid (PAA), at a concentration of 120 ppm can decrease the contamination of *Brettanomyces* by 90% within one week.
- Chlorine dioxide is effective for stainless steel tanks, but not barrels.

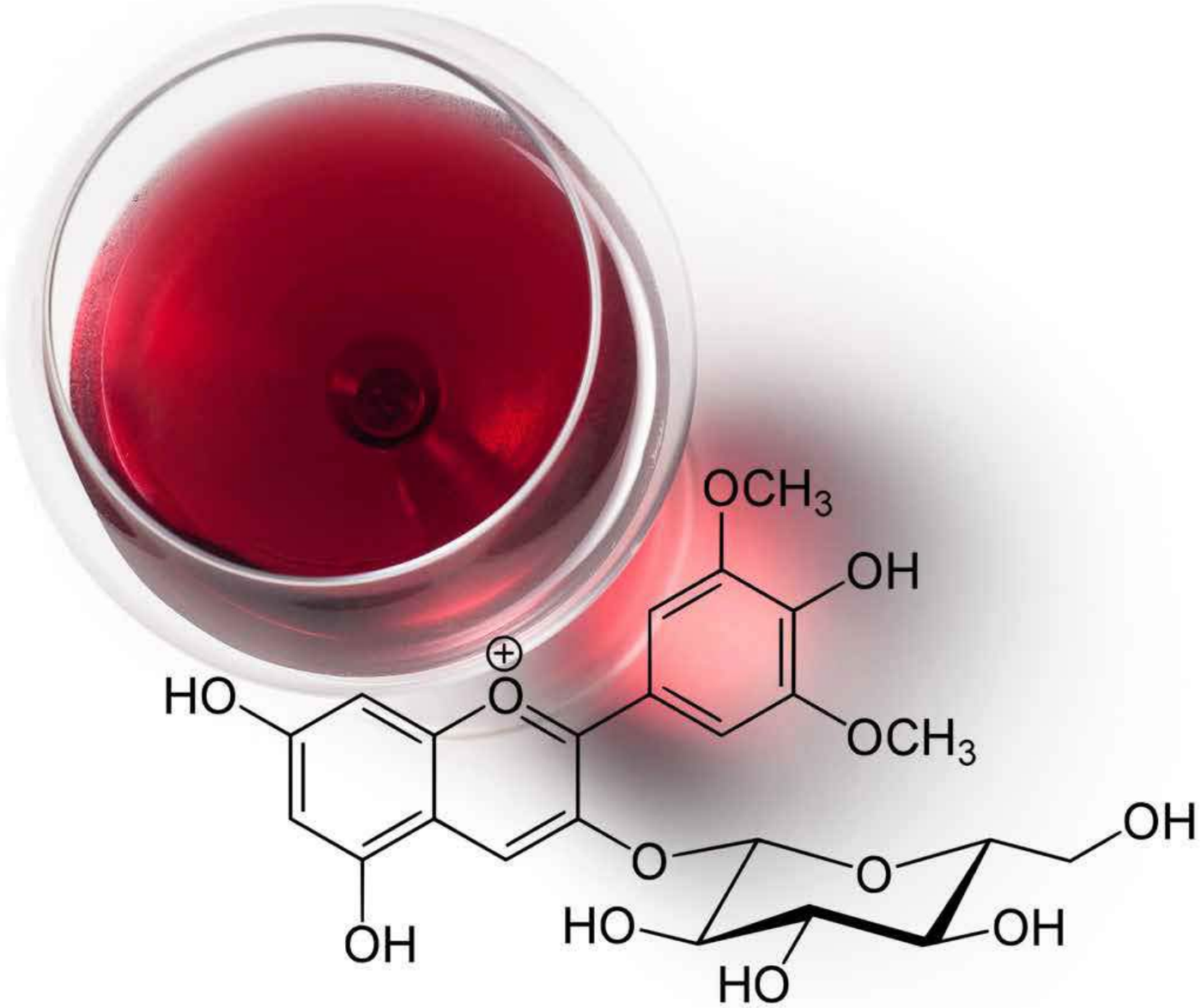
The cleaning and sanitation of sampling equipment are also important to prevent cross contamination. PAA, citric acid and alcohol can be used for it.

The sanitation and monitoring of the filling bowl and filling nozzles are the most important actions prior and during bottling. If mobile bottlers are used, it must be ensured that they apply effective sanitation and control before bottling commences to prevent contamination from other cellars.

Different anti-microbial products and processes can be used during the different stages of winemaking to prevent the development of microbes, decrease the micro population or eliminate it. Sulphur dioxide is probably the most used. It is however important that the molecular sulphur dioxide concentration is more than 0.8 ppm. Different factors determine it, but the pH of the wine is the most important factor. Lower pH increases the percentage. Chitosan is a polysaccharide extracted from *Aspergillus niger* and kills micro-organisms. Lysozyme, extracted from egg white, is effective against malolactic bacteria and is mainly used to prevent MLF or stabilise wine microbiologically after MLF. Dimethyldicarbonate (DMDC), also known as Velcorin, is very effective against yeasts and is mainly used to prevent secondary fermentation in bottled sweetish wines. Sterile filtration with membrane filters using a pore size smaller than 0.45 micron prior to bottling, is the final process to sterilise wine prior to bottling (Rieger, 2015).

REFERENCE

Rieger, Ted, 2015. Microbial monitoring and winery sanitation practices for quality control. *Wine Business Monthly*, October 2015: 42-46.



The influence of malolactic fermentation on the colour of red wines

MARCH 2016

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KEYWORDS: Malolactic fermentation, red wine.

The conversion of malic acid to the weaker lactic acid during malolactic fermentation (MLF) is not the only important change that occurs during this process. Other intermediate and co-products are also formed, which will have a secondary influence on the wine composition. The decrease in the colour is one such product.

Inoculation with pure MLF culture.

MLF causes lighter red wines.

Wine quality and its sensory description are determined by factors like flavour, taste and mouthfeel, but especially in the case of red wines the colour is also an important factor. This is especially applicable in the case of light coloured red wines like Pinot noir. Much research was consequently done to determine which viticultural and cellar practices will optimise the colour. The colour of red wines is not only determined by the anthocyanin concentration of the wine. After being extracted from the skins, the anthocyanins can react with different compounds in the wine to form more complex colour compounds. They may for example react with tannins to form polymeric pigments. This formation can be accelerated by reactions with acetaldehyde. Stable anthocyanin derivative products can also be formed by reactions with pyruvic acid. All these pigments are more resistant to oxidation and bleaching by sulphur dioxide (SO_2), and also tend to increase during wine maturation.

It is known that winemaking factors like fermentation temperature and extended maceration influence the formation of polymeric pigments. Yeast can also influence the colour of red wines by the adsorption of anthocyanins to their cell walls or by the formation of acetaldehyde and pyruvic acid. Yeast strains differ regarding the acetaldehyde concentration which is formed and will consequently have different effects on the colour of red wines. Other micro-organisms can also influence the colour of red wines. It is for example known that *Oenococcus oeni* decomposes acetaldehyde and pyruvic acid during MLF.

Winemakers have always experienced a decrease in the colour of red wines after MLF. It can partially be attributed to the pH increase during MLF, but the influence of *Oenococcus oeni* has never been researched intensively. Recent research in the USA tried to clarify the influence of MLF on the colour of Pinot noir. The wine was made with standard cellar practices and sterile filtered prior to MLF. After the filtration it was inoculated with three different *Oenococcus oeni* strains and compared with a non-inoculated control. After completion of MLF, different colour components were measured and the wine was also matured for nine months. Wine was also made with simultaneous alcoholic and malolactic fermentation. Lower colour (measured as absorbance at 520 nm) was observed in all the wines with MLF, in comparison with the control sample. The reduced colour persisted throughout ageing. The lower colour in the MLF wines corresponded with decreased polymeric pigment and higher concentrations of the more unstable monomeric anthocyanins. Analyses also confirmed that acetaldehyde and pyruvic acid are decomposed by *Oenococcus oeni* during MLF. When acetaldehyde was added to the wine after MLF, the colour difference between the control wine and MLF was smaller.

Winemakers believe that colour losses during MLF can be prevented by delaying the MLF. In the American research it was delayed for up to six months, but it did not have a significant influence on the colour loss (absorbance at 520 nm) during MLF. The polymeric pigment and monomeric anthocyanin concentration did however differ less from the control. It was most probably due to the acetaldehyde remaining in the wine for a longer period. From a practical point of view the delay of the MLF for several months can be risky, because SO_2 additions cannot be made before MLF is not completed. Wine must consequently be kept at a low temperature to minimise microbial growth (Osborne & Burns, 2015).

REFERENCE

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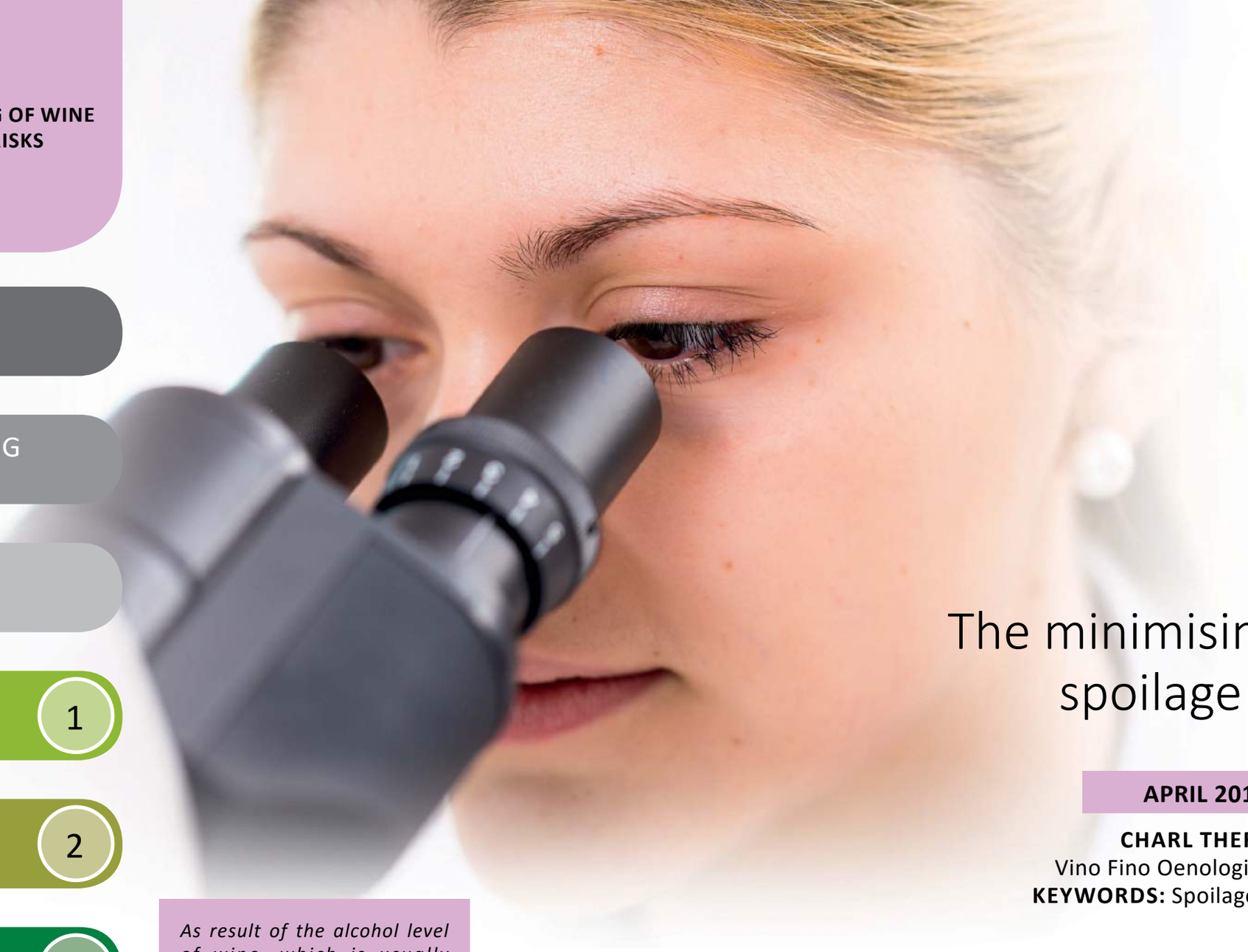

The minimising of wine spoilage risks

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KEYWORDS: Spoilage, organisms.

As result of the alcohol level of wine, which is usually more than 12% A/V, its pH which varies between 3 and 4 and the absence of sugar in dry wines, it is usually not so exposed to microbial spoilage as fruit juices and fresh products. The microflora of wine cellars consists of some organisms which proliferate under such conditions if the necessary precautions are not implemented.

The microflora occurring in wine cellars originate from grape berries, cellar equipment and the cellar environment. Some of these organisms are classified as spoilage organisms, because high volatile acid concentrations can be formed by them. The occurrence of microflora on cellar equipment is influenced by cellar sanitation. *Saccharomyces cerevisiae* is the yeast species that is mainly responsible for the alcoholic fermentation of spontaneous and inoculated fermentations. During the beginning of spontaneous fermentation, *Picchia*, *Candida*, *Hanseniaspora*, *Torulaspora*, *Hansenula*, *Issatchenkia*, *Metschnikowia*, *Kluyveromyces* and *Zygosaccharomyces* species can also contribute. Bacteria can be advantageous or not. In case of malolactic fermentation (MLF) it is essential, but *Acetobacter* species are generally defined as spoilage bacteria.

The chemical composition of wines plays an important role in its spoilage potential. The so-called low alcohol wines with alcohol levels as low as 4.5% A/V have increased in popularity and many of these wines also have a high residual sugar concentration, which typifies it as high risk wines from a spoilage perspective. Alcohol (ethanol) is a very good anti-microbial agent and some of the spoilage species, which are present during the beginning of alcoholic fermentation,

TABLE 1. The free SO_2 concentration required at different pHs to maintain 0.825 mg/ℓ molecular SO_2.

pH	Free SO_2 required (mg/ℓ)
3.20	22
3.30	27
3.40	33
3.50	41
3.60	51
3.70	65
3.80	81
3.90	102
4.00	129

cannot survive at alcohol concentrations higher than 6% A/V. This is also the reason why some pathogenic species are not a spoilage risk in alcoholic beverages.

In the case of low alcohol wines, *Candida*, *Saccharomyces* and *Zygosaccharomyces* species can metabolise sugar and form carbon dioxide. The use of dimethyldicarbonate (Velcorin) can prevent the secondary fermentation in bottles of low alcohol wines with high sugar concentrations. Sulphur dioxide, benzoic acid (illegal in South Africa) or sorbic acid can also be used in combination with low pH to prevent secondary fermentation in bottles.

Many micro-organisms prefer pHs higher than 4 and lower pH wines consequently have more resistance against spoilage. Wines with pH lower than 3.5 are preferred, because the percentage active molecular form of sulphur dioxide is also higher at a lower pH (see Table 1).

Irrespective of the form of sugar, whether it is glucose, fructose or sucrose, the presence thereof in wine can be problematic. The concentration of sugar in wine will determine which micro-organisms can metabolise it. Concentrations higher than 0.2 g/ℓ can promote the growth of *Brettanomyces bruxellensis.* This species can proliferate under the aerobic conditions of bottled wines and off-flavours like 4-ethylphenol and 4-ethylguajacol can be formed. *Zygocaccharomyces* species can cause problems at high alcohol concentrations. It is also resistant against preservatives like sulphur dioxide. *Zygosaccharomyces rouxii* is particularly osmotolerant and can cause fermentation in grape juice concentrate. *Zygosaccharomyces bailli* is resistant against preservatives and often a contaminant of food and beverages.

The process stage in wine cellars also plays an important role in spoilage risks, because conditions differ during the different processes. During the initial stage of alcoholic fermentation, most micro-organisms can proliferate as result of the high sugar and low alcohol concentrations. Spontaneous fermentation with *Saccharomyces* or non-*Saccharomyces* species can impart desirable sensory characteristics to the wine and also save costs, but if it is not managed properly high volatile acid and aldehyde concentrations can be formed. The formation of the undesired Brettanomyces flavours usually takes place during the latter stages of winemaking, because it can resist high alcohol and sulphur dioxide concentrations and is also a slow growing yeast. Although *Pichia* species can also form these undesirable flavours, it can fortunately not grow at alcohol concentrations above 6% A/V. The use of sulphur dioxide in the vineyard and prior to alcoholic fermentation can eliminate many of the non-*Saccharomyces* species. Stuck fermentations can be problematic, because residual sugar can, in the absence of sulphur dioxide, be metabolised by *Acetobacter* species and lactic acid bacteria to form significant concentrations of volatile acid.

After the completion of alcoholic fermentation and MLF, *Brettanomyces bruxellensis*, *Acetobacter* species and to a lesser extent lactic acid bacteria are the sources of potential spoilage. It is unknown at which stage the first-mentioned forms the specific off-flavours and oak barrels are frequently a source of contamination. Sterile filtration followed by the addition of sufficient sulphur dioxide should prevent potential *Brettanomyces* spoilage. A molecular sulphur dioxide concentration of 0.825 mg/ℓ is recommended by the AWRI (Australian Wine Research Institute) to prevent the growth of undesirable micro-organisms (see Table 1). Seeing that *Acetobacter* is aerobic, its spoilage can be limited by keeping containers on ullage and maintaining a sufficient sulphur dioxide concentration. Lactic acid bacteria can be problematic if MLF is not completed and it happens spontaneously in containers or after bottling. It is consequently important that the MLF status of wines is monitored regularly, in order to implement necessary actions to prevent or facilitate it.

Bottling is the last stage in the production process and it is important to differentiate between different wine types. Low alcohol wines with a high sugar concentration and a low concentration of anti-microbial agent or preservative have a high spoilage risk and except for inline sterile filtration sufficient sulphur dioxide levels must be maintained and oxygen uptake be limited. It is also essential that the micro status of wines are monitored regularly during storage (Seabrook, 2015).

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Natural cork is the traditional material for bottle closures.

The choice of bottle closures

MAY 2016

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KEYWORDS: Cork stoppers, bottle closures.

Winemakers are dedicated to make the best wines by using suitable quality grapes and applying the best winemaking practices with suitable cellar equipment. The entire process in the cellar is usually under their control and they must also bear the responsibility for it. The packaging of the wine is however often another person's or institution's responsibility and the dedication of several years can be destroyed instantly. Irrespective of the responsibility for the packaging, the combination of the wine, container and the closure of the container must be executed optimally to ensure that the wine reaches the consumer in the best condition. The choice of the bottle closure is only one of the decisive factors.

Natural cork is seen as the traditional closure of bottles. The physical properties of cork contribute to its suitability, but due to its natural nature a considerable variation can occur between different corks. Problems with the corking machine or bottle necks can also cause certain problems, but it is mainly the incidence of the cork mouldy character, caused by trichloroanisole (TCA), which has been a setback for the image and popularity of cork stoppers. The well-known International Wine Challenge Wine Competition in London monitored the incidence of wine faults in entries from 2006 to 2010. Over this period 6 to 7% of entries had obvious faults and 27% could be attributed to cork spoilage. During the tasting of wines for *Wine Spectator* magazine in 2005, 7% of the samples exhibited cork spoilage. The market share of natural cork closures declined over the last decade from 95 to 70%, while 20% of all table wines were closed with screw closures and 10% with synthetic corks in the same period. It is however wrong and unfair to attribute the incidence of all mouldy character in wine to natural corks, because other production additives or factors can also cause such character.

The problems with natural cork are mainly contributed to the development of alternative bottle closures. In order to evaluate and compare different bottle closures, the properties of the most suitable closure must be clear. It must close bottles effectively, in spite of the variation of bottle necks. The removal of the closure must be easy and the romantics of the process must be retained. In order to facilitate the optimal wine maturation in bottles, sufficient air (oxygen) must migrate into the bottle. If sufficient oxygen does not migrate, the so-called reductive flavours can develop in the wine and excessive air can lead to the oxidation of the wine. Bottle closures must also not impart foreign flavours like mouldiness or synthetic flavours to the wine and be aesthetically acceptable to consumers.

Technical cork closures like Diam use natural cork as material, but by using the Diamant® technology it is ensured that the closures are free of TCA and other off-flavours. It has individual uniformity and different products in the range have different OTR specifications (Oxygen transmission rate), which makes it suitable for different products.

Synthetic corks are made from plastic compounds to resemble natural cork stoppers, but without causing the TCA contamination of wines. Some of the closures do however, have excessive air permeability and are sometimes difficult to remove from bottles.

Nomacorc can be described as a synthetic bottle closure. It is manufactured by using a two-stage co-extrusion process. The raw materials are mixed and according to specifications, two separate blends are combined to form two parts of each closure. A foam core with uniform cell size and density is extruded and a flexible elastomeric outer layer is thermally bound around the foam core

to ensure a tight leak-proof closure. The contact between the outer layer of the closure with the inside of the bottle neck prevents any oxygen migration between the two, but the inside of the closure, consisting of the foam core provides a consistent, predictable oxygen permeation to the inside of the bottle. Nomacorc offers different products with different oxygen transmission rates (OTR) which can, depending on the requirements, be used for different wines. The different Nomacorc closures differ regarding their oxygen permeability over different periods after bottling.

Stelvin is the most widely known screw cap and is made from aluminium, which threads onto the bottle neck. It forms a tight seal and less air migrates into the bottle than natural cork stoppers. It is supplied with two different liners which differ in terms of their oxygen permeability. The Saran tin liner has a high barrier level, allowing less oxygen migration into the bottle, while the Saranex liner allows more oxygen to pass through. The Saran liner is consequently recommended for white wines or wines with a maturation potential of more than five years and the Saranex liner is recommended for red wines or wines to be consumed within two to three years.

The general quality and maturation potential of wine is consequently maintained. A possible disadvantage of screw closures is however the potential incidence of reductive flavours like sulphur compounds (rotten egg) which can especially occur in Sauvignon blanc and the belief of some consumers that screw closures are only used for cheap wines. The use of screw closures in bottled wines increased considerably over the last decade. In the United Kingdom it is already accepted by 85% of regular consumers, while 70% of the USA wine consumers also accept it.

VinPerfect bottle closures were developed resulting from the assumption that wine should not start oxidising within five years after bottling. OTR experiments with present screw caps indicated that the closures with a tin liner or Saranex liner will take respectively 150 and 80 years to allow the migration of 5 ppm oxygen. This can consequently cause reductive flavours. The VinPerfect closure uses a 30 x 60 Stelvin screw cap with the top of the cap having microscopic holes to allow a consistent oxygen access to the inside of the bottle. The liner of the closure is a multi-layer laminate which allows a predictable, consistent OTR. The liner has two PET films with a partial aluminium coating. A two millimetre high density polyethylene foam on it provides elasticity to seal the bottle. The measure of the aluminium coating of the closure determines the OTR of the closure.

Many factors determine the decision about which bottle closure should be used for a specific wine. A variety of screw closures or other closures are available and it is important to determine how the different closures will influence the oxygen control after bottling.

Vinolok (also known as Vinoseal) is a glass closure with an inert O-ring, which seals the bottle hermetically to prevent oxidation and no TCA-contamination can occur. It is an elegant seal with a good image, but is very expensive and cannot be used for standard bottles and closed by existing bottling equipment.

Zork is an alternative closure for still wines and seals like a screw closure, but pops like a natural cork. It consists of a tamper-evident clamp that locks onto the band of a standard cork mouth bottle, an inner metal foil which provides an oxygen barrier similar to a screw cap, and an inner plunger which creates the ‘pop’ on extraction and reseals after use (Cohen, 2012; Rieger, 2012; Sullivan, 2015).

Traditional cork companies and their countries of origin have spent millions of dollars over recent years to improve the image and quality of their products. The Spanish government for instance banned the use of alternative closures in some of their known wine regions in 2006.

The variety of available bottle closures complicates the choice for winemakers and a perfect closure actually does not exist. The applicable wine and cost of the closures are factors that influence the decision. The chosen closure(s), must be the best for the applicable cellar’s products and requirements, and cellars must sometimes adjust their sulphur dioxide adjustments prior to bottling according to the bottle closure (Sullivan, 2015).

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A well-organised maturation cellar.

Factors influencing a wood maturation program

MAY 2016

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KEYWORDS: Barrels, wood, flavour, maturation.

Due to their physical properties, wood barrels were used in the past as containers, whether during winemaking or for transport. Mild steel or stainless steel has however as material many advantages in comparison with wood. Unlike wood, it is inert and tight, which prevents the adding of foreign character to the wine or exposure to air. As result of the exchange rate decline, barrels are a very important input cost of winemaking. The use of wood during winemaking must consequently be managed sensibly with the aid of a maturation programme. In order to compile a suitable maturation programme certain factors must be borne in mind.

A good barrel requires strong and resilient wood. It must not be too porous and should not shrink excessively under dry conditions to prevent potential leakage. It must also not contain too much extractable phenols. The three white oak species, which are usually used for barrels, are the European species, *Quercus sessiliflora* and *Quercus robur*, and the American species, *Quercus alba.*

During barrel maturation a slow migration of oxygen into the wine occurs. In red wines free monomeric anthocyanins occur in colourless and red form. During maturation the monomeric anthocyanins combine with the wood tannins to form tannin-anthocyanin compounds, known as polymeric pigments. These polymeric pigments are more stable and the ratio thereof in the coloured form increases with aeration. This causes a more intense, stable colour. The tannin changes occurring during barrel maturation, lead to the softening of wines as a result of the polymerisation of the tannins and tannin-polysaccharide combination, which

soften the tannins and add more body to wines. Extraction from the barrels also gives complexity to the wine flavour, which can be particularly complimentary to full, complex red and white wines. Wood contains volatile and non-volatile wood extract, which can be modified by the ageing and toasting processes during barrel making. This modification continues during the development and ageing of wine.

Cellulose, hemicellulose, lignin and tannin are the most important components of oak wood. The long chain polymer, cellulose, is mainly responsible for the strength of the wood and changes minimally during coopering and also contributes little to wine flavour. Hemicellulose is smaller polymers of mainly xylose and arabinose and can be hydrolysed into individual sugars and acetic acid. Wood toasting causes the breakdown of hemicellulose which can form aromatic compounds likes maltol, cyclotene and ethyl lactate. Lignins are branched chain polymers, which play a significant role in wine flavour. The thermic degradation of it during wood toasting liberates flavour compounds like vanillin, eugenol, guaiacol and phenols. Oak wood also contains tannins, which are polymers of gallic acid and ellagic acid. It is liberated during barrel maturation when the tannins are hydrolysed as result of the wood contact. These compounds can as a result of their astringency and bitterness contribute to the mouthfeel of the wine. Oak lactones increase during toasting and can impart a pleasant, sweetish wood flavour, but in high concentrations can become overwhelming and unpleasant.

Different factors like botanical and geographic origin, wood structure, individual trees, stave seasoning, toasting degree, maturation period and the previous use of barrels can cause a variation in the eventual flavour profile of matured wines. The flavour and impact of oak barrels are influenced by the oak wood species and wood origin. In Limousine *Quercus robur* is for example dominant and the wood has a coarser grain, which will add more tannin and flavour to the wine; contrary to wood from Alliers and Nevers from Central France with a tighter grain. The seasoning and drying of the wood are also important. Green wood has low concentrations volatile compounds, but oxidation during drying increases the aromatic aldehyde concentrations. Longer drying of wood is usually more acceptable, because shorter drying will add more smoky flavours and astringency to wines.

The degree of toasting also plays an important role. More toasting causes higher phenolic aldehyde concentrations and other favourable sensory compounds, but decreases the tannin concentrations.

If abovementioned factors are considered, it is obvious that wood can play a significant role in wine character. It is essential that barrels are coopered properly from high quality wood and winemakers must consider all the facts before barrels are bought. The style of the wine to be matured and what is expected after maturation, will determine the applicable barrels that will be chosen. Full, tannic Cabernet Sauvignon will for example be complemented by a high percentage new tight-grain French oak, while Rhone blends will need less prominent wood character and consequently also a lower percentage new oak. In both cases the toasting degree of the barrels, maturation period and wood origin will play another role, because the flavour profile and intensity thus imparted will be noticeable in the final wine. Barrels are however not a commodity bought from the shelf and choice of cooper will determine the eventual barrel. This choice is mainly influenced by reliability and consistent quality, although knowledgeable sales staff, adjustable payment and delivery terms also play a role. It is consequently a sound practice to buy barrels from different coopers and also to apply an evaluation process continuously (Cohen, 2015).

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The minerality of wines

JUNE 2016

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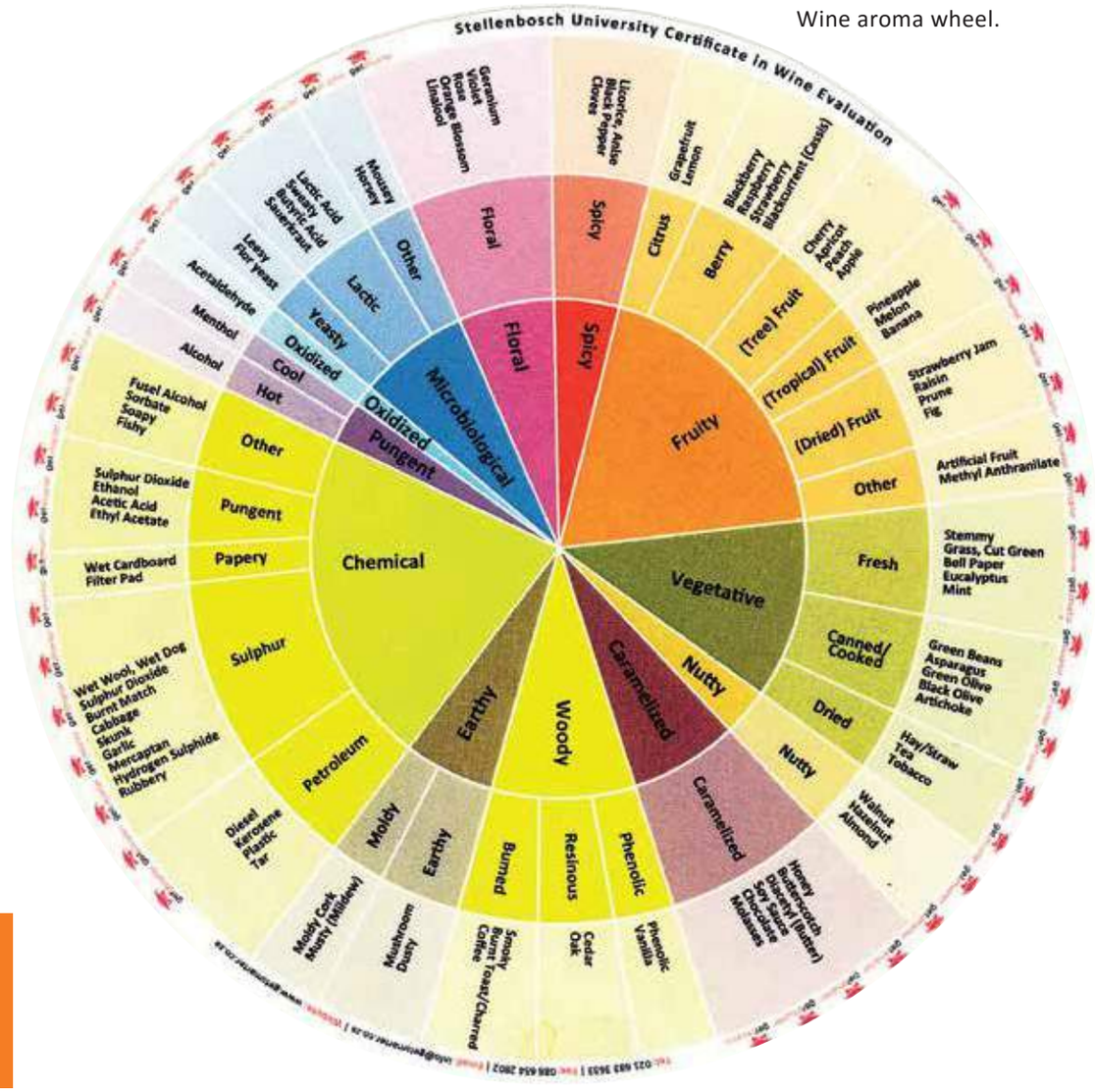
KEYWORDS: Minerality, wine, taste.

Although sweet, acidic, salty and bitter are the basic taste descriptors, the originality of copywriters has ensured that the back labels of wines sound like landscape, flower or garden descriptions. Many of these descriptions are meaningless or even misleading and actual wine descriptions are not used.



Wine aroma wheel.

The use of “minerality” or “mineral” in wine descriptions became very popular over the recent decade. It has actually become more popular than the well-known “fruitiness” or “fruity”. The question arises whether the interpretation of minerality by wine experts is alike and if so, what is meant by it. It appears that the increase in use may rather be attributed to sociological than practical viticultural or enological reasons. Limited research has been done about the meaning of “minerality” and it is unclear whether the word has a specific meaning for consumers or professional wine personalities. Its meaning is also complicated, because different sensory attributes like flavour, taste and feeling are addressed by it. Some wine writers link it to aromas like gun flint, oysters or iodine: tastes like acid, salt or bitter and a feeling like chalk. Minerality is also sometimes expressed in metaphors like crystalline minerality, mineral purity or mineral tension. It is consequently often used in association with terroir in terms of soil and climate descriptions. A few winemakers and wine writers believe that minerality is the sensory expression of mineral salts present in wine which originate from the soil. This can however not be supported by scientific research. In summary it can be stated that different sources report that minerality is an aroma or palate experience or a combination of both.

In 2014, French researchers tried to determine whether wine experts have the same interpretation of minerality. Their aims were to determine whether it was

mainly an aroma and palate sensation and secondly how the wine experts define their perception of minerality. Sixteen different Chardonnay wines from Burgundy with vintages that varied from 2007 to 2009 were used for the research. Half of the wines were described as mineral in wine guides or on websites and the rest were not. Two panels were used for the research. One panel consisted of 34 wine experts from Burgundy and the other panel consisted of 33 persons, trained in wine descriptors.

The words used by the panels to describe their perception of minerality, varied considerably and may create the impression that consensus about minerality does not exist. If the semantic relationship between words is however considered, the majority of the words can be clustered in three groups, as indicated in Table 1.

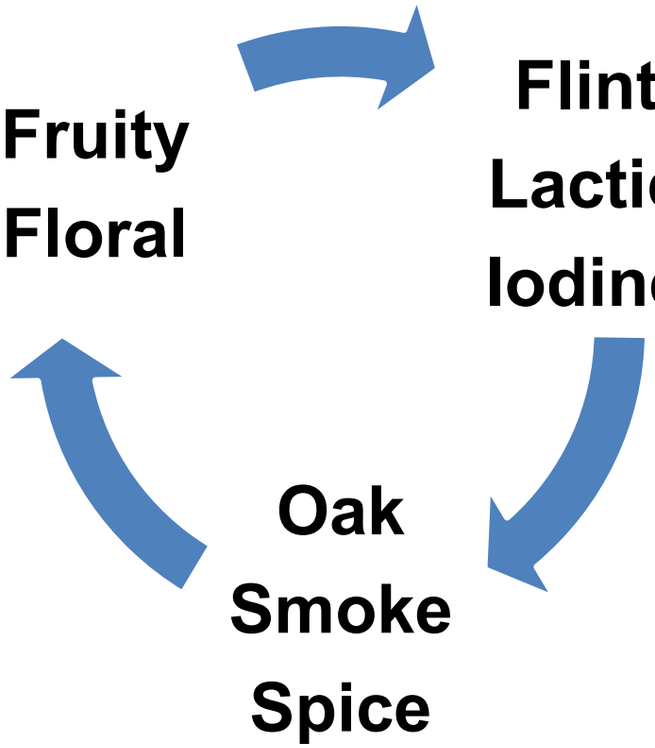


FIGURE 1. Minerality perceptions of different panels.

TABLE 1. Words to describe minerality in wines.

Related word group	Individual descriptors
Stone related	Gun flint
	Flint
	Stone
	Chalk
	Wet stone
	Hot stone
Acid related	Acidity
	Freshness
	Tension
	Vivacity
Sea shore	Iodine
	Salt
	Shellfish
	Algae

Statistical grouping of the panels regarding the words used to indicate high minerality in wines, is illustrated in Figure 1.

The panel that typify it as flint, lactic or iodine, also described it as reductive. It corresponds with the words that are the most used by panels and wine writers. The grouping that describe minerality as oak, smoke or spice, also indicated it as reductive. This corresponds partly with other research results. The perception of the grouping that associates minerality with fruity and floral is unexpected and the only possible explanation for it is that they associate minerality with quality and consequently typified such wines as fruity or floral.

In spite of the apparent consensus in the verbal description of minerality, strong disagreement exist between wine experts regarding the minerality intensity and concentration of wines (Ballester *et al.*, 2014).

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The grain of the wood plays an important role in the maturation period.

Latest trends in barrel maturation

JULY 2016

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KEYWORDS: Barrel, toasting, wood, grain.

As result of their nature, one of the most important problems experienced with wood barrels is the variation which exists between individual barrels. It is easy to specify physical properties like capacity, wood species, stave diameter, number and type of hoops, but factors like toasting degree and grain density are not easy to quantify and cooperages also differ with regard to their classification of different degrees.

Coopers initially used the geographic origin of forests to differentiate between barrels. Nevers, Vosges, Troncait, Limousin and Allier were consequently part of barrel specifications. Winemakers have also based their expectations of the matured wines on the influences of these differences. It was only during the 1980s that the toasting degree became part of barrel specifications. By varying the toasting duration and intensity, coopers could supply barrels, which impart predictable flavours like vanillin, dry nuts, roasted almond and smoke to wine. Winemakers could add more complexity to their wines, which also created a new dimension for consumers. Cooperages continued research to offer more diversity to winemakers. This included inter alia the wood origin, wood leaching, wood drying and toasting. An important resulting action, was the selection of timber according to the grain, instead of the traditional origin classification. It contributed mainly to more uniformity amongst barrels delivered to cellars.

The toasting degree of the barrel plays an important role in the flavour of the resulting wine.

The different wood grain classifications can be summarised as follows:

- **Coarse or wide grain:** Is mainly used for spirits.
- **Semi-fine or medium grain:** Is used for shorter maturation periods like eight to 14 months.
- **Fine or tight grain:** Is used for maturation periods from 12 to 18 months.
- **Extra fine or extra tight grain:** Is used for longer maturation periods like 18 to 24 months.

Generally longer maturation periods are required for increasing grain intensity.

The extra fine or extra tight grain is the best choice for premium wines. Seeing that the timber used for it originates from slow growing trees, it leads to subtle and elegant flavours, which need a longer maturation period to balance the flavours. During the first 12 months the wine will exhibit a dusty oak, pencilly, timber-like character, which appear unbalanced. It is consequently important to choose the grain according to the required maturation period. The stage of optimal oak maturation is also influenced by the cellar temperature, the body of the wine, variety and analyses like alcohol concentration, pH and acid concentration. As soon as the oak maturation appears balanced, it must be terminated, seeing that further maturation will lead to excessive wood character, which can overwhelm the fruitiness of the wine.

Wine styles have also changed over recent years. Consumers prefer wines with soft tannins and delicate wood character, which are immediately drinkable, because most consumers do not mature wines in their own cellars before drinking it. The most wines are not matured for a long period and the maturation period must be kept as short as possible to improve the cash flow of the cellar. Winemakers must plan their wood maturation to comply with the requirements of consumers and business. It can be done by considering the following aspects:

- The use of semi-fine or medium grain wood, which requires a shorter maturation period.
- The use of water instead of traditional open fire to bend the staves. It decreases the impact of the wood on the wine.
- The use of 300 or 500 ℓ barrels instead of 225 or 228 ℓ barrels, which decreases the surface-volume ratio of the barrels.
- The use of American oak. In combination with French oak it can lead to wines that are acceptable at an earlier stage of maturation.
- The degree of toasting must suit the wine and it must also be borne in mind that the toasting degrees differ between coopers.
- The outside seasoning and maturation of the wood can decrease the impact of the wood if it is extended.
- The use of alternative wood products offers possibilities to shorten the wood maturation period.

The decision to balance the wood character of wine, is mostly a compromise decision regarding the different factors which influence it (Little, 2015).

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The mixing of skins and juice is essential to extract colour and tannins.

Colour and tannin management practices for red wines

JULY 2016

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KEYWORDS: Tannins, astringency, colour.

Seeing that skin contact is a standard practice of red wine making, the orderly management thereof is important to ensure that the colour and tannins of the eventual wine are balanced. Colour is mainly of visual importance, but tannins play a determining role in the taste of wine. A lack of tannins leads to thin, insipid wines, while an excess will cause unbalanced and excessive astringency.

Winemakers have different winemaking techniques to manage phenols, tannins and colour during the making of red wine. Phenols are important, because they contribute crucially to the colour, aroma, taste, flavour, body and texture of wine and also play a role in wine stability and maturation potential. The most important red grape and wine phenols are flavonoids, which contribute to colour and astringency. These include amongst others the anthocyanins, which are responsible for the red colour and flavan-3-oles like catechin, epicatechin, epigallocatechin and epicatechingallate. Polymers of flavan-3-oles are also called proanthocyanidins or condensed tannins. Tannins originate from grape skins, seeds and stems and skin tannins are extracted easier than seed tannins. Colour or anthocyanins originate from the skin.

Skin and seed tannins are formed before *veraison*, but anthocyanin synthesis and tannin ripening occur after *veraison.* The bitterness of seeds decreases with the ripening of the seed, but the opposite happens with the astringency thereof. Viticultural practices like more sunlight exposure, higher nitrogen levels, less soil moisture, moderate canopy, moderate yield, lower soil fertility and smaller berries increase the phenol concentration of grapes and the resulting wine. More light exposure increases the skin tannins, sugar concentration and colour, but decreases the green character, acid concentration and disease potential, although sunburn may occur. It is consequently better if light exposure occurs as early as possible during the ripening cycle. In the cellar the phenolic evolution and consequently the phenol extraction is influenced by variables like time, temperature, concentration gradient, cell permeability, berry ripeness, press programmes and maceration practices.

Cold maceration of the crushed grapes for a few days at 10 to 15°C is used to prevent spontaneous fermentation with potential advantages regarding phenolic extraction. Published data do however vary. The most research found no or a negative influence on colour and some results showed an increase in seed tannins. Cultivar differences also occur with cold maceration with Cabernet Sauvignon and Merlot gaining the most, in comparison with Pinot noir. Draining of juice prior to fermentation increases the skin-juice ratio, which will increase the phenol concentration. In some cases the increase disappears after bottle maturation for six months to a year. Thermovinification increases the skin tannin and anthocyanin concentration, but it can lead to a decrease in cultivar character. It can however be used beneficially for Pinot noir and lower quality grapes. Colour enzymes break down the skin cells and can thus promote tannin and phenol extraction. Enzyme additions are, however, not necessarily beneficial for quality grapes. Higher temperature and alcohol concentration during fermentation cause more extraction of colour and phenols, but lower temperatures will promote more fruitiness and a balance must be found between the two extremes. Phenolic extraction increases during fermentation, whereafter it can decrease again as result of colour adsorption by lees and re-adsorption by the skins at the end of fermentation and extended skin contact. The processing of the cap during fermentation is necessary to keep the skins wet for fermentation, ensuring a more uniform fermentation and optimising the skin and juice contact for extraction. The choice of skin contact process is influenced by the cultivar, experience and the extent of required air contact. The different extraction techniques in order of lower extraction potential are rotating tanks, mechanical punch downs, pump overs, manual punch downs and *delestage.* Extended skin contact after fermentation tends to increase the seed tannin concentration, but does not increase the colour. An investigation to determine the influence of fermentation temperature and pump overs on tannin extraction, found that skin tannins are extracted early, while seed tannins like catechin are extracted later during fermentation. The extraction of seed tannins is also influenced by temperature, but it is not clear whether the volume of pump overs has an important influence on extraction.

Phenol analyses can be utilised during *veraison*, ripeness, harvest, fermentation, juice drainage, pressing and blending to ensure that soft, but full-bodied red wines are made. Colour intensity, a tannin index and a ratio between the two analyses can be used (Rieger, 2014).

The required balance between sufficient colour and tannins is consequently the result of different viticultural and enological processes and techniques, which can be managed with the aid of available analytical methods.

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The evolution of wine containers

AUGUST 2016

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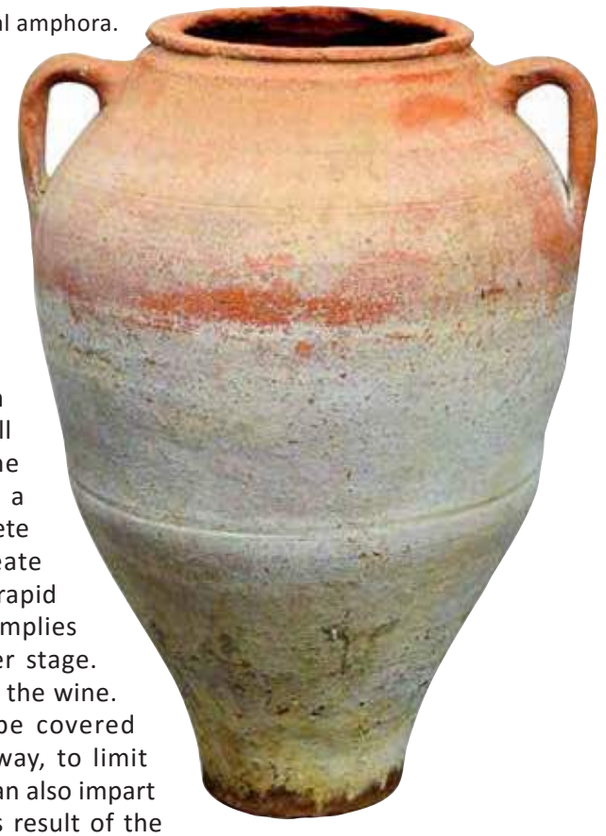
KEYWORDS: Wine, container, amphora.

Due to the physical nature of juice and wine, the containers that are used for them need to comply with certain requirements and it sometimes does not only serve as container, but can also contribute to the development of the product or influence the quality detrimentally. Containers have also sometimes been used in the past without the scientific evaluation of their disadvantages or advantages. This does sometimes lead to the re-use of containers which have become unpopular. Concrete tanks which were most commonly used in the past, were initially replaced by mild steel tanks, followed by stainless steel tanks, but concrete ovums have for different reasons become popular again. Amphorae made from clay, were most probably the first containers used for wine and have after several years become popular again.

Clay containers were used in the wine industry since the seventh century BC and the ruins in Pompeii confirm that Roman amphorae were used for the fermentation, preservation and transport of wine. Amphorae are handmade clay containers, which take days to build and months to dry, before they are fired in a kiln. The making thereof can consequently rather been seen as a craft than manufacturing. Different cellars in different wine countries show interest in the use of it, because its properties and influences are better known. Different providers also contribute to its general availability to cellars.

Amphorae are usually 25 mm thick, in comparison with the 125 to 150 mm of concrete tanks' walls. As result of this thickness, steel fortification is usually required and coils are used for the heating or cooling of the contents. Contrary to that, clay is a good isolator and has a natural evaporation transpiration, which can cool the contents. Fermentation will consequently be slower due to the lower temperature. Clay also has a higher porosity than wood or concrete and more air (oxygen) will permeate through it. It can lead to a more rapid development of the wine, which implies that it can be bottled at an earlier stage. More minerality is also imparted to the wine. The inside of the amphora can be covered with bees wax in the traditional way, to limit permeability and wine losses. This can also impart interesting flavours to the wine. As result of the shape and mass of amphorae, they are more difficult to clean and move. It is also difficult to remove the pomace from them and they cannot be stacked.


A traditional amphora.

The motivation for the use of amphorae differs between cellars. It especially finds approval with winemakers who concentrate on unique wine characteristics rather than volume. It suits boutique wineries making wines in a traditional way from scarce or lesser-known cultivars and focusing more on the softening of the wines, than adding excessive wood character to the wine. If wood is used together with amphorae, the bigger Italian *bottis* are preferred. It is especially popular using Italian cultivars in the same way the Romans used to do it. More cultivar aroma is retained in amphorae, which adds more freshness to the wine if it is bottled at an earlier stage. Winemakers usually focus on only one or two cultivars to ensure that the amphorae are utilised optimally. It can however be white or red cultivars and also in different styles. Wines, other than white or red Italian cultivars, which are already made in amphorae, include Sauvignon blanc, Chardonnay, Riesling, Viognier, white wines with skin contact known as orange wines, Pinot noir, Shiraz, Grenache, Cinsaut and Cabernet Sauvignon (Ness, 2016).

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Spheres (“xoakers”).

Improved alternative wood additives

SEPTEMBER 2016

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KEYWORDS: Alternative wood additives, chips, blocks, staves.

Alternative oak additives have been used in the wine industry for approximately 30 years. The price of new barrels was the initial motivation for their development, but the image of alternative products was previously negative and especially traditional wine purists were also negative about their use. The suppliers of the products became very innovative, however, which led to different forms of alternatives. Winemakers also experienced over the course of time that alternative products can comply with certain requirements that cannot be met by barrels. The magnitude of the market consequently increased considerably and their use has become a standard practice for specific aims at the most cellars. This is also confirmed by the increase in the number of local and international suppliers of the products.

One of the main advantages of the manufacturing of alternative additives for cooperages, is the better utilisation of timber in comparison with barrels. The physical specifications of alternative wood additives ensure that different sizes thereof can be used, but this advantage for cooperages can be an important disadvantage for cellars, because the quality of the wood used for the products cannot be verified easily and the integrity of the cooperage is crucial. Contrary to the variation of individual barrels, the specifications of alternative wood additives can be maintained easier, which improve the consistency of their use.

Seeing that the contact between wood and wine ought to be better with alternative wood additives than barrels, their physical measurements are important to ensure optimal wine contact with subsequent extraction from the wood. It is illustrated by the specifications data in Table 1.

TABLE 1. Measurement specifications of alternative wood additives.

Product	Specifications
Powder	None
Chips	None
Cubes	10 mm x 10 mm x 10 mm
Spheres (“xoakers”)	Diameter 25 mm
Dominos	50 mm x 30 mm x 13 mm
Blocks	50 mm x 50 mm x 13 mm
Chains	25 cm x 25 mm x 13 mm
Barrel staves	73 cm x 6.5 cm x 13 mm
Tank staves	91 cm x 6.5 cm x 13 mm
“Winewood”	1 m x 10 cm x 13 mm

Although alternative wood additives will most probably never replace traditional barrels, the suppliers' goal is to get as close as they can. It must rather be seen as an alternative wood treatment than a barrel alternative. The suppliers consequently reacted innovatively in different ways. Most suppliers of both French and American oak products, due to the quality of timber used, are much closer to barrels than 10 years ago. Apart from wood quality being better, cutting-edge technology is also used to ensure that optimal wood contact is obtained. The spectrum of flavour profiles that can be obtained, was also extended considerably by the better understanding of toasting techniques and development of new ones. The required extraction period was also shortened in order to obtain the required wood flavours quicker. These innovations went with a product price that is still considerably cheaper than barrels. As result of the lower price, it is consequently also possible to create a required wood character in cheaper wines, which are sold at a younger stage. Many of the changes were initiated by winemakers. The younger generation of winemakers are not so tradition-bound and are consequently willing to experiment with new products like alternative wood additives (Siegel, 2016).

The changed use and perception of these products are also confirmed by winemakers:

- Although their price remains a significant factor, the influence of alternative wood additives on the style and quality of wine has also become a determining factor in the choice to use such products. Winemakers realise that specific products and variations thereof can impart required specific wood characters to wine. It can range from a subtle wood character in Chardonnay to a sweet, outspoken vanilla character in red wines.
- Different products like staves, smaller products or chips are used during the different stages of winemaking like prior to or during alcoholic fermentation or during malolactic fermentation (MLF) or maturation.
- Both French and American oak are used.
- Alternative wood products are also used for premium wines, especially if wood contact is required during MLF or prior to maturation.
- More expensive alternative wood additives are also acceptable, if the required results are obtained with them.
- Certain wood brands or types suit specific cultivars or wine styles and it is important to evaluate it or request the experience of other winemakers, before it is used commercially.
- The use of alternative wood additives has different advantages. Commercial red wines can be marketed sooner, more structure can be added to wines, colour stabilisation can be improved and more oak flavours, but less tannins are extracted over a shorter period.
- Staves must be at least 9 mm thick, but preferably 12 mm or more to impart sufficient structure to wines.
- The duration of wine contact must be managed cautiously, seeing that results occur much faster than with barrels. Wine balance is a critical issue of wine quality and it is consequently important to prevent excessive wood character.
- Winemakers are willing to talk about the use of alternative wood additives, because the stigma attached to them has decreased.
- It will be welcomed if alternative wood additives can be connected to specific wood origins (Roundtable, 2015).

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Risk management for wine cellars

SEPTEMBER 2016

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KEYWORDS: Risk management, wine cellars, risks.

Since the beginning of the wine industry during the Middle Ages, continuous changes have led to risk management becoming more important. The risk exposure of wine cellars ranges from the vineyard to the wine consumer. All actions executed during this production cycle, as well as factors influencing it, contain potential risks. Production substances and the equipment used during the production chain increase these risks, which are not only limited to the health and safety of employees.

Risk management is a specialist field, which usually does not receive sufficient attention during the training of winemakers. It compels wine cellars to use consultants. In order to execute risk management effectively, certain measureable results must be interpreted. One of these is Total Cost of Risk (TCOR), which expresses the indirect and direct risk management costs versus sales. If it is charted over time, it is an indication of the efficiency of the specific risk management programme. Risk management consultants usually recommend a committee consisting of managers over the full management spectrum and it usually consists of the Chief Executive Officer (CEO), Chief Financial Officer (CFO), production, operational, safety, logistics and sales personnel.

The eventual aim of risk management is the decrease of uncertainty, which implies that the cellar's activities and its results must be sustainable. Examples of risks that wine cellars are exposed to, include resources like electricity and water, product tampering, product recall, earthquakes, floods, cyber threats, intellectual property, copyrights, trademarks, media liability, product liability, pollution, production substances and packaging material. After the potential risks have been identified, it must be decided how to manage them. Ways to limit the risks or minimise their results, is the first option and insurance is usually only recommended for disastrous risks. One of the main potential risk costs is the compensation of employees for workplace injuries. It can however be decreased, if it is managed correctly. Injuries in the cellar do not only influence the safety and health of employees, but also the profit of the cellar. It can usually be prevented and 90% of it can be attributed to human behaviour and only 10% of workplace accidents can be attributed to threats and equipment. The magnitude of it can be diminished by loss control training of employees or the use of temporary workers, in which case insurance is not required. The latter can however be detrimental, because temporary workers are not necessarily properly trained or exposed to a work culture. The work culture of wine cellars is critical to prevent such accidents and management training of managers and supervisors, as well as the empowerment of personnel regarding safety, is essential to maintain such work culture.

Although traditional insurance companies are active in the wine industry, alternative risk financing must also be considered. It has loss incentives and premiums are linked to claims. Large cellars can also consider self-insurance and the creation of an insurance profit centre can be part of this.

If cellars have a risk management programme, it must be monitored regularly and modified, if necessary. Insurance of buildings, tanks, bulk and bottled wine, equipment, business interruption, vehicles, aviation, injuries, emergency and disasters are all part of any form of risk insurance (Goldfarb, 2016; Niebuhr, 2016).

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Moving parts can cause serious injuries.

Bottling is the final process to ensure a safe product.

Wine must be an expression of the cultivar and the environment.

The myth of natural winemaking

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KEYWORDS: Natural wine, winemaking, philosophy.

A myth can be defined as a popular viewing of a natural process. If grapes are consequently left to ferment without any additives to form wine, it can be seen as a myth. Contrary to such viewpoint, natural wines are regulated in South Africa to have an alcohol content of 4.5 to 16.5% A/V, without referring to any process or additives. The use of the term "natural" therefore has no limitations and can easily be used as a marketing gimmick.

The viewpoints of some Californian winemakers and a sommelier regarding natural winemaking lead to some interesting aspects. In general it is stated that the naturalness of wine increases with decreasing inputs by the winemaker. If the different processes during winemaking are analysed to evaluate whether they are really necessary, some of them, like for example sulphur additions, yeast additions, enzyme additions, finings, filtration, stabilisation and rackings can be eliminated. This does not necessarily imply better or inferior wine. The grape character must however, be retained and not be overpowered by excessive wood or other additives. Such wines can be seen as more pure.

The expectation of supporters of "natural" wine is the expression of the grape cultivar and the site where it is cultivated. Although it can be expected that the same cultivar will exhibit different characteristics on different sites, the difference must not be so much that the unique recognisable cultivar characteristics are not exhibited. The role of the winemaker is thus limited to the managing of the winemaking process, without disguising the origin and cultivar.

In order to realise the abovementioned expectations, the following actions are implemented:

The vineyard must preferably have already moved from the growth phase into the ripening phase. Basal leaves must already have started discolouring and seeds are ripe and brown. The acidity of the grapes, but not necessarily their total acid concentration, and pH are seen as the most important chemical indicators to decide when the grapes are harvested. The sugar concentration of the grapes is not critical and harvesting can be over a wide spectrum. No additives are added when the grapes are received. The grapes are stamped by feet or pressed, without destalking or crushing them. If the grapes are spoiled, a low dosage of sulphur dioxide, depending on the pH can be considered, but sound grapes should rather be used. Fermentation control is actually not applied and the alcoholic fermentation can last for months. Varying temperatures during fermentation are seen as associated with specific vintages and possibly contribute to the vintage characteristics. Practices like cold soaking during red winemaking are seen as artificial. If fermentation problems occur, pure culture inoculation can be used to resolve these problems. Post-fermentation procedures are also limited, because yeast lees contact can protect the wine. Rackings are only applied according to personal judgement. A low sulphur dioxide dosage is usually added prior to bottling.

From a scientific enological viewpoint, various wine problems can occur with abovementioned approach. To commence with low pH grapes, is therefore rather important. Supporters of the concept however, view moderate volatile acid concentrations and Brettanomyces character as part of the surprise results of such wines and do not make such wines with specific specifications in mind, but rather in expectation of the eventual wine (Cutler, 2016).

Winemakers can therefore be divided into two groups. The majority are those who make sustainable wine scientifically according to specific prescriptions and complying to measureable characteristics. These wines form the greatest part of the international wine market. Contrary is the group who only selects the grapes and nature makes the wine. The latter is therefore rather a philosophy of life than winemaking. This will obviously deliver unpredictable, interesting wines to consumers, who rather look for excitement than standard global wine portfolios. This however, does not imply that only such wines are "natural".

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Solar panels at Lourensford Winery, Somerset West.

Water and energy saving in the wine industry

NOVEMBER 2016

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KEYWORDS: Water, energy.

Power shedding in South Africa has forced various cellars to buy or rent generators to continue with their normal activities. Although the capital expenditure related to this is considerable, the running costs amount to several million rand for an industry, which is already continuously exposed to increasing input costs. The drought in various wine regions has also set back the size and quality of the 2016 vintage, which once again emphasised the importance of sustainable natural sources in the wine industry.

The west coast of the USA has experienced serious drought over various years. A survey was done in the Californian wine region to evaluate which actions were or could be implemented to address the drought problems. The local industry can learn quite a lot from this survey.

The cellars were grouped in small, medium and large cellars. 61% of the cellars are dependent on well water and 33% on municipal water. Only 43% of the cellars dependent on well water, do however monitor their water consumption. It is mainly the small cellars that do not monitor their water consumption. Many of the

Solar panels at Neuras Winery, Namibia.

small cellars that do monitor their water consumption, are doing it because it is required. Seeing that this trend remains constant for three years, it indicates that the official requirements were not adjusted much during the increasing drought conditions, especially when it is borne in mind that surface water, rather than snow is increasingly becoming the most important water source.

The actions of cellars to limit water consumption, vary from steam sterilisation, which is used instead of warm water for washing purposes, the installation of flow meters and low flow water pipe valves or the training of personnel in the saving of resources. The latter is the action that is mostly implemented by cellars in order to save water and energy. Small cellars have implemented the most innovative actions like the recirculation and treatment of cellar waste water, which is afterwards used for irrigation or the catchment of rain water, which meets the total water demand of the cellar.

Approximately 60% of the cellars do not monitor their energy use, which implies that they also do not monitor their energy expenditure. This is however unwise, bearing in mind the increase in energy tariffs, which is a considerable cellar expenditure. In spite of this trend, various cellars act very innovative regarding their energy savings. It includes amongst others the isolation of walls and roofs and the installation of low energy lighting. Cellars with more substantial financial resources also install movement monitors, which switch lights on or off automatically.

Although the mentioned Californian survey does not include the use of solar panels, they are installed by various cellars. This can include roof panels or the roofs of parking areas (Lasky, 2016).

The sunlight reaching the earth continuously, contains enough potential energy to meet the total global energy demand. It is also an inexhaustible energy source, often noise free and can also supply energy to distant areas. Presently solar energy does however supply less than 0.1% of that demand, but the present global reserves can only supply energy for the next 30 to 40 years. Over the past 15 years the use of solar energy has consequently increased annually by 20% and Japan, USA and Germany are the most important markets. Solar energy can be used in different ways to generate electricity. It can for example separate electrons from atoms in sun cells to generate an electric current or solar panels can be used to convert water into steam, which can be used to drive steam turbines for electricity generation. In spite of all the advantages, solar power also has a few disadvantages. Solar panels and cells are relatively expensive and during darkness or cloudy conditions they do not function. In such cases energy, stored in batteries, must be used. These batteries are also expensive, bulky and heavy and must be replaced over time.

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The use of barrel technology for uniform barrels

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KEYWORDS: Barrel variation.

All winemakers know that individual barrels differ. Although it may sometimes be beneficial and offer variation to wines, a barrel can influence the sustainability of standard wine blends. These differences are mainly due to chemical and origin differences. The wood species differences are usually simplified to only French and American origin, but are much more complex than that.

Different cooperages have introduced new barrel types over recent years in order to limit the variation between barrels. The Essencia (Demptos), Icône (Sequin Moreau), Pure T (Taransaud), Oakscan (Radoux) and Generation 7 (Vicard) are examples of this. The science of barrel technology is used by them to limit barrel differences. Although the natural wood characteristics of barrels are acknowledged, the management thereof is used to limit the barrel variations. It is however a complex matter, seeing that wine and maturation differences also contribute to the variation of the final product. Concerning the barrels, it is mainly the wood itself and the leaching and toasting of it that contribute to the barrel variation.

American oak (*Quercus alba*) or more specifically American white oak, because many different American oak species exist, differs distinctly from French oak. French oak is however not only one species, but rather two related and hybrid species, namely European sessilis oak (*Quercus petraea* or *Quercus sessiliflora*) and European pedunculate oak (*Quercus robur* or *Quercus pedunculiflora*). Both are used by French, European and Hungarian cooperages. Although it is expected

Toasting temperature plays an important role.

that considerable differences exist between different wood species, French oak barrels were in the past mainly sold according to geographical origin, without focusing on the species differences in the same origin. The environmental differences of different origins can however possibly be a greater contributing factor than species differences. The wood variation is however much more complex than only origin and species differences, because timber originating from the same tree can differ. Irrespective of the species and origin, the seasoning and toasting of the staves will also change the chemical composition of the wood.

The control of variables is complex, because the assembly and treatment of barrels is a craft and not an application of theoretical knowledge. The assembly of barrels is mainly of physical nature, but the visual judgement concerning the use of staves with a uniform grain and degree of toasting is the result of experience over years. During the past 30 years much research has been done regarding the influence of toasting on the chemical composition of wood. The temperature and duration of toasting are of determining importance. The concentration of vanillin, one of the most important flavours formed during toasting, will for example be too low if the toasting temperature is too low, or it will evaporate if the temperature is too high. This wood chemistry research forced cooperages to consider methods other than visual judgement by the cooper as a way to produce barrels of greater uniformity. Most cooperages started using infrared technology in combination with computer technology as a more absolute way to measure and control the degree of toasting. Better toasting control will however not eliminate the individual barrel variation, because different staves will differ as result of the environment where it originates from. In order to classify uniform staves, the ellagitannin concentration of different staves can be analysed with near infrared spectroscopy.

In conclusion, the following aspects therefore play a role in the variation between individual barrels:

- The variation in the chemical composition of wood and the unscheduled irregular sensory evaluation of wine during maturation can lead to a considerable variation in wine styles and quality after maturation.
- The selection of oak wood on the basis of forests must be dealt with prudence, seeing that the variation in the wood composition is not limited by it.
- Selection according to the grain of the wood offers a way of uniformity, but considerable variation can still occur.

Sorting and selection of staves play an important role in the assembly of barrels.

- Classification according the tannin analysis of staves offers a better solution to limit barrel variation.
- The rationalisation of coopers' wood by means of tannin analyses and better toasting control are supported by chemical analyses and sensory evaluation.
- The rationalisation of wood classification will become more important to utilise wood sources better and produce more uniform barrels according to specific requirements (Philips, 2016).

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The use of basket presses

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KEYWORDS: Presses, basket press.

The effective recovery of the most possible juice from grape skins is one of the most important actions to ensure that cellars are financially successful. Pressing of the skins is one of the ways to achieve this. Different types of presses were developed over years to execute it and the basket press is one of the types that can be used. Over the course of time it fell into disuse, but with technological advancement equipment was improved, which led to the use of different basket presses by different cellars.

The use of wooden beams, capstans and windlasses to exert pressure on skins in order to extract juice or wine from them, dates back to the Greeks and Romans. Further development of this equipment led to the general use of wooden presses during the seventeenth and eighteenth centuries. The vertical basket press with a metal capstan, which cellars are still familiar with, was introduced in the 1830s and were the presses mostly used in the nineteenth century. Some of these presses are still in use at cellars, that prefer to keep their winemaking traditional. The press action can be executed manually or hydraulically.

Basket presses, which are presently used or available, have different capacities and can consequently be used in the laboratory or wine cellar. It consists of wooden strips or perforated stainless steel and the press action can be executed in different ways. It can be done by a rotating action on a vertical, central metal capstan or by means of hydraulic pressure on a disk on top of the skins. The press action or program used to implement it, can be controlled manually or with a PLC program.

Small scale manual basket press.

As result of the capacity differences, basket presses are mainly used in small to medium size cellars. In latter cellars it is often used together with a final press to extract the most juice from the skins.

On small scale it is usually filled manually, but containers with skins can otherwise be emptied by tipping them or the cellar can be designed to use gravitation for filling the basket press. Like any other separation action the free run juice or wine can be collected separately. Basket presses can be used for white or red wines, although they are mainly used for red wines. The free run can vary from 450 to 500 litres per ton, depending on the cultivar and preceding winemaking procedures. Further juice or wine recovery will depend on the pressure and duration of pressing and can be an additional 200 to 250 litres. The total press cycle can last 1.5 to 24 hours. As result of the liquid's movement through the skins, a clarification process can occur leading to juice or wine with a relatively low solid percentage. The emptying of basket presses is laborious, before the dry skins can be removed from the cellar. Basket presses with wooden slats are more difficult to clean, because seeds and skins can be fixed between the slats. High pressure water and caustic soda, followed by an acid and water rinse, like for other cellar equipment is however usually used.

Basket presses are usually used by cellars that believe in traditional winemaking and better quality. It is claimed that the better quality can be attributed to the following:

- The press action is soft and the skins are not crushed or disintegrated. This can lead to lower tannin concentrations resulting in soft, better balanced wines.
- The lower solid percentage, as result of the clarifying action by the skins, results in more delicate wines.
- The aeration during pressing creates more colour stability in red wines, but if it is for example not required during white wine making, the press can be covered with canvas and carbon dioxide blown underneath it.

The disadvantages related to basket presses are mainly their limited capacity and required manual labour. Their use is consequently limited to boutique or relatively small cellars (Howard, 2016).

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5 General

New look at fruit industry (Part 1) FruitLook technology assists farmers

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KEYWORDS: FruitLook, technology, farmers, satellite.

A couple of satellite eyes in the sky are casting a new look on the local fruit industry.

Together this constellation of satellites – some orbit 800 km high up in the sky – allows producers to look at their farms and its management in a different light.

This is thanks to remote sensing and satellite-based spatial data products used in innovative ways, channelling data through an open web portal called FruitLook. This tool – hailed as cutting edge internationally – provides a new generation of agricultural intelligence for the fruit sector.

Says André Roux, from the Western Cape Department of Agriculture, “FruitLook is basically an online tool that uses the latest satellite technology to help farmers precisely manage crop productivity, growth and water use. It has proved to be a useful tool to enhance sustainable farming practices.”

What started out as research mainly to look at how grape farmers could increase their water use efficiency in 2007, has since expanded in scope. Roux, departmental director of sustainable resource management, says the technology can be applied on other crops and across climatic regions. “The satellites can for instance tell you how well your crop is growing, how much water it is using and also how effectively it is doing that,” says Roux. “The system then enables you to identify areas with weak growth or even pinpoint the particular area in your vineyard or orchard with water shortages.”

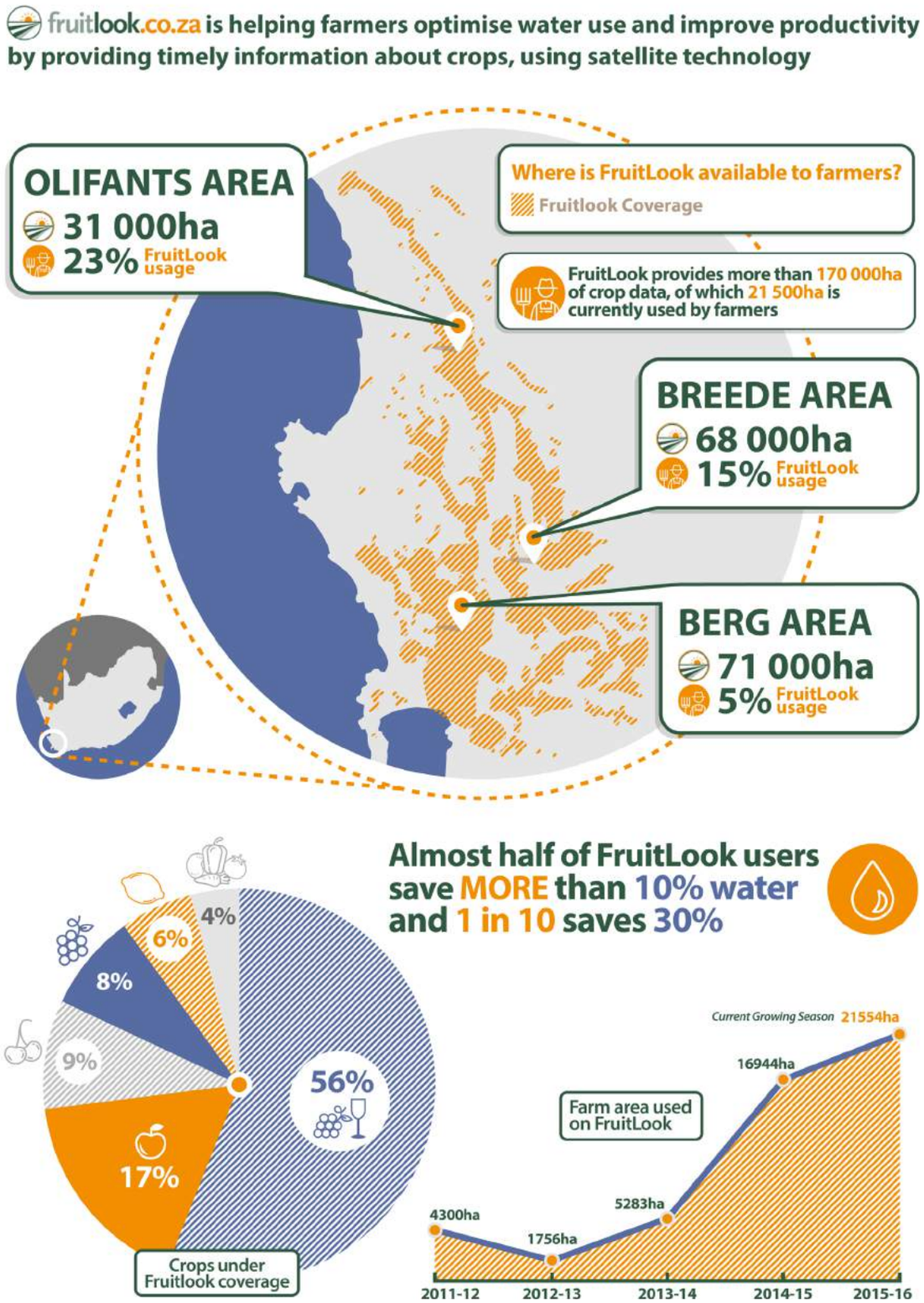
This can potentially save producers lots of money, especially on irrigation costs.

Currently FruitLook is only available to fruit and wine grape producers in the Western Cape. The area under satellite scrutiny stretches roughly from Lutzville (in the north) to the Hemel-en-Aarde Valley (in the south) and Robertson (in the east).

“FruitLook offers producers an innovative and up-to-date service that helps them understand what is happening on their farms on a weekly basis, throughout the growing season,” says Dr. Caren Jarmain, an independent researcher. “It provides a complete picture of the plant within its growing environment.” This is possible thanks to FruitLook’s unique architecture that integrates satellite data with geographical data and weather information in complex models (see graphic).

The satellites zip past the fruit producing region daily, gathering growth, water and nitrogen related data for orchards or vineyards. The raw data then gets digested using various techniques, including algorithms. Local researchers also verify the data through field measurements to ensure its credibility. All this information is then integrated into user-friendly maps and graphics that can be accessed for free and online.

Roux believes FruitLook, supported by a Dutch company called eLeaf, offers win-win options for producers. His department currently subsidises its use, spending R3,8 million on it annually. Says Roux, “The optimum use of our scarce water resources will save water, reduce irrigation water return flows, reduce pollution of our rivers and streams, as well as save on electricity and fertiliser costs. “Ultimately it will make farming operations more viable.”



Jarmain, a specialist agricultural meteorologist and research associate at Stellenbosch University, agrees. “FruitLook offers a tailor-made technology in support of precision viticulture and horticulture in the Western Cape,” she says. “It provides producers with spatial data based on the latest satellite information to analyse crop growth and water status over time and space.

EYE ON AGRICULTURE

Over the past decades, technologies to assist farmers in managing their water use have advanced substantially. There is much interest in this field, as agriculture is by far the biggest consumer of fresh water in the country. In the Western Cape, 43% of the available water resources are used for irrigation. Fruit crops are in general rather thirsty. Fruit crops, for example, have irrigation requirements of between 7 000 m^3 and 11 000 m^3 water per hectare, depending on the type of crop and locality.

The indications are that farmers will in future also have to do more with less. Says Roux, “This is especially true given the increasing conflict between water users. We (agriculture) need to find ways to farm more efficiently and precisely. Within the context of climate change and the predicted changes in rainfall distribution, water wise management is key.” Roux says FruitLook provides intelligent and timely adaptation responses given growing water pressures.

Jarmain agrees, “It requires precision agriculture, especially given rising input costs. In order to use water efficiently, growers need solid information on crop production and water consumption.” FruitLook for instance monitors how much water vineyards use during transpiration using elements of satellite images.

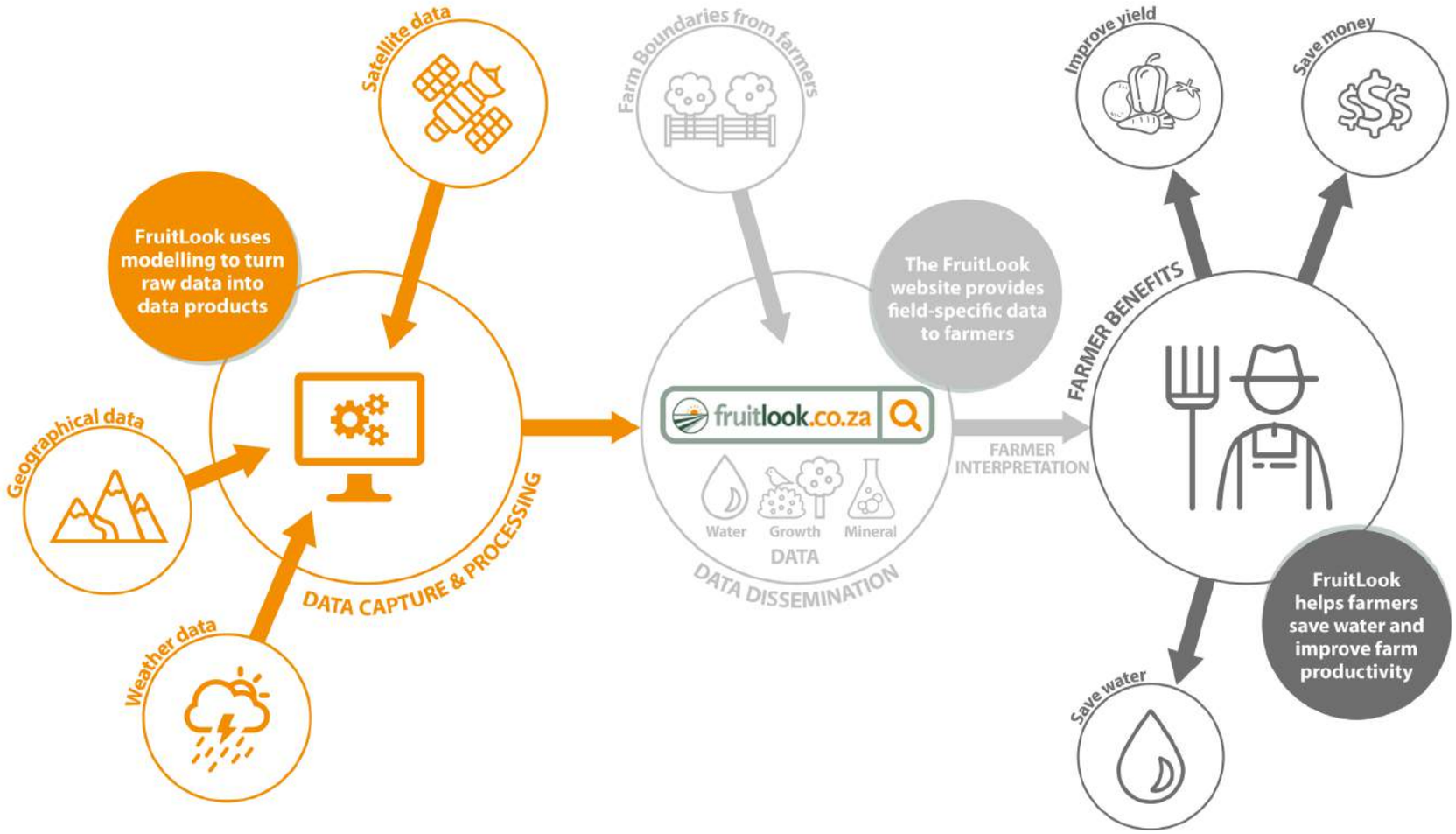
FruitLook monitors just how much water is released from the plants during evaporation and how efficiently water is being used overall. But, unlike aerial pictures taken by drones, FruitLook also overlays layers of quantitative data for every pixel at frequent intervals throughout the season.

The satellites capture details that are not always visible to the naked eye. Remote sensing outside the spectrum visible to humans therefore actually extend our visible field. This is because remote-sensing measures the solar radiation reaching the crop, reflecting off the plants and soil surface, and being emitted from the surface.

Jarmain explains, “The satellites are taking pictures that contain much more data than what you can observe with the naked eye, for instance of surface temperatures. This can be extrapolated to see how actively plants are using and losing water and if a particular block or orchard is experiencing water stress.”

The satellites are for instance able to do moisture readings of a high spatial resolution of 20 m x 20 m blocks of vineyards or orchards. The different images captured by satellites are then merged and

fruitlook.co.za is a tool that helps farmers improve crop productivity and water use by turning raw data into useful information



turned into site-specific images, graphics and rich content. This enables producers to keep an eye on biomass production, detect diseases and use actual indicators of evapotranspiration to detect under-irrigation in their vineyards or orchards. This enables FruitLook to provide additional information about the physical world surrounding us, explains Jarmain.

Just like the pictures on your television screen, the satellite imagery is made up of tiny squares called pixels. These picture elements – each have a different grey share or colour – represent the relative reflected and emitted light energy recorded for that part of the image. Every picture therefore has meaning. Each of these 20 m x 20 m pixels for instance contain data on evaporation and plant growth. This means FruitLook can detect how much water is lost in a pixel through transpiration. It can also document how much plant material is produced above and below the ground (in stems, leaves, vines and roots). It can even give producers an idea of weeds and cover crops growth.

Currently FruitLook's data covers an area of about 170 000 ha (see graphic). Less than 20% of the available area covered is currently utilised. Says Jarmain, "We are only scratching the surface about the useful and far-reaching impacts it can have for local producers."

All this is done without any effort on the producer's side. Says Jarmain, "Normally farmers have to do a lot of visual assessment or have measurements done. Now

they can use technology that captures information in a different way to better understand for instance water use. In addition to sticking probes in the ground to measure the irrigation applied, they now have access to satellites that capture information in the electromagnetic spectrum."

But it is not all about just taking images from the sky. The fact that FruitLook provides spatial variation in picture format is only one of its benefits. Says Jarmain, "FruitLook offers pixel intelligent mapping, as well as intelligent information that farmers can interpret and apply. This data enables farmers to analyse their crop growth and water status over time and space. At the moment we have weekly data for the last five growth seasons from October until April, hence it's offering a nice database to evaluate past seasons.

FARMING WITH TECHNOLOGY

The growth of the industry will be limited by the amount of water it can access, says Hugh Campbell, general manager of Hortgro Science. "We are all pertinently aware that water is our most finite and limiting resource that we will have to optimise now and into the future."

Campbell considers FruitLook "a unique tool that allows growers to evaluate and benchmark their water use efficiency and correlate it to the performance of their block in terms of growth (biomass increase). Campbell says the objective must be to maximise the productivity of every single drop of water. Research has shown that there is a correlation between water use and yield up to a point where the marginal utility per unit of water flattens off.

FruitLook is a tool that has the potential to demonstrate that water has and is being used responsibly by a particular industry or region throughout a growing season, he believes. Says Campbell, "In the realm of water allocation per hectare we need to make sure that a high producing block is not allocated 6 000 m^3 per hectare when it should get 10 000 m^3 of water to optimise the productivity of the water and the other resources utilised."

FruitLook offers tailor-made technology for farmers that can support their efforts towards precision agriculture, Jarmain believes. This translates directly into extra rand and cent for farmers. A 10% saving in input costs, together with a 10% increase in production, will translate into earnings of about R33 860 more per hectare for table grapes, R7 036 per hectare for wine grapes and R25 630 per hectare for deciduous fruit. This is according to estimates done by agricultural economists from the Department of Agriculture.

"You can say it is farming for the future in action already," says Jarmain.

A growing number of users say they benefit from this online tool. Farmers are using the data in different ways. Many use it to determine how the water status on their farms relates with their irrigation plans or to strategically place soil moisture probes. Almost half of the producers using it indicated they have cut their water use with a tenth. One in every ten producers say they are using almost a third (30%) less water than before.

Anton Muller, Kromco's technical advisor, says many producers are embracing this technology as it provides new spatial dimensions and insights into production. He finds it particularly useful to identify plants under disease threat and to determine how successful irrigation management is (compared to previous seasons). The farms in the Grabouw region using it have reduced their early-season water use with up to 30% since using the data.

Luca Bein, from Bein Private Cellar in Stellenbosch, says FruitLook also supports efforts towards precision viticulture and managing variability within his vineyard. He has been using it since 2010 in combination with soil moisture probes to estimate his vineyard's irrigation needs. Bein says the tool is sensitive enough to help prevent and monitor water stress, in particular to identify areas of water stress. Gideon Burnett, a vine grower from Vredendal, has been using FruitLook the past two seasons. He uses it as an additional tool (along with water probes) to determine his vineyard's water needs.

Distell, South Africa's biggest wine producer, has used FruitLook during the last season on Plaisir de Merle and Alto to determine different growth patterns in and between vineyards. This information was used in combination with Maselli (instruments to help measure optimal ripeness). Grapes were pressed separately according to differences in vineyards, as far as possible. Different wines will now be produced to determine how differences in growth vigour affects wine quality from different cultivars.

At Môreson Trust, situated in the Vygeboom region near Villiersdorp, FruitLook has also proven really useful to inform irrigation management in orchards. Says manager Kobus Swanepoel, "We find the data especially useful to determine if we need to irrigate more or less and where to place the soil moisture probes. We also use it to detect drainage problems and to evaluate our irrigation practices." Swanepoel says they also use FruitLook to determine the position of soil moisture probes for new block developments.

This article was first published in the *SA Fruit Journal* and is published with permission from Hortgro.

New look at fruit industry (Part 2) Fruit farms become tech-savvy

AUGUST 2016

JORISNA BONTHUYS
Freelance journalist

KEYWORDS: FruitLook, technology, farmers, satellite.

Today we live in a world of interconnectedness, the dawn of the "internet of everything" and shifting technological frontiers. Even the use of robotics on farms are on the (not so distant) horizon.

The face of farming changed significantly over the past few decades and technological advances took fruit farming to a new level (see timeline).

Whether it is planning a new farm or harvesting grapes, new technology is constantly employed. Although drafting farm plans are nothing new, spatial data (whether elevation maps/topography, slope and aspect and soil maps) is playing a bigger role. So says independent researcher Dr. Caren Jarmain. Many datasets are taken into account to produce these. These days, most farm maps are also digitally produced.

Part of farming is using a good medium (soil) for growing your crop. Quite often growth anomalies in blocks reflect soil differences (soil samples are often taken from distinctly different areas). Normalised difference vegetation index (NDVI) maps have been used extensively to identify "growth zones" in fields and sampling have taken place within these. Based on the soil chemical analysis, corrections in nutrient/mineral applications have been done.

Says Jarmain, "Plant breeding and genetic engineering has also resulted in a wide range of new cultivars available throughout the world. The most suitable combinations can these days be utilised in vineyards. This is paired with available rootstocks, farm plan details on aspect, slope, soils, etcetera."

Technology has also played a major role in the way in which crops are cultivated. Says Jarmain, "Mechanisation is already playing an important role in thinning fruit trees and robotics might lightly play roles in other aspects in future. Simple systems of leaf sampling to determine nutrient deficiencies and corrections based on this, has become common practice in recent years."

With computers and electronics being so readily available and integrated in so many systems, it is deeply integrated in water management systems; whether through monitoring soil water content and managing irrigation. Says Jarmain, "Systems like FruitLook (see graphic) is also utilising computers and combining these processing facilities with satellite information, a spatial view is possible of water management in field and on farm level.

"Harvesting of grapes and fruit are also influenced by spatial technology, by providing information on unique sampling zones. Research into the use of robotics for harvesting fruits are also advancing and will likely play a bigger role here in future."

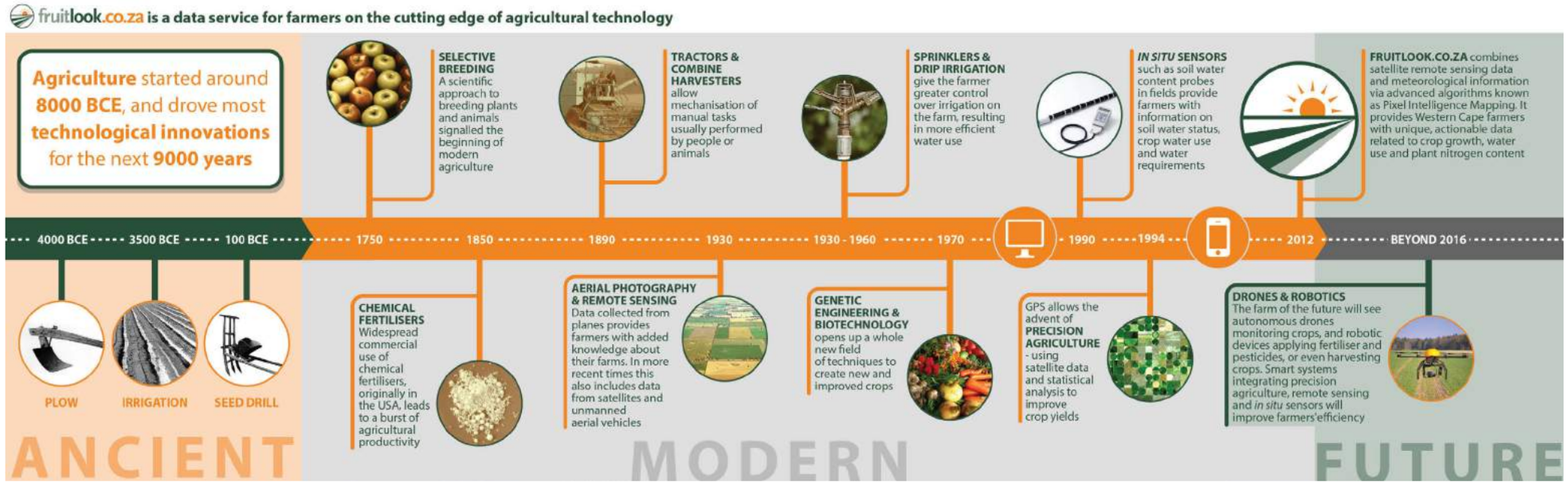
Will the orchard of the future be managed through wireless centre networks? Are machines changing the face of farming in the future?

"The speed of technological change will necessitate local producers to rethink their investment and skills if they don't want to be left behind," says Martin Butler, senior lecturer in Information Systems Management at Stellenbosch University's Business School. He is also a Research Associate at the Institute for Future Studies.

Artificial intelligence is already enabling computers to behave like they did in science fiction movies 40 years ago. Says Butler, "The indications are that fruit farmers will not be able to farm like their forefathers did given increasing resource pressures."

Every farm has again become frontier country in the technological wild. How do you navigate farming in this (frequently digital) landscape?

Since the earliest times, forward thinking farmers have been trying to increase their understanding and management of their crops (see timeline). Over recent decades local farmers have for instance benefitted from many technological advances, for example allowing mechanisation of processes like thinning. Furthermore mass



automation, customisation biometrics and genetics have offered some key areas of technological change. Precision farming (the idea of optimising every square meter of land) is on the rise due to new sensors, (airborne) imaging technology and mobile devices with sensory, as well as location awareness technologies. Butler believes these and other technological advances can potentially steer the sector into a more computerised, information-based world.

Currently farmers are embracing various new generation tools to better understand their block development in space and over time. In recent years satellite remote sensing has also proven very useful. These "eyes in the sky" have already given rise to a myriad of applications, mainly aimed at increasing yield and the need to produce more with less water.

FruitLook, an online tool that has received recognition internationally, has been gaining good traction in the local fruit sector since 2012. FruitLook is funded by the Western Cape Department of Agriculture and currently available for free. It uses the latest remote sensing satellite technology to help farmers manage crop productivity, growth and water use more precisely (see graphic).

These technologies like remote sensing and geographic information systems (GIS) are designed to help farmers get greater control over the management of farm operations and improve the efficiencies of resource use, says independent researcher Dr. Caren Jarmain. "The full potential of individual and a combination of technologies are far from being fully grasped," says Jarmain. She is also a research associate at the Centre for Geographical Analysis in the Department of Geography and Environmental Studies at Stellenbosch University.

Jarmain believes remote sensing technology integrated into precision agriculture represents "the most promising new frontier for the technology since applied environmental resource monitoring was initiated with Landsat in the early 1970s". The current information age allows for integrating technological advances into precision agriculture, she says. Precision agriculture was developed in the 1990s for arable crops and in mechanised fruit crops like grapes for winemaking. It has only been evolving the last decade for handpicked fruit crops.

FARMING FOR THE FUTURE, TODAY

When it comes to new technology, context is everything, reckons Dr. Albert Strever, viticulturist doing research at Stellenbosch University. Spatial data has the potential to assist farmers to assess and improve their resource use. Says Strever, "The rise of computing power exponentially increased the ability to develop technologies based on artificial intelligence and capable of autonomous action. The local agricultural sector is only starting to deepen its understanding of the implications of these technological advances."

The challenge, however, is about much more than catching up with technology and using all kinds of nifty gadgets on farms, reckons Dr. Elmi Lötze, horticultural researcher at Stellenbosch University. Says Lötze, "Generating heaps of data for the sake of more data makes no sense without interpreting it. We need relevant technologies that are integrated into (fruit) farming practices to be really meaningful. A spade is not a useful tool if you don't know how to use it. The same goes for new technologies."

Hugh Campbell, general manager of Hortgro Science, believes spatial information has the value of "representing relevant information in a visual format that allows for objective decision-making". Says Campbell, "Large chunks of data can be converted into a visual expression of what is happening in an orchard. A spatial view of an orchard coupled with GPS allows you the option of optimising the vineyard of orchard. If one looks at pest control, there is a growing need to manage pests on a wide-area basis. Spatial information allows one to effectively manage and plan at farm and regional level."

It should be evident to anyone who has been following the MegaBoere TV-series (on KykNET) that successful farmers are those that operate close to the cutting edge of new technology, he believes. Says Campbell, "The new technologies like robotics and intelligent systems, satellite monitoring and other technologies provide unique opportunities to problem solving and increasing efficiency. This includes becoming more cost-effective and improving worker safety." The challenge in a South African context is to utilise these technologies to optimise activities and in the process increase labour productivity. The cost of implementing new technologies is also often a problem for local producers.

PICKING FRUIT OF SPATIAL ANALYSIS

If the theme of the 2000s was mass data capture, 2016 and beyond will be all about data driven design, reckons Butler.

This kind of "design thinking" is at the heart of FruitLook. Says Jarmain, "It captures the physical nature of a vineyard or orchard in a spatial manner and yet provides more than just pictures. FruitLook gives access to additional information about the physical world surrounding us." This is because the satellite sensors capture details that are not always visible to the naked eye, she explains. This online tool provides producers with spatial data based on the latest satellite information to analyse crop growth and water status over time and space. Says Jarmain, "You can say it is farming for the future in action already. All this is done without any effort on the producer's side."

FruitLook derived data – it has a spatial resolution of 20 m x 20 m – can show the spatial variation in data very clearly. However, it cannot directly explain the causes for variations visible, whether at field or regional level. The user has to make his or her own interpretation. Says Jarmain, "Farmers can relate to their vineyards and find the variation in actual crop water use very interesting and helpful."

This is possible thanks to FruitLook's unique architecture that integrates satellite data with geographical data and weather information in complex models. Researchers verify the data through field measurements to ensure its credibility. All this information is then integrated into user-friendly maps and graphics.

According to Nelius Kapp, soil and horticultural scientist from Prophyta, FruitLook provides both grower and technical advisor with a "useful mechanism" to evaluate and interrogate the specific actions that have to be implemented. "One of the biggest problems in the local fruit sector remains the lack of monitoring on farms. Many people are doing the basics (including leaf and soil analysis), but people don't know what is happening on a bigger scale," says Kapp. "Technology like FruitLook enables you to determine growth variation in your blocks and provides a more complete picture over time."

Mico Stander, soil scientist from Agrimotion, agrees. "FruitLook offers you a timescale to look at your orchard or vineyard and see if there was a problem, for instance by looking at the biomass at a specific time compared to previous seasons. It is a technology that can help producers to farm more effectively."

FruitLook also allows a grower to see where a section of vineyard or orchard has been over or under irrigated. Says Campbell, "So it has the opportunity to save water, but more importantly to ensure that it stays within the required norms to optimise production."

Capturing information and assessing this data are crucial to determine best practices and achieve increased production. This been the experience of Ernst Heydenreich, general manager of the fruit section of Oak Valley Estate in Grabouw. He uses it to determine the placement of soil moisture probes, detect drainage problems and evaluate irrigation post-seasonal.

This is why André Roux, the Western Cape Department of Agriculture's director of sustainable resource management, believes FruitLook offers "valuable technological applications" for local farmers. "If you improve your farm monitoring, you can diagnose problems better and also act quicker," he says. "It can help detect and manage field variability to save costs and determine the reasons for this."

A BRAVE NEW WORLD OF FARMING

Researchers, consultants and producers alike seem to have consensus: Technological advances like FruitLook are changing the face of local farming.

"The brave new world of farming is upon us," says Prof. Wiehann Steyn from Hortgro Science. "A new technological wave is on the horizon." For the agricultural sector this could potentially lead to farm management practices which only require human interaction when necessary.

The next big technology wave to hit farming is said to be robotics enabling unmanned farming in orchards. Researchers like Prof. Salah Sukkarieh from the Australian Centre for Field Robotics says intelligent robots harvesting in orchards and doing the work of a farm labourer is not entirely as far-fetched as it would seem. He has already designed a robot that can select the correct flower, remove other flowers with a jet of air and at the same time pollinate the selected flower with pollen. Although it will take many years before this kind of technology will be commercially available, the possibilities are potentially endless.

Says Butler, "There are many technological changes happening, but certainly mass censors, genetics, robotics and artificial intelligence hold a lot of promise."

Is there then still a role for humans in the vineyard or orchard of the future? "A huge role, but not doing the mundane," he believes. Says Butler, "Computers are incredibly fast, accurate and stupid. Human beings are incredibly slow, inaccurate and brilliant. Together they are powerful beyond imagination."

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New look at fruit industry (Part 3) FruitLook employs satellites to monitor crops

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KEYWORDS: FruitLook, technology, farmers, satellite.

Satellite “eyes in the sky” have become really useful tools to keep watch over crops.

This technology and its applications are considered increasingly useful by a growing number of producers and consultants alike who use FruitLook to help monitor conditions in vineyards and orchards.

FruitLook is an online tool that helps producers improve crop productivity and water use. Its satellite-derived data can for instance tell you how well your crops are growing, how much water it is using and also how effectively this is done. This is because the satellites “employed” capture details that are not always visible to the naked eye.

“FruitLook provides producers with spatial data based on the latest satellite information to analyse crop growth and water status, over time and space, as well as in different seasons,” explains independent researcher Dr. Caren Jarmain. The tool enables producers to identify areas with weak growth or crops under stress, she says. It can also help pinpoint particular areas experiencing water shortages.

This is possible thanks to the way FruitLook combines satellite-derived data with complex algorithms and weather data. The tool then “translates” it into user-friendly maps for producers. This offers unique “farming intelligence” to producers that is currently available for free, thanks to a subsidy from the Western Cape Department of Agriculture. At the moment FruitLook covers an area of about 170 000 ha and focuses on local deciduous fruit producing areas.

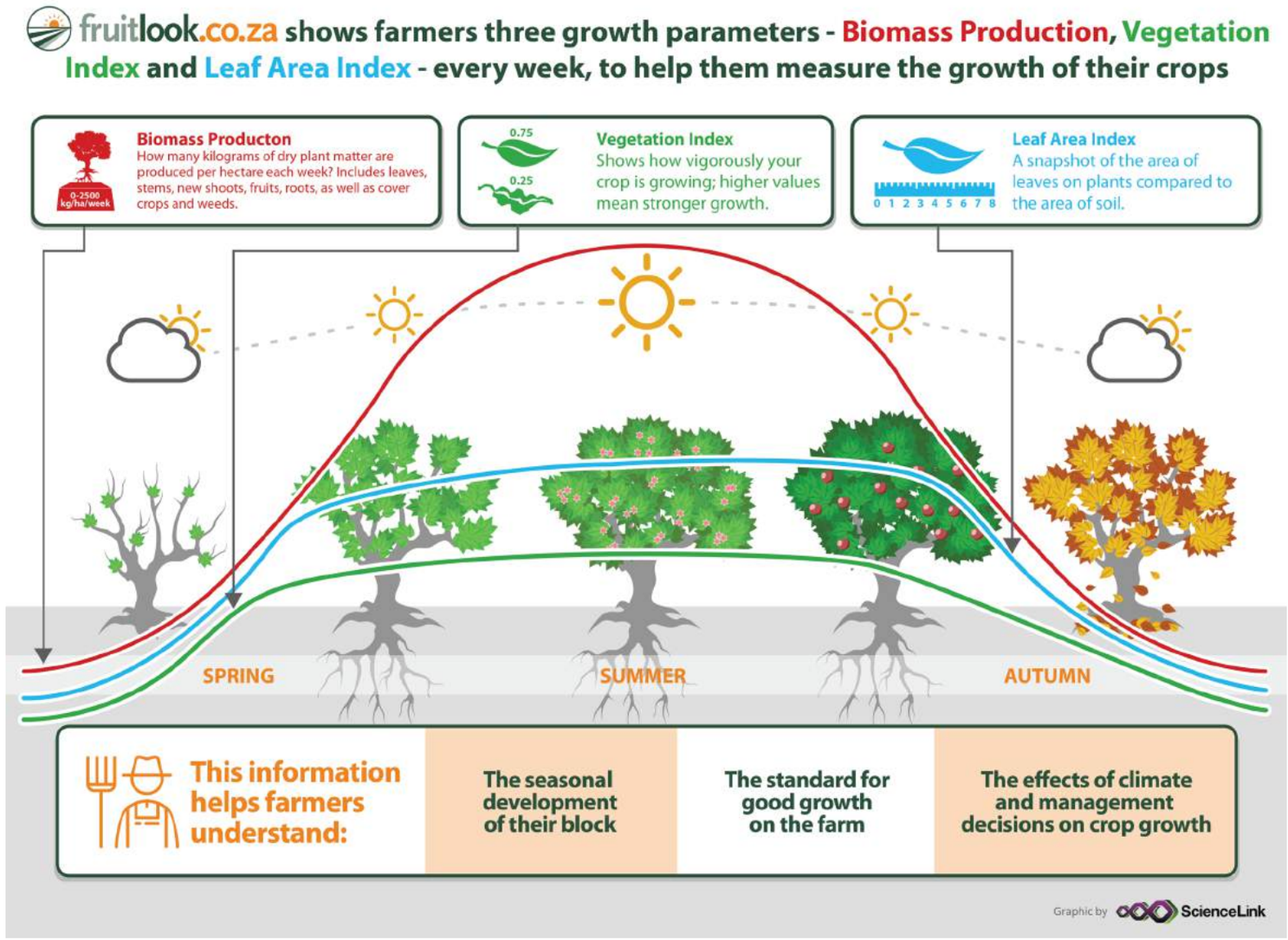
FruitLook’s datasets (growth, water and nutrient related) have proven valuable to many producers who use it to inform their farm management decisions, says Dr. Jarmain. Some use it to detect disease-ridden spots in orchards. Others employ it to evaluate water use and efficiency over large areas. Many also testify that it is helping them to reduce costs, including what they spend on pesticides and irrigation.

FruitLook-derived data is provided at a 20 m x 20 m spatial resolution, showing detailed variation across orchards and vineyards. It measures different features that basically visualise the growth and status of your crop (see graphic). This include indicators of crop performance, how vigorously fruits or vines grow and how much foliage it carries. Variations in biomass production between and within different blocks are also visible in these datasets.

The data on biomass production presents the total dry matter increase of your crop in kilograms per hectare during a specific week. This includes the growth of roots, shoots, fruits, leaves and even the cover crop or weeds in your field. “It is strongly influenced by crop management and block characteristics, as well as the weather,” explains Dr. Jarmain. “Younger crops will produce less biomass than full bearing crops. Biomass production is also normally lower at the beginning and end of the season: at the beginning of the season the crop is not yet fully developed. At the end of the season the leaves start colouring, chlorophyll breaks down and the fruit crop enters a period of dormancy. Weather conditions and seasons also have influence: in the midst of summer solar energy, used for photosynthesis, is more abundant than in spring and fall.”

USING FRUITLOOK’S BIOMASS DATA

FruitLook’s index of biomass production of a single block should have a uniform outlook throughout the season. “Strong variation in a block might indicate part of your block is responding less well to your block management,” explains Dr. Jarmain. “This could for example be due to underlying variation in soil conditions or irrigation issues. In-season variation in biomass production can also be caused by crop hazards like disease or pests.”



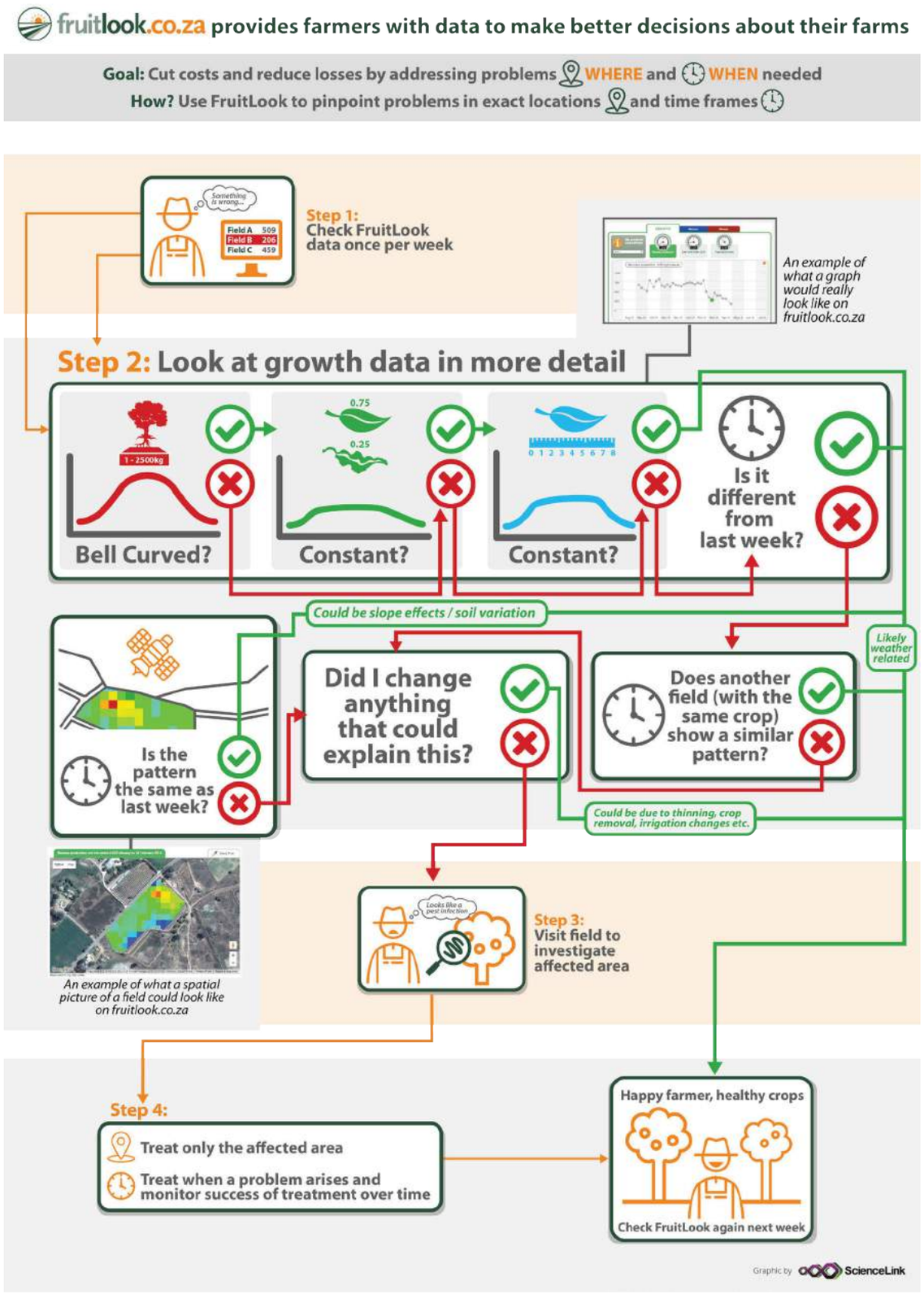
Môreson Trust, a large fruit producer situated near Villiersdorp, has used FruitLook to help inform irrigation practices and to help tackle pest problems in some of its orchards. For an example of the kind of steps involved in this process, see graphic. By keeping an eye on biomass production (and other FruitLook datasets) it helps Môreson's employees to monitor seasonal changes, impact of treatments, as well as growth in different areas.

Says Kobus Swanepoel from Môreson Trust, "We have used FruitLook to delineate areas where diseases affected plant growth. We also targeted our sampling and pesticide treatment in that area. FruitLook for instance showed relatively low biomass production in a certain block in 2012 which corresponded to the spread of nematodes in it. This helped us to only treat the affected area and ensure savings of maybe 75-80% of pesticides used in that block."

Jacques Crous, a horticulturist from First Fruits Consulting, has had a similar experience with FruitLook helping to identify problems in his citrus orchard successfully. He considers FruitLook's growth data a "useful indicator" of an orchard's or tree's ability for photosynthesis.

Crous experienced problems within a particular Eureka orchard established in 2013 on a family farm in the Nuy Valley. "The biomass in the orchard dropped for no apparent reason," he says. "I was away on leave for 10 days and on my return clearly noticed this trend in the data. We investigated and discovered high infestation of thrips (a citrus pest) in the orchard.

"Although we immediately applied a pesticide, the biomass production, according to FruitLook's parameters for it, was still down for about three weeks after this.



This was because of the loss in new growth, as well as permanent damage to the remaining young flush. FruitLook definitely helped unravel the mystery behind the biomass loss at the time. It also helped to quantify the long-term effect of this pest if not controlled in time," he says.

Although FruitLook cannot tell you what causes lower biomass production on your farm, it can give you a very good idea where to look for reasons for your problems. Says Crous, "FruitLook enables producers to compare orchards or vineyards with similar profiles, as well as at different times with each other. Monitoring is key and this is why looking at growth data weekly is useful to show any outliers." Every crop also has a unique seasonal growth curve and this is reflected in the FruitLook data. "If this curve changes there must be a reason why the tree cannot photosynthesise to its optimum capacity," he points out.

Crous considers FruitLook "particularly useful" to help eliminate variation in orchards. "Farmers generally strive for homogeneity within a block, since this would suggest uniformity in production. FruitLook helps identify the extent of spatial variation in an orchard. However, it cannot directly explain the causes for the variations visible. The user has to come up with his or her own interpretation."

WATER USE AND BIOMASS

In the Breede Valley region Petrus Steyn from VillaCrop Protection has used FruitLook to monitor growth in young orchards, as well as identify sections in blocks that are receiving too much or too little water due to irrigation practices. FruitLook's data can also help producers to identify poorer producing vineyards or orchards and improve its management accordingly, he says.

"We had an example in the Robertson region of a new peach orchard with stunted growth in the early season, for no apparent reason. FruitLook's data showed its most vulnerable spots being very similar to those in an old infested orchard in the same area that was removed three years earlier. We interpreted this as an indication of replanting problems and it helped us to identify the reasons for it. Taking nematode samples then confirmed our suspicions. Thanks to FruitLook we treated the (infested) area early enough and none of the crops were damaged."

In this case FruitLook in combination with block inspections and sampling helped to detect a difference in the growth vigour of the new trees early, he says. "This enabled us to focus on the area that was also previously infested, adjust our pesticide use strategy accordingly and also to prevent fruit damage early in the season. The area now has good production and FruitLook contributed to this through timely observations."

Another application of FruitLook's data on biomass growth is to use this information in combination with its datasets on evapotranspiration deficit (an indicator of plant water stress). This is useful for the placement of for instance soil moisture probes.

Jaco Engelbrecht, viticulturist from Paarl, looks at FruitLook's evapotranspiration data (related to water use) in addition to its growth features. He uses it to identify event-based problems within the vineyards (for instance irrigation problems). "FruitLook helps us to adjust our management practices at the time when the plants need it the most," he says. "We try to adjust our irrigation scheduling (in relation to FruitLook's data) to avoid spikes or drops in biomass production." FruitLook can also help clarify why yields in one vineyard are higher than in the other or what the effect is of irrigation scheduling on crop growth.

Others like Karin Cluver, manager of the fruit section on the De Rust farm near Grabouw, employ FruitLook's parameters for biomass production, water use efficiency and evaporation deficit. Cluver compares these datasets weekly and uses it to identify any emerging problems, including non-efficient growth in blocks throughout the season. "I find it particularly useful to look at historic data from previous seasons and compare blocks and the effect of different management practices in them. If you understand your farm and monitor it regularly, it is easier to find reasons when something changes significantly."

Using biomass and water use parameters have enabled De Rust's team to use approximately 20% less water than before, she points out. This is because they can now monitor if the orchards are for instance irrigated too much, especially during the early season. "The data enables us to constantly re-evaluate our crops' growth development and how much or how little water we are giving."

Cluver also considers FruitLook useful to help interpret direct farm observations and to inform management decisions when it comes to disease control and irrigation scheduling. "It has given us access to a bigger picture, but you still have to interpret the data. You have to go into the field to ascertain why particular results were achieved," she stresses.

Distell uses FruitLook's growth indicators to buy grapes of the required quality, as well as to keep certain grapes apart for making top quality wine. According to Isabel Habets of Distell it is also useful to monitor growth differences between blocks and press grapes accordingly. Distell uses it to monitor if a block or a section of it is not doing too well and to make suggestions about changing irrigation practices.

These and other examples emphasise how FruitLook can help inform producers working towards precision agriculture, Dr. Jarmain believes. "In the process it can help producers to improve efficiency on farms, something that is increasingly important given resource scarcities and production pressures. "FruitLook helps farmers understand the effects of farm management on their crop, since information is updated on a weekly basis and farmers can see the results of actions taken."

In Part 4 we will look at how FruitLook is used to inform water management in local vineyards and orchards.

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New look at fruit industry (Part 4) Satellites aid irrigation on fruit farms

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KEYWORDS: FruitLook, technology, farmers, satellite.

In future farmers will have to produce more with less, especially when it comes to fresh water supplies. We take a closer look at how an online tool called FruitLook is helping farmers manage their "open-air factories" better.

WATER AND FARMING

- Eight percent of South Africa's landscapes provide more than half of its fresh water.
- Two-thirds (66%) of all water consumption countrywide is by farmers who are its biggest direct users. Already water issues are compounded by the fact that demand outstrips supply in many parts of the country.
- In the Western Cape, 43% of the available water resources are used for irrigation.
- Fruit crops need between 7 000 m^3 and 11 000 m^3 water per hectare, depending on the type of crop and locality.
- Approximately 2 406 000 ha is under irrigation in the Western Cape. This includes 186 400 ha under fruit crops and 8 000 under vegetable crops.
- Rainfed agriculture is also considered important in the province. Approximately 468 000 ha dry-land grain crops (like wheat, canola and barley) are annually planted in the Western Cape. Another 400 000 ha lucerne is produced with more irrigation.
- Last year's drought caused an estimated loss in wheat production of about 200 000 ton.
- FruitLook measures evapotranspiration (the sum of evaporation and plant transpiration from the surface to the atmosphere) in mm per week. 1 mm evapotranspiration per day is the same as 10 m^3 per hectare per day.
- 1 ℓ evaporation per second per hectare is the same as 8.640 mm per day.

Sources: FruitLook; WWF-SA; DAFF; FAO.

It has been a really dry summer in many parts of the country, mostly due to El Niño causing a serious drought. Many fruit producers are among those still counting the cost of related water stress during critical phases of crop development.

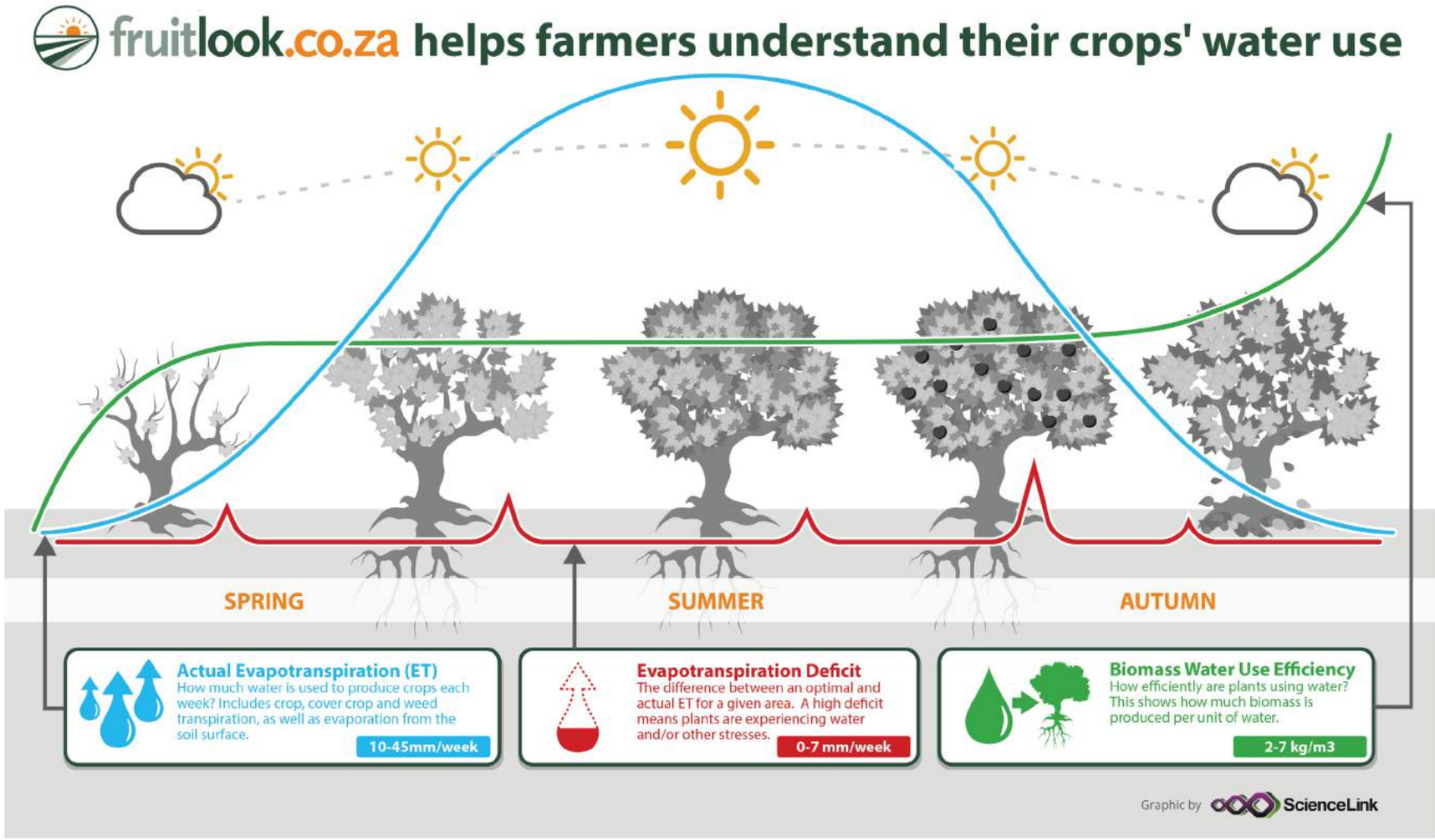
The need to use reliable information tools and best practices to enhance producers' ability to inform farming decisions, is clear.

Luckily, becoming a "water smart" farmer is already possible with FruitLook, says André Roux of the Western Cape Department of Agriculture. FruitLook helps producers to understand how their crops respond as part of the bigger water cycle of their region. By knowing more about the transfer of moisture from the earth to the atmosphere, producers are better placed to efficiently manage their farms. Many producers are using this online tool to provide insights into water use on their farms, helping them to become more resource efficient.

Almost half of the producers using FruitLook have indicated they have cut their water use with a tenth. One in every ten says they are now using almost a third (30%) less water than before. The majority have also indicated it is very useful in the detection of over- or under-irrigation, with over half of them highlighting it could be used to detect irrigation problems (for example pipe leaks).

But how does it work? "Basically, FruitLook employs the latest satellite technology to help farmers manage crop productivity, growth and water use more precisely," explains Dr. Caren Jarmain. She is an independent researcher and research associate at the Centre for Geographical Analysis at Stellenbosch University.

This cutting-edge data service provides remote sensing derived data products to the deciduous fruit producers in the Western Cape, currently free of cost. Says Dr. Jarmain, "Cameras mounted on satellites capture characteristics of the crop which cannot be seen by the eye, providing farming intelligence normally not



GRAPHIC 1

available to the farmer. It can help producers to better understand how their crops use water and how much is needed where."

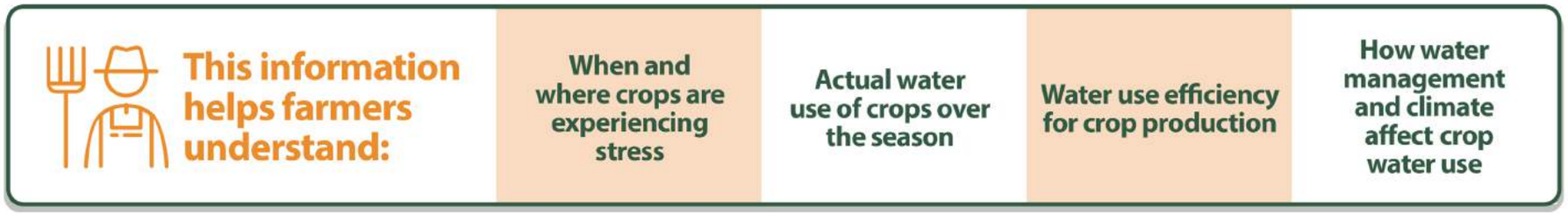
The satellites can, for instance, tell you how well your crop is growing, how much water it is using and also how effectively it is doing that. This allows for a more targeted approach to irrigation with farmers able to monitor their water use. "It is a system that enables you to identify farm-specific areas with weak growth or even pinpoint the particular area in your orchard or vineyard with water shortages," Dr. Jarmain says.

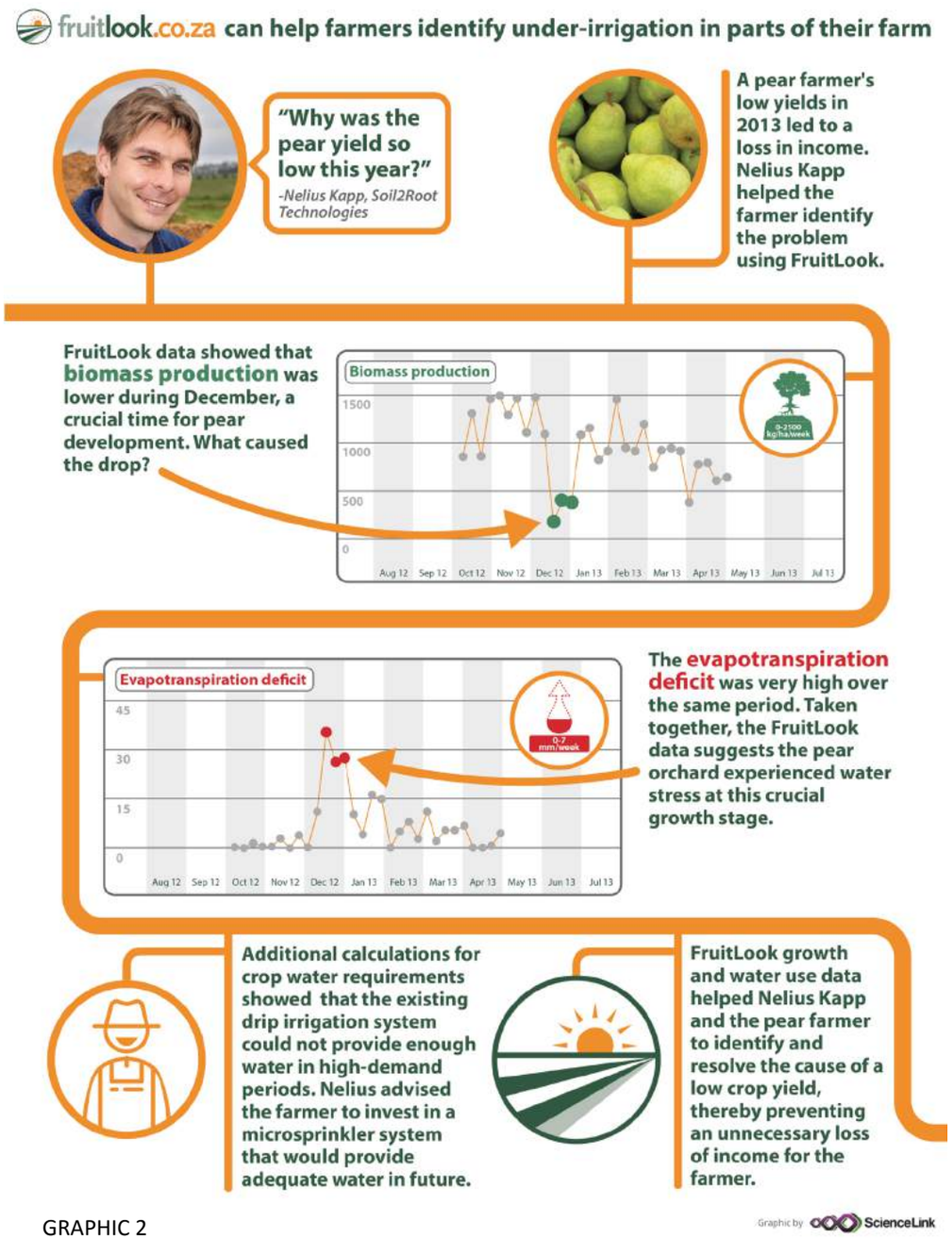
FruitLook integrates the latest in remote sensing technologies by satellite with weather data and complex algorithms and offers three water-related datasets. These relate to actual evapotranspiration, evapotranspiration deficit and biomass water use efficiency in orchards and vineyards. Data about the evapotranspiration deficit, for instance, can give clues about whether plants are under-watered or stressed in some way (see graphic 1 and 2). This helps to take some of the guesswork out of farming and could save producers lots of money, especially on irrigation costs.

Many users say this tool has proven useful to analyse the "bigger picture", especially when it comes to their water needs.

Farmers know that irrigation schedules and probes have their limitations and that you need to adjust your "recipe" according to specific conditions, including plant development and stress factors. This is where FruitLook has often proven useful to check irrigation system performance, indicates soil scientist Nelius Kapp. Kapp, director of Soil2Root Technologies, has been using FruitLook for several years. For instance, he looks at its data on biomass production along with its datasets on evapotranspiration deficit (an indicator of water stress in plants) to unravel what is happening in a problem area.

Anton Müller, Kromco's technical advisor, considers FruitLook especially useful to detect irrigation issues, for the placement of soil moisture probes, the detection of drainage problems and to evaluate just how efficient irrigation regimes were during a "post-mortem" of the season. "FruitLook also helps clarify what the effects are of irrigation scheduling on crop growth," he adds.





GRAPHIC 2

Both under- and over-irrigation have a negative impact on water use efficiency and yield, thus it is important that users understand and improve on both.

Says Müller, "FruitLook allows growers to see where in the orchard trees have been over- or under-irrigated. This information helps farmers understand the actual crop water use over the season, water efficiency on their farms and fields and how crops respond to changes in irrigation and climate. Thanks to FruitLook, producers also have farm-specific data compiled over past growing seasons that can act as a reference point. I find that very useful."

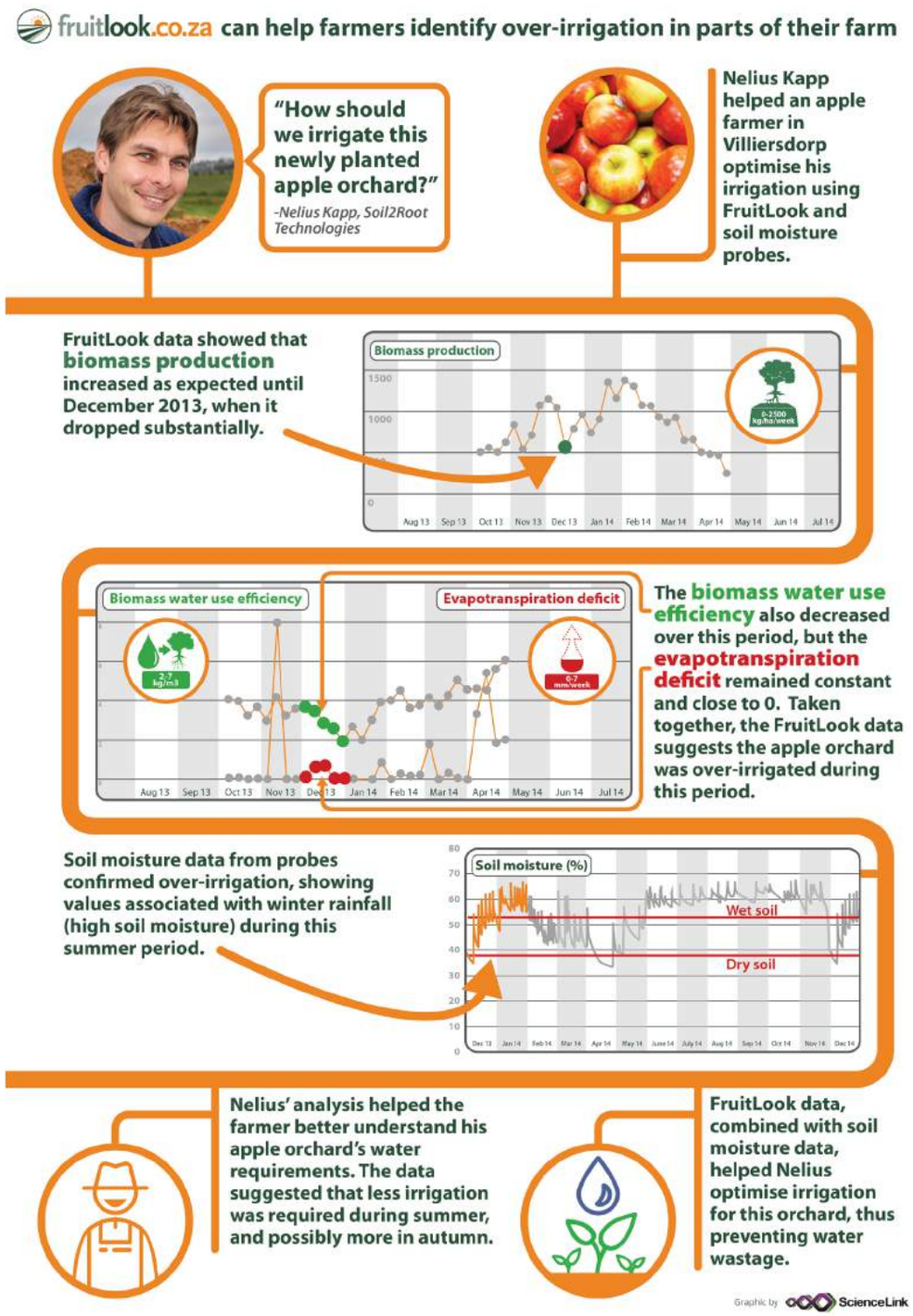
Some farms in the Grabouw region have for instance been able to reduce their early-season water use with up to 30%. This was done using FruitLook in combination with soil probes.

Sometimes issues related to irrigation in an orchard are first noticed thanks to unusual changes flagged by FruitLook's growth data. Müller gave an example of a 5 ha apple orchard in the region that developed a real problem with red spider mite infestation late January. "The evapotranspiration deficit data showed clearly that the trees were under stress during early January. Considering integrated pest management on the farm, the problem was resolved by biological control after it was decided to adjust water scheduling, rather than spray in masses. Adjusting water management helped reduce stress in the orchard. The pest issue at hand was an irrigation issue."

Jaco Engelbrecht, a viticulturist from the Boland, has had similar experiences. He often also considers FruitLook's evapotranspiration data. Engelbrecht uses it to identify event-based problems within the vineyards. "FruitLook helps us to adjust our management practices when the plants need it the most," he says. "We adjust our irrigation accordingly to avoid spikes or drops in biomass production."

Kapp often employs FruitLook's biomass data in conjunction with its moisture parameters. He also uses it in conjunction with information from soil profiles to decide where to for instance place soil moisture probes.

"FruitLook is useful to identify areas that are experiencing evapotranspiration deficits, but it won't tell you the reason. Sometimes there are other reasons why this dataset would for instance show changes in your orchard. This could be because of the wind, pests or atmospheric stresses like a heat wave. Relying on a satellite image alone will not necessarily give you the answer. You still have to interpret the data." The data must be assessed given unique block, farm and regional conditions, he emphasises.



GRAPHIC 3

The real value of FruitLook, Kapp believes, emerges when used in collaboration with soil moisture probe data, information from weather stations and hands-on investigations on the ground. "FruitLook not only helps you understand the current season, but also to look back at conditions in the seasons. To interpret decisions around water scheduling you have to think farm specific and integrate all your measurements and observations," he says. See graphic 3 for some practical examples from Kapp.

Len van der Merwe, who manages the Ratelfontein Boerdery near Villiersdorp, agrees. He has been using FruitLook for approximately two years to inform farm management decisions. "FruitLook offers me the opportunity to look back over the season and to assess some of the irrigation decisions we made. I access the data weekly and adjust scheduling accordingly, along with soil moisture probe readings." The probes give detailed information over time and the depth of soil and the other data provides a picture of the entire orchard.

He considers FruitLook a "handy" tool to use in pursuit of more precision-type agriculture. It is also valuable when trying to calibrate irrigation scheduling and helps to visualise trends in soil drying. "FruitLook offers a nice way to check if what you have been doing last week and earlier in the season are having the desired results. Our farms have different soil conditions. We have to, for instance, consider factors like slopes and drainage to make sense of why one section grows slower or dries out quicker. It has proven useful to compare different areas with each other."

At Spier near Stellenbosch, FruitLook is employed to help with qualitative assessments of the vineyards. Orlando Filander from Spier says it helps them ensure that their irrigation is optimal. "FruitLook's maps clearly show difference in the blocks, whether growth is weak or good or whether dry or wet conditions are present. It also gives an indication about what is wrong even before you sometimes see the symptoms with the naked eye."

JC Goosen from DP and JC Goosen Boerdery near Ceres monitors 300 ha orchards with this tool. Goosen looks at FruitLook and soil data weekly to establish whether or not there were incidents of over- and under-irrigation during the previous week. He says FruitLook helps him save a lot of time. "It enables me to look at all my blocks in one and a half hours. It also helps to look at certain blocks in a targeted way during field investigations."

FruitLook reflects farm specific and regional conditions. And therein lies the beauty of using this kind of remote sensing tool that can look back in time and space. "A better picture emerges of what is happening on the whole farm when you integrate this with your existing farming practices," Müller concludes.

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New look at fruit industry (Part 5) FruitLook offers bigger picture

NOVEMBER 2016

JORISNA BONTHUYS
Freelance journalist

KEYWORDS: FruitLook, technology, farmers, satellite.

FruitLook is increasingly gaining traction from local users. We asked producers how they use and integrate it with other technologies and information.

Producers are embracing various new-generation tools to better understand their block and orchard development.

FruitLook employs satellite remote sensing and this is becoming increasingly popular. This online tool has been tailor-made for local use. It offers a unique view on different aspects of a block or orchard, including water use and crop production. This is possible thanks to FruitLook's ability to collate useful information that is not always visible to the naked eye.

FruitLook's data is made available in the form of a range of user-friendly maps of production units and can be used by the producer in many ways. See graphic for examples. Producers can, for instance, use it to monitor growth differences in their production units by using FruitLook's indicators on biomass production over time. It can also be used to decide where to place soil moisture probes and even to harvest selectively.

"Each one of FruitLook's data sets (on growth, water use and nitrogen) shows a different picture and provides insights on your block," explains independent researcher Dr. Caren Jarmain. "The more picture elements available, the more comprehensive the puzzle that can be built with it."

As part of FruitLook's dataservice to producers, it is now also possible to automatically let them know if the satellites detect huge differences in blocks compared with the "normal" picture. This electronic "watchdog" feature that automatically alerts producers of such changes via email, is called FruitSupport.

A healthy vineyard-block will look rather uniform throughout the season when it comes to FruitLook's growth indicators. If this picture changes suddenly, it can be because of pests, water stresses and other issues related to crop-growth in the block. "This enables producers to adjust their management decisions or if there is a problem to correct it to ensure optimal growth in the block," says Jarmain.

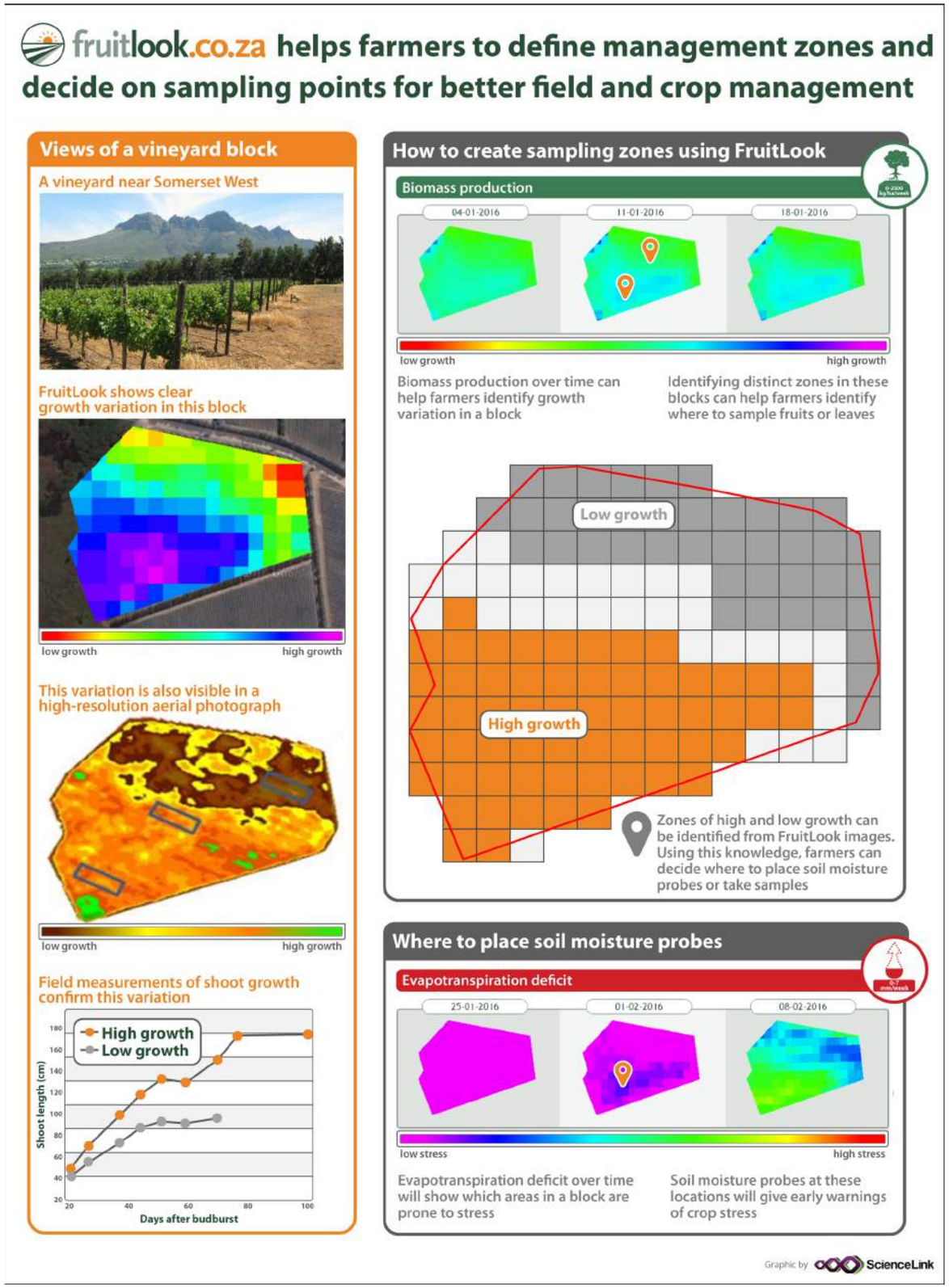
Nigel Cook, a consultant from Prophyta, considers FruitLook a handy tool when it comes to irrigation scheduling. "FruitLook offers an independent tool to measure what is happening in the block and can inform decisions on scheduling."

Cook adds: "Before FruitLook was available, it was much more difficult for a producer to get a bird's eye view of a block or a farm. You can compare it with someone playing golf without a scorecard. FruitLook is also not only useful in-season, but to look back on a season to see how well you fared (from a management perspective)."

He believes it is really important to consider soil conditions when making decisions on irrigation scheduling on a farm. Integrating this information with FruitLook's water indicators provides unique farm-based intelligence.

Dr. Albert Strever, a viticulturist from Stellenbosch University, considers FruitLook a valuable tool for producers, especially when it is integrated with other technologies. The difference in the growth of areas in a block can, for instance, be useful to provide information on its management, given that variations are often linked to soil differences related to water and growth.

"It can be used to assess resource use on farms and make it more efficient if possible," he says. "It, however, depends on the readiness of a producer or



consultant to interpret and/or apply the data that is being generated," he says.

Strever is involved in a research project on how FruitLook can be used to do yield projections. The team, with Jarmain as its project leader, is looking at phenological, as well as yield data and employs statistical and machine learning techniques for this. The project employs both statistical analysis and machine learning to discover relationships between remote sensing data and yield data, in order to model yield in future.

FruitLook's unique datasets can also be used in combination with other technologies and information, for instance, when it comes to soil moisture samples.

Tiaan Snyman, a soil scientist from Agrimotion, says this process of integration can yield interesting information for producers. "If a soil moisture probe, for instance, shows the soil is dry and we see that the orchard or block is using water in a less efficient way, we can say with certainty that this is due to under-irrigation. The more data we integrate, the better our answers and recommendations.

"Growth differences in blocks often indicate soil differences and soil samples are therefore taken in different areas," he says. "FruitLook, along with soil and plant samples allows us to compile a comprehensive picture."

He considers FruitLook a very efficient tool to understand variation in a block. "Consider data from a warm week in December, for instance. FruitLook's indicators on how efficient water is used, as well as what type of variation is visible along with soil moisture meters will provide the most representative measurement."

Distell has been using FruitLook in season in combination with measurements on optimal ripeness. At Plaisir de Merle (near Franschhoek), grape samples were taken at different places in the blocks based on growth differences established with FruitLook. They tried to harvest the grapes separately in different blocks where possible.

Distell aimed at harvesting a block as soon as the Maselli index indicated that the grapes were optimally ripe. Their blocks are generally homogeneous, but sometimes strong and weak growth areas are evident. They want to experiment with making different wines from this separately harvested grapes and to establish how growth differences affect wine quality for different cultivars.

Dirk Sutherland from Omnia says FruitLook is also very useful to identify weak areas in blocks when it comes to soil health, if used together with other applications and measurements. "It is

becoming increasingly important to evaluate soil health. We combine a physical, chemical and biological assessment to determine why growth in a certain area is weak and compare it with assessments from better sections in the block.

He marks these “weak spots” with GPS and combines it with other applications used by Omnia. It is very interesting how soil life differs between the weak spots and the better ones, he says. In this experience FruitLook helps a lot to take accurate samples with much less effort when it comes to applying fertilisers in general. It also gives you an idea of how water moves through the block.

He uses FruitLook to identify two GPS points per block that he considers as being representative when he does his sampling. This enables him to revisit the same places next year to do follow-up measurements. “This enables me to say with greater certainty whether or not the fertiliser programme I employed worked or not, seeing that my sampling can be standardised.”

Sutherland believes FruitLook’s technology, also when integrated with other technology and information, enables farmers to manage water, fertilisers and pest control in an economically more sustainable manner. “I believe FruitLook will enable producers to micro-manage on a bigger scale.”

When it is used in collaboration with other technologies and information that producers have access to, including their current knowledge of their farm and production units, it also unlocks more possibilities to use and manage available resources more efficiently.

Snyman also adds that FruitLook is especially valuable to producers given that it is independent, available for free and also updated weekly. “It offers a fantastic way to look at a block or orchard in an entirely new way,” he says.

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New look at fruit industry (Part 6) FruitLook offers bigger picture

DECEMBER 2016

JORISNA BONTHUYS
Freelance journalist

KEYWORDS: FruitLook, technology, farmers, satellite.

The face of agriculture and natural resource management is changing with satellites and remote sensing playing an increasing role to inform decision-making. We look at how a service called FruitLook is strategically placed to help support local fruit producers, and agriculture in general, on their journey towards more efficient water use.

MORE ABOUT FRUITLOOK

- FruitLook is an online tool that helps farmers improve yield and save resources. It helps farmers monitor their crops and optimise irrigation.
- FruitLook integrates satellite data with geographical data and weather information in complex models and produces user-friendly, farm-specific maps and information for producers and consultants alike.
- FruitLook offers a unique view on different aspects of a production unit, including water use and crop production. This is possible thanks to its ability to collate relevant information that is not always visible to the naked eye.
- FruitLook allows a grower to see where an orchard or vineyard has received too much or too little irrigation. It provides the opportunity to save water and to ensure that a production unit stays within the required norms to optimise production.
- Almost half of the producers using it indicated they have cut their water use with a tenth. One in every ten producers says they are using almost a third (30%) less water than before.

It has been a really challenging year in the agriculture sector with the effect of the recent drought being felt widely, including in the local fruit sector.

Last season's drought (the worst in 112 years according to the weather gurus' records) has emphasised yet again just how important efficient resource use is to local farmers.

Even before last season's drought hit, water availability was already set to be the single biggest factor in limiting agriculture production. Demand is already outstripping supply in many catchment areas.

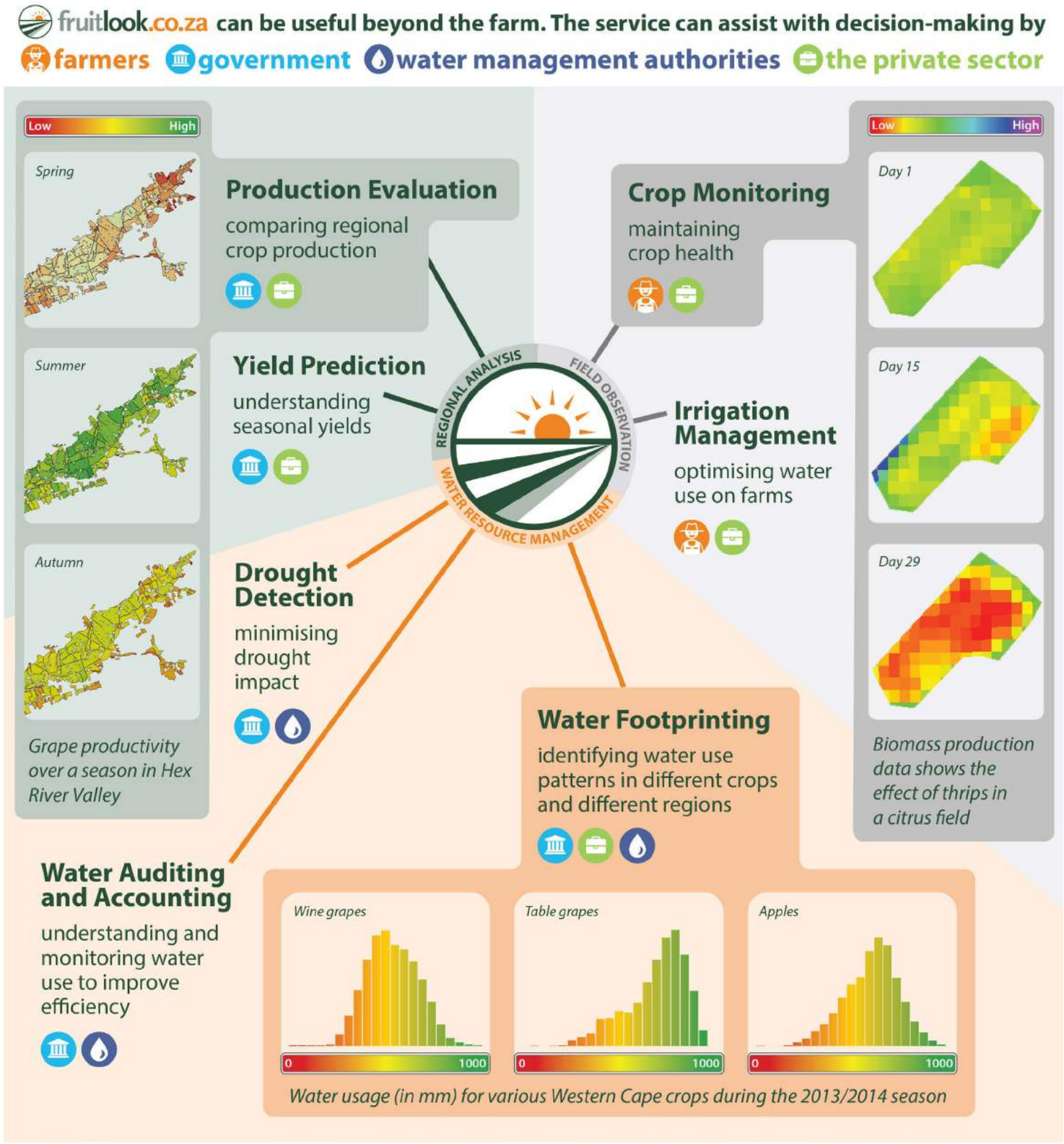
In the Western Cape – the heart of the country's fruit and wine producing region – water allocations to the agricultural sector have already been capped. This means that water efficiency measures like precision agriculture and "satellite farming" will be crucial for any further agricultural expansion that requires irrigation.

There is an increasing need to improve the efficiency of resource use to mitigate impacts of climate change, says André Roux, Director for Sustainable Resource Management in the Western Cape's Department of Agriculture. Farmers will need to produce more crop per drop, especially given rising input costs and more erratic weather patterns emerging.

Rising input costs (especially energy) and increased competition for water compel producers to improve their water-use efficiency in terms of agricultural yield per amount of water consumed. It is imperative to measure crop water use in order to achieve high water productivity and food security, he believes. "You can't manage what you don't measure," says Roux.

This is where a data service called FruitLook has proven especially useful to many producers. It employs the latest satellite technology to help farmers precisely manage crop productivity, growth and water use. FruitLook uses satellite-derived information to help farmers decide about optimal timing, extent and location of inputs such as water and fertiliser.

As an example, FruitLook monitors just how much water is released from fruit trees and vineyards through evapotranspiration and how efficiently water is used for crop growth. Says Roux, "The satellites can, for instance, tell you how well your crop is growing, how much water it is using and also how effectively it is doing that."



Water user associations are also seeing the benefits of FruitLook and are encouraging their members to use it. Quinton Brynard from the Winelands Water User Association says: “We encourage our members to use FruitLook, not only to use water more effectively, but also to do more with the water that is available. Our resources are under incredible stress and this is not going to change in the foreseeable future. We need to find ways to do more with less.” Christiaan Olivier from the Hex Valley Water Users Association believes producers can benefit from using it. “The user must, however, understand the technology and spend time analysing it.”

Due to the expected crises with the limited irrigation water available during the 2016/17 season, the area covered by FruitLook was recently extended. The area under its satellites’ scrutiny now roughly stretches from Lutzville/Vredendal (in the north) to the Hemel-en-Aarde Valley (in the south) and Montagu and Bonnievale (in the east). Roux’s department is currently subsidising farmers to use this high-tech remote sensing information service, free of charge.

BEYOND THE FARM GATE

The data behind FruitLook can also be useful beyond the farm gate. It can be used for regional analysis (including research into yield prediction) and water resource management (including drought detection, water audits and water footprinting). See graphic for more examples.

In the case of FruitLook, the application of remote sensing is very specifically aimed at providing farmers with the right information to help them increase their water use

efficiency (enough water at the right time). There are several 'by-products' from FruitLook's work that help to improve the efficiency of water use in agriculture in a different way.

FruitLook's datasets have shown many linkages, overlaps and potential areas of collaboration with other satellite and remote sensing research underway, says independent researcher Dr. Caren Jarmain. The work that underpins FruitLook is an example of "where the science of agriculture and water meets", she says. Jarmain is involved in a research project on behalf of the Water Research Commission (WRC) performed by Stellenbosch University (SU) to determine the actual area under irrigation and the volume of water required annually for this. FruitLook's data is used to determine the actual crop water use in the Western Cape and is used to calibrate models that are being developed to estimate actual crop water use across the country.

FruitLook is considered "one of the most advanced remote sensing tools" applied to local agricultural practices.

Shelly Fuller, WWF-SA's Fruit & Wine Programme Manager says FruitLook and other remote sensing technologies have "enormous potential" to help the agricultural sector become more water-use efficient. "Remote sensing and satellite data services offer a valuable option for farmers," she says. "It enables producers to know what to use where and when and save costs. The government can use it to establish realistic baselines in terms of water usage figures per crop and region and to establish risk areas for future (where to shift crops across the country)." FruitLook could be used for a catchment level view of water efficiency.

GreenCape, a sector development agency that supports businesses operating within the green economy, is using some of the results from FruitLook to validate the crop water requirements they have calculated as part of their own water research project in the Western Cape. Although they are not currently using FruitLook for research purposes, this data could according to Claire Pengelly from GreenCape have application in water footprinting models as it provides information about crop water requirements.

Following this line of thought, David Black from the consulting service Blue North has been involved in efforts to use remote sensing data to determine the water footprints of stone fruits. He considers FruitLook "a powerful and cost-effective water management tool". Blue North has used FruitLook's evapotranspiration data to calculate the "green" and "blue" components in their water footprinting work as part of efforts to curb water risk on farms. Says Black, "Because it is modelled on real data, crop water use figures should be more accurate than those obtained through a crop water balance model (given the range of input assumptions), and obviously far more practical than actual in-field measurements."

The output of a water footprint is expressed as a volume per mass of product figure (i.e. m^3/ton) and is comprised of the "green", "blue" and "grey" water components. This is not grey water in the conventional sense, but an indicator of the negative impacts of contaminants on local water resources. Says Black, "It is an effective means of identifying water-related risks and opportunities within agri-value chains, and communicating these risks and opportunities with stakeholders further up the value chain."

Regular and consistent recording of remote sensing data, combined with withdrawal quotas and records, can also be a powerful tool for driving catchment-scale water management discussions, especially in agricultural regions. Black adds, "The capabilities of remote sensing seem to be constantly evolving as new algorithms are developed and tested, and is no doubt the future of resource-efficient agriculture. I'm sure FruitLook, and its product offering, will continue to evolve accordingly, and it would be great to see a country-wide roll-out of FruitLook."

REMOTE SENSING FOR THE FUTURE

Although satellite products have been around for decades, producers and consultants alike now have free and timeous access via the Internet to products that previously would have been prohibitively expensive.

Remote sensing of the earth's surface for observation is as yet in many ways an untapped suite of possibilities, according to Mireille Lewarne from WWF-SA's agricultural team. Says Lewarne, "If remote sensing offered us a language to interpret what is happening on the earth's surface, we are still in the learning-the-alphabet stage, maybe putting a few simple words together."

Remote sensing is applied in various ways. A number of researchers are mapping irrigated areas with it and doing research on the water footprint of crops. Others are interested in river discharge, improving efficient water use on farms or identifying just how much of our precious water resources are currently being used by water thirsty alien invasive plants and trees. Collective efforts are under way to gain a better understanding of what this means for water availability in future. Heaps of data is collected in the process that needs to be interpreted.

Prof Adriaan van Niekerk from SU says "big data" and remote sensing can help a great deal to help make agriculture more water efficient in future. Prof Van Niekerk, director of the Centre for Geographical Analysis at the university, is the project leader on mapping South Africa's irrigated agriculture for the WRC. He is also involved in "machine learning" exercises for FruitLook.

"Big data/remote sensing is critical for agriculture, but its employment in South Africa is still in its infancy," says Prof Van Niekerk. "Five years ago the main challenge was to obtain suitable imagery to support farming practices, but that has changed dramatically over the past few years. The volume of remotely sensed imagery is exploding, with high spatial and temporal resolution data becoming available free of charge (e.g. Sentinel-2 satellites)."

"The challenge now is to process and make sense of this data. It is impossible for the human brain to analyse the enormous volumes of imagery, and relate it to what is going on on the ground. The only way to effectively use this data is to make use of machine learning (also called "deep" learning). By supplying a small set of known data (e.g. poor crop condition measured in the field) to these sophisticated algorithms, relationships with satellite imagery (and other spatial data) can be identified and exploited."

Remote sensing also offers a possible viable way of mapping constantly changing environments for seasonal variation, as well as for threshold changes.

One of the highest risks to both conservation and agricultural production remains the invasion of alien plants. Various institutions, including CSIR, is driving research

projects using remote sensing on this. This includes the application of FruitLook data to better understand water use by invasive plant species. A challenge, however, remains identification of invaded areas. Says Lewarne, “Numerous attempts have already been made to distinguish the spectral signature of alien plants from natural veld or forests. This remains an area of keen focus, as it costs the government and private landowners more and more to control or remove alien plants. They are the number one threat to water availability in many areas, and pose a high risk for fire damage to crops and infrastructure, and if the fires are too frequent, to the longevity and diversity in natural habitats.”

Dr Mike Wallace, specialist scientist at the Western Cape Department of Agriculture’s Research and Technology Development Services, is also using remote sensing in dry land crop and vegetation monitoring and modelling. This enables him to assess vast areas without visiting them and assess drought situations.

Although an extensive arable farmer cannot do much about supplying more water to his crop, there are important management decisions that can be supported by this work, including about predicted yield adjustment and making decisions about fertilisers. Likewise, a grazing farmer or their advisor will be supported by these technologies on decisions regarding stocking rates and local distribution of drought impacts, he says.

How does FruitLook fit into these regional and nationwide strategies to deal with water and environmental issues? It provides valuable information on actual crop water use of the previous week of the selected crop, orchard or vineyard.

Roux explains, “This can guide the irrigators to optimise the water use in the current week. Water savings in excess of 10% are quite common for the users of the FruitLook data and saving water also results in electricity savings. Reduced irrigation water run-off reduces/eliminates the pollution of our rivers and streams with fertiliser enriched water, which has a tremendous positive impact on the environment.”

Roux believes the optimal use of the limited irrigation water available this summer season due to the drought will be crucial for farmers to produce a quality crop with the water restrictions in place. “FruitLook’s data will greatly contribute towards an increase in agricultural water use efficiency. This all starts by assisting irrigators to manage their limited water resources in the upcoming irrigation season.”

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For more information visit www.fruitlook.co.za or contact Caren Jarmain or Ruben Goudriaan at info@fruitlook.co.za.

What is the SmartAgri project?

MAY 2016

STEPHANIE MIDGLEY
African Climate & Development Initiative (ACDI), University of Cape Town
KEYWORDS: SmartAgri, climate, agriculture.

The Western Cape government's SmartAgri project recently reached a milestone with the completion of the Climate Change response framework and implementation plan for the Western Cape agricultural sector.

The province has noticed an increasing demand for support to farmers and agribusiness to become more climate resilient. The current drought emphasises the sector's vulnerability to climatic extremes, and climate change will increasingly create conditions that are detrimental to farmers.

Although farmers have always reacted to climatic variability, in future it will be necessary to pay more attention to innovative solutions to confront long term changes. The sector will have to collaborate to implement tangible and co-ordinated actions so as to prevent climate change from destabilising agriculture and food security and undermining economic and rural development.

In a first joint effort the Western Cape Departments of Agriculture and Environmental Affairs and Development Planning launched the SmartAgri project (*SmartAgriculture for Climate Resilience*) in August 2014. Under the leadership of the University of Cape Town's *African Climate and Development Initiative* (ACDI) a

Floods can cause severe damage to vineyards.

Vineyards will require more water in a future warmer climate.

consortium developed a provincial framework and accompanying implementation plan for reaction to climate change for the Western Cape agricultural sector. The plan identifies and prioritises practical and relevant reactions specifically for the Western Cape agricultural sector.

Weather data show warming has already taken place (generally 1.0°C over the last 50 years), especially in mid- to late summer. The data show a decline in the number of rain days across the province and especially in autumn, but with some increasing trends in the western regions in spring. This may indicate a progressively later start and end to the seasons. So far there have been no noticeable trends in total precipitation during the winter season or annually across the province.

Climate modelling studies indicate that the average annual temperatures will increase further by between 1.5°C and 3°C by mid-century, compared to the annual temperatures experienced from 1976 - 2005. The biggest increases will probably be experienced in the interior, and the lowest along the coast. Consequently we can expect higher minimum and maximum temperatures, while the number of cold days and cold nights will decrease and the number of very hot days and nights will increase. For fruit cultivation it is important to note that cold units will decrease significantly, with a bigger impact in the warmer regions. Warmer conditions also result in fruit quality issues.

The studies show with a high level of certainty (in other words almost all models agree in this regard) that the western parts of the province will experience a decrease in winter rainfall by mid-century and thereafter. In the short term, however, the influence of mountains and oceans may result in increased rainfall along the wind aspect of the mountains, or cause precipitation to move to spring and summer. Higher levels of evaporation and plant water usage will place more pressure on water resources.

To react to climate related risks requires decision-making in a changing and uncertain world. The Western Cape agricultural sector adapts to the changes by reacting to the demands of the current climate variability and extremes in terms of socio-economic pressure. If producers and their value chain partners have access to a wider range of suitable options, they will be better equipped to innovate and adapt their practices. This may include improved water and soil management, identification of modified harvest times with accompanying cultivar and marketing implications, and planning for droughts and other climatic disasters.

The Western Cape Department of Agriculture undertakes to help optimise the sustainable use of water and soil resources and further increase climate smart agricultural production. Various initiatives have been implemented for many years, such as for example the FruitLook programme and the conservation agriculture focus. Such programmes and others should be upscaled to a larger group of beneficiaries, and have been integrated into this sector-wide strategy.

Several information booklets aimed at farmers, including one for wine farmers, are available in English and Afrikaans.

For more information about the SmartAgri project and the Climate Change framework and implementation plan, visit the Western Cape's new GreenAgri information portal at http://www.greenagri.org.za.

Lourensford PV (photovoltaic) installation.

Guideline for energy management in the South African wine industry

JUNE 2016

ALAN BRENT
Centre for Renewable & Sustainable Energy Studies, Stellenbosch University
KEYWORDS: Energy management, electricity, carbon footprint, EnMS.

The South African wine industry has had an increased focus on energy efficiency, contributable to a number of drivers.

RISING COST OF ELECTRICITY

Two factors influence the cost of energy in South Africa: the security of electricity supply (capacity), and the rising energy resource prices due to limited availability. The majority of electricity in South Africa is generated from (old) coal-fired power stations. Recently, Eskom has been under pressure to guarantee security of supply, and the addition of new assets that are dependent on variable resource prices has meant the inevitable increases in electricity prices.

CLIMATE CHANGE

The South African wine industry is vulnerable to climate change as this will affect grape growing directly, since the result of climate change is the increase in extreme weather events, such as droughts and floods. Our electricity has a high carbon emissions factor, compared to the rest of the world. These factors make it essential for the energy users to adopt more sustainable methods of utilising energy.

POLITICAL LANDSCAPE

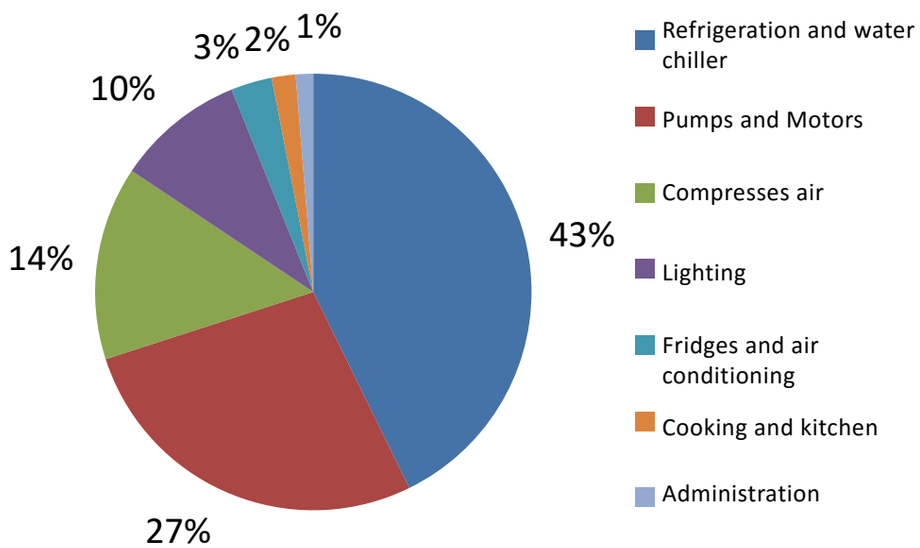
The South African government has set carbon emissions reduction targets through the National Development Plan. This has led to stricter energy regulations, as well as subsidies and rebates that have been made available through green economy initiatives. Internationally, carbon taxation is a threat for South African wine producers as our wine inherently has a high carbon footprint due to the electricity from Eskom, and the distance to market.

CONSUMER AWARENESS

Consumers are increasingly becoming more aware of the impact of their product choices. Consumers are then asking more questions than ever, while the internet and social media have made ethical consumer behaviour easier. The growing prevalence of “green marketing” is indicative of this trend. Informed consumers are likely to give preference to a product produced by a company who is making an effort to use sustainable production methods.

TABLE 1. Key aspects for a winery to engage with a service provider.

Energy efficiency plan	The winemaker or operations manager should have a written energy efficiency plan that details the methods of energy consumption reduction within the wine operations. This should include energy consumption reduction strategy, training of personnel to improve energy consumption, and monitoring programmes. A documented energy efficiency plan will assist the wine farm to achieve the energy efficiency and cost reduction in a formal manner. A plan will document how, when and where energy use will be monitored and how the results will be used to improve energy efficiency. • Do you have an executive level commitment to a successful energy management? • Have you assigned an in-house energy manager to oversee management of energy at your winery? • Do you track costs and benefits of energy management? • Have you developed an inherent system for continuous improvement of energy management?
Energy monitoring	Energy consumption data should be collected to enable detailed analysis of the winery energy usage. Real time data will allow the winemaker or operations manager to identify peak demands and high energy usage processes that could be targeted for energy reduction. The energy consumption records could be in the form of monthly financial statement or in the form of: electricity usage (kWh), diesel usage (litres), petrol (litres), coal (tonnes), etcetera. • Have you established baseline using appropriate measures of performance for each system? • Have you quantified current energy used and losses? • Do you shift electricity consumption into less expensive off-peak times? • Have you evaluated using alternative and/or renewable energy like wind, solar, biofuels and others? • Do you incorporate carbon footprint information in all energy management evaluations?
Equipment servicing and optimisation	Energy efficiency should be put on high priority when purchasing new equipment. Also, the existing equipment should be serviced regularly and repaired whenever necessary to ensure optimum energy consumption.
Optimisation of refrigeration and cooling efficiencies	Refrigeration and cooling systems provide an opportunity for the cost reduction and energy efficiency. This can simply be achieved by optimising the existing equipment. • Have you reduced suction pressure to reduce compressor power and save energy? • Do you variably adjust condenser set-point temperature to optimise compressor pressure difference for varying ambient temperatures? • Have you reduced excess heat gain from: interior lights (replace with LED), inadequate defrosting, inadequate insulation, excessive air exchange, worn weather stripping, etcetera? • Have you insulated refrigeration lines? • Do you optimise tank volume? • Have you installed variable speed controls (where necessary) on condenser and evaporator?
Are you using the most efficient lighting sources?	Space lighting provides an opportunity for cost reduction and efficient energy use. This can be achieved by replacing the existing light bulbs with more energy efficient light bulbs. Also, the existing energy equipment can be optimised for better operation. • Have you established necessary light levels for specific areas when used and unused? • Have you reduced lighting levels where appropriate? • Do you utilise lighting controls (time clocks, by-pass/delay timers and motion detectors)? • Do you make use of natural lighting (daylighting – windows and skylights)?
Utilisation of programmes to maintain and operate all motors, belts, drives, fans, pumps and compressors for optimum energy use	Overall, there should be programmes in place for all the energy consuming equipment on the wine farm. Energy reduction and cost cutting will not occur homogenously, however, the combination of these programmes may result in breakthrough improvements. • Have you installed properly sized premium efficiency motors? • Have you installed timers and sensor controls to turn off during idle time? • Have you installed properly sized energy efficient pumps and fans? • Do you perform regular preventative maintenance?



Typical winery energy consumption distribution.

COMPETITIVE ADVANTAGE

Improving energy efficiency will reduce the cost of production, which, in turn, will increase the profit margin on the sale of a bottle of wine. Wineries that are taking action to become more energy efficient may be eligible to benefit from financial incentives provided by the government, such as subsidies and rebates. By investing in renewable energy, a winery is also likely to improve energy security in the long term.

TOWARDS BEST PRACTICE ENERGY MANAGEMENT

These drivers then mean that the energy performances in the wine industry need to be improved. Typically, better energy performance can be attained in two ways: behavioural change, and technical interventions.

However, it is often difficult to sustain improvement efforts over a period. Common pitfalls when attempting to improve energy performances, include:

- Not enough resources are allocated – there is not enough time to focus on energy or limited finance is available for energy efficiency projects.
- Improvements are focused on technical interventions only – employees are not aware of energy or the influence they have as end users.
- One person is responsible for energy in the winery – all knowledge resides with one person and that knowledge is not easily accessible to the rest of the winery.
- Improvements are not measured – there seems to be no change in the energy consumed although energy savings projects have been implemented.

The need was then identified to develop an appropriate guideline to implement an Energy Management System (EnMS), based on best practices elsewhere, but tailored for the South African context.

The objectives, and benefits, of such an EnMS, include:

- It is a system designed to save money;
- Based on world-class, best-practice, and tried and tested methods;
- Based on a continuous improvement (Plan – Do – Check – Act) cycle;
- A focus on improving energy performance and not on developing a management system; and
- Sustainability is built into the system.

In contrast to other international energy efficiency guidelines for wineries, the developed document of Stellenbosch University, which was funded by Winetech, is an attempt to explain the principles and steps of an EnMS, as outlined in the ISO50001 standard, and illustrates how an EnMS may be applied to a winery situation. Furthermore, it shows by way of case studies from South African wineries, typical energy savings opportunities that exist. However, the energy efficiency and cost cutting initiatives can be capital intensive. It is then advisable that the winemaker approaches the EnMS systematically. The first step would be, after identifying low hanging fruit (potential savings), to engage with a service provider. This would be on a high level where the winery can pose all the relevant questions that would assist in decision-making. The key critical aspects (summarised in Table 1) would be useful to the winemaker or operations manager in deciding whether to go ahead with a project; and thus to engage with the service provider. The full document can be downloaded at http://www.sawislibrary.co.za/dbtextimages/BrentA.pdf or on Winetech's website www.winetech.co.za.

Wine cellars and producers are also urged to make use of the carbon calculator to measure their carbon footprint in preparation for the implementation of carbon tax. Visit www.climatefruitandwine.co.za.

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Crimson Seedless (PHOTO: Renier Louw, Vititec.)

La Rochelle (PHOTO: Jennifer Wayland, Pretoria University.)

The benefits of certified, virus-tested, true-to-type plant material

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INTRODUCTION

The benefits to the primary producers of establishing vineyards of certified, virus-tested, true-to-type plant material are not well understood. This article presents the results of a research project which assessed the lifetime economic and non-economic benefits for table and raisin grape vineyards in South Africa. It is a summary of a longer article which also includes the results for fresh plum, apple and pear orchards and canning peach orchards. The full research report include Midgley and Vermeulen, 2015; and Midgley *et al.*, 2015 and 2016.

MAIN VIRUSES, SYMPTOMS, SPREAD, AND CONTROL MECHANISMS

For grapevines the most important viruses are Grapevine leaf-roll associated viruses (GLRaVs). The GLRaV-3 is the main strain of this virus responsible for Grapevine leafroll disease (GLD) in South Africa. There is a progressive reddening and rolling of the leaves in red cultivars as the season progresses, often beginning in mid-summer when vines are under water stress. Very few of the white cultivars show any noticeable symptoms, but may do so if very heavily infected for a long time. Most vineyards in the Western Cape are infected with GLRaV and the disease

spreads very rapidly. If left unmanaged, all plants may become diseased within 10 to 13 years due to the exponential spread. The GLRaVs can be transmitted by grafting and several insect vectors, such as mealybugs and soft scale insects. GLD can be managed by controlling insect vectors with a combination of systemic and contact insecticides. This is seen as the primary response, together with the planting of certified virus-tested vines. Roguing can be performed based on a visual selection of symptomatic plants in red cultivars. When infection levels are high (>ca.25%) the removal of the entire vineyard can be considered.

ESTIMATES OF INCIDENCE AND IMPACTS IN THE WESTERN CAPE

In table grape vineyards the incidence of GLRaVs can be up to 30% and sometimes higher. Yield losses are estimated at approximately 21 to 30%. The quality of the table grape bunches may be reduced by berries being less pigmented, smaller, and on smaller bunches with poor form. Bunches may have larger off-cuts to improve the bunch form, leading to loss of productivity in packing and yield reductions. The price reduction could be up to 80%. The price may also be reduced in cases where an early market window is not achieved due to delayed ripening associated with GLRaV infection. The potential loss in raisin grape production and value due to virus infection is not known, although it is thought that the raisins from infected grapevines are smaller, because of a lower sugar content in the berries. The price reduction for smaller raisins could be about 50%.

There is a lack of data on GLRaVs supported by laboratory virus tests to support an accurate understanding of the extent of the problem in commercial vineyards, and in uncertified plant material used by some producers. The industry should consider moving away from the term “visually free” by adopting future testing of all plant material for the viruses identified in the Certification Scheme.

USING A MODELLING APPROACH TO ASSESS IMPACTS OF VIRUSES AND BENEFITS OF CERTIFIED MATERIAL

Against this background, the long-term (vineyard lifetime) financial benefit to the producer of using certified virus-tested plant material was estimated by comparing various scenarios under a model. The model of Fuller *et al.* (2013) for grapevine production in California was used as a basis; see also Fuller *et al.* (2015). The model was set up for table and raisin (dried) grapes and their key virus (GLRaV), and is not at this stage cultivar or area specific. A wide range of scenarios was modelled to capture the range of possibilities and identify the most critical factors, rather than to depict the current situation in the industry.

To estimate the value of losses incurred by grape growers as a result of GLD, and the benefit from using certified virus-tested plant material, we estimated differences in net income from a representative hectare of grapes between several scenarios. For each host-virus combination, we compared scenarios for various aspects of disease pressure: initial disease incidence linked to whether or not the vineyard was planted using certified virus-tested plant material, rate of disease spread, yield loss in diseased vines, and reduction in price obtained for fruit harvested from diseased vines (reflecting reductions in berry and bunch quality due to the disease). Details of the model are presented in Midgley *et al.* (2016).

RESULTS OF MODELLING

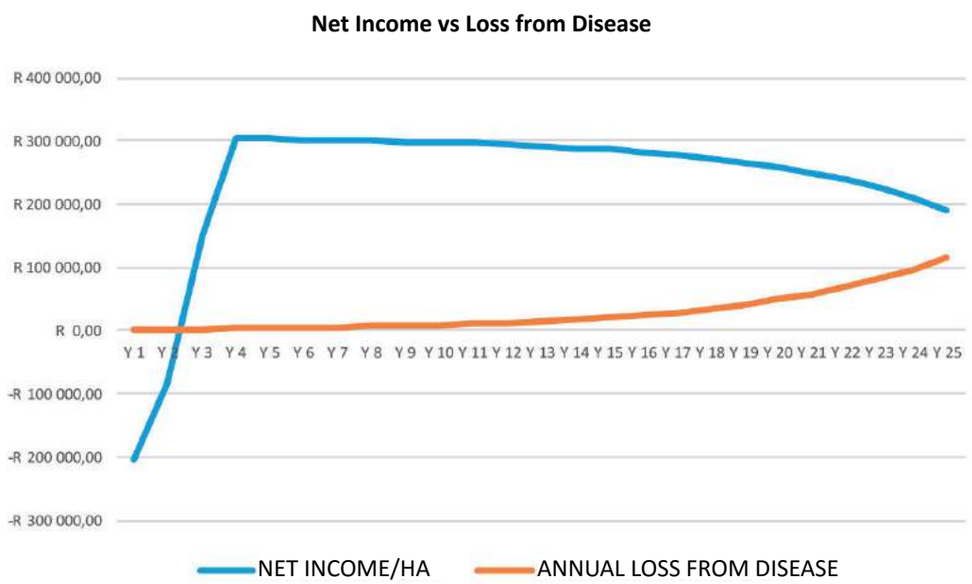
For table grapes/raisin grapes and GLRaV, infection and spread rates can rapidly reach 100% in a number of scenarios and the cumulative benefit can be up to R2 414 732/ha (table) or R1 957 528/ha (raisin). It is important to note that the results of the modelling study need to be verified with field data from commercial nurseries and vineyards.

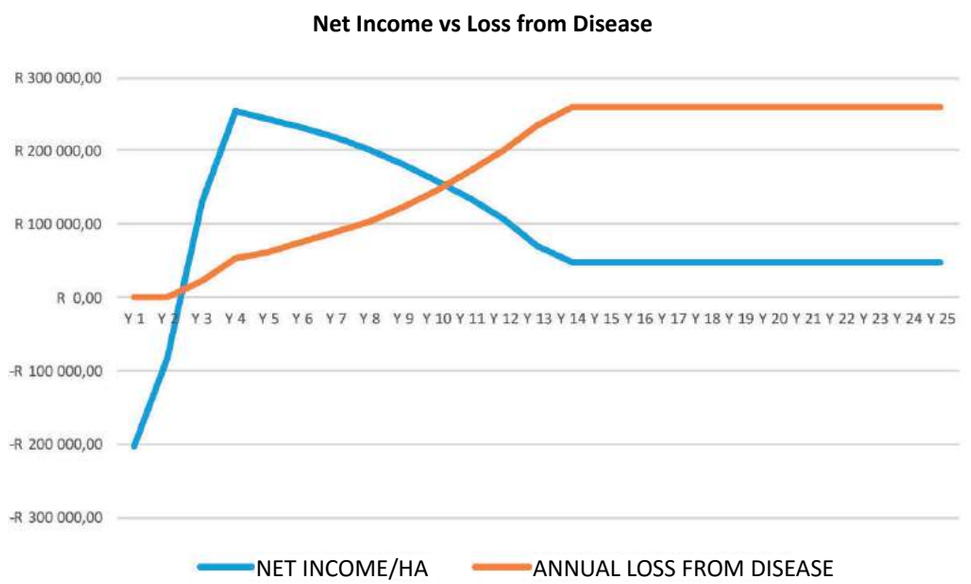
Financial benefits to the producer are maximised primarily where the difference in initial virus incidence between certified and uncertified plants is greatest, and secondarily where the rate of spread is high. A minimum hypothetical difference in infection level at planting between 0.5 to 1% (certified) and 2 to 5% (uncertified) already yields benefits, but the benefits are significantly increased as this difference widens. This can be explained by the compound nature of the impacts over the vineyard lifetime.

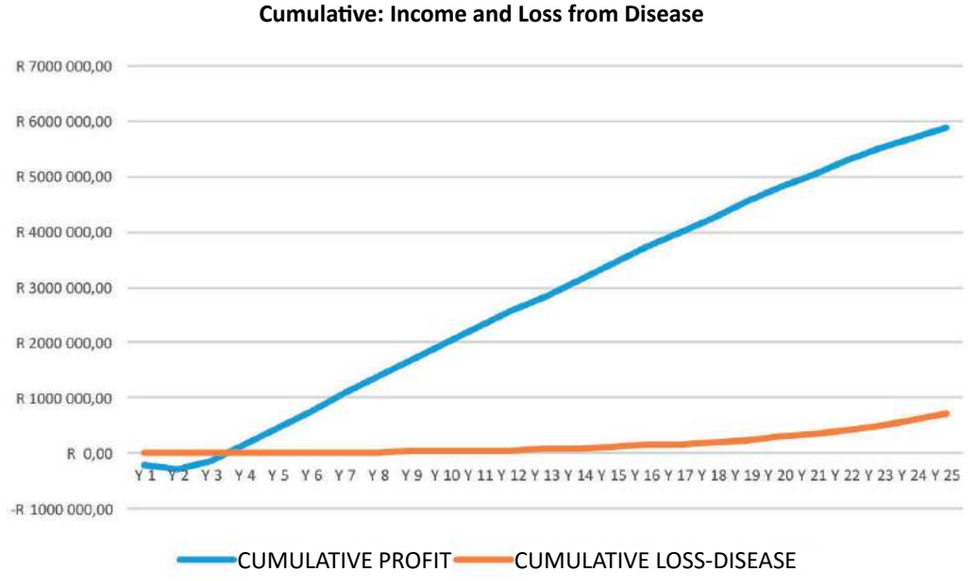
The model results show an increase of 34% (table) and 37% (raisin) of the financial benefit of using certified virus-tested material, given the expectation that at least one stressful year will be experienced over the vineyard lifetime giving rise to a doubling of infection impacts. This would be expected to multiply with every additional “stress year”.

Currently, only approximately half of table and raisin grape producers are estimated to be using certified virus-tested material. There is a belief that this technology is cost effective within 10 years of establishment, but not over the lifetime of the vineyard due to high levels of disease pressure from infected neighbouring farms. This is partially supported by the results of the scenario based on 0.5% incidence in planted certified vines and a high spread rate of 20% per year. These show a reduction in annual profit from around R292 000 (table) or R311 000 (raisin) in Year 13, to R191 000 (table) or R229 000 (raisin) in Year 25 (Figure 1 and 2). Uncertified vines (10% starting infection, 20% spread per year) reached 100% infection in Year 14 and had a final annual profit of R48 000 (table) or R112 000 (raisin), since diseased plants still produce marketable fruit. The annual benefit of using certified vines peaked in Year 14 at R241 000 (table) or R197 000 (raisin) and decreased thereafter to R143 000 in Year 25 (table) or R117 000 (raisin). However, the lifetime cumulative benefit kept increasing to R3 600 000 (table) or R2 991 000 (raisin). There may be an opportunity cost in not replacing the entire vineyard with fresh plants every 15 to 20 years in response to market pressures, as indicated by some industry experts. This could be modelled in future.

This study has provided some initial quantified analysis showing that the use of healthy certified virus-tested plant material in the industry is the basis for an integrated strategy for managing viruses, together with best practice monitoring and vineyard management. Given the expected increase in climatic stress in future brought about by climate change, it is becoming even more important to manage







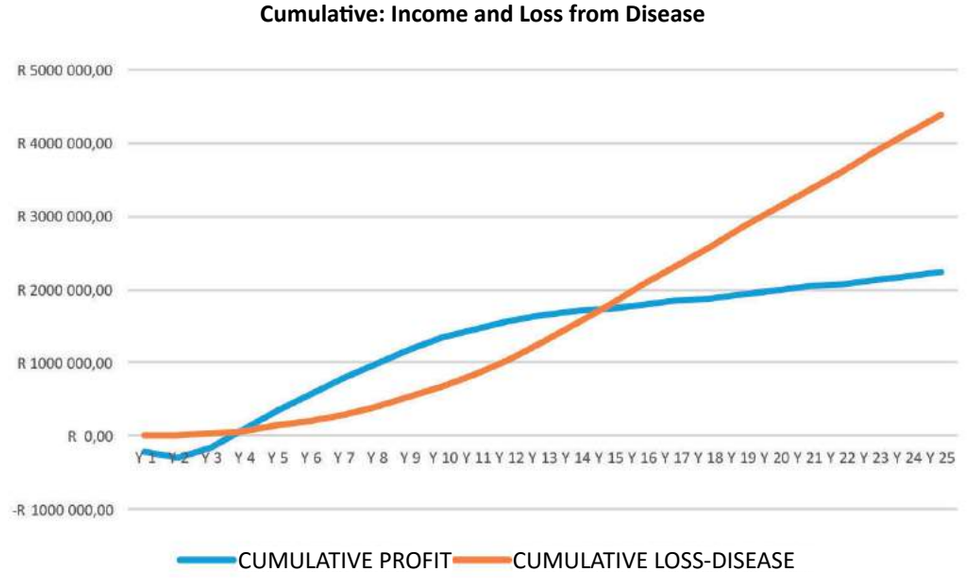


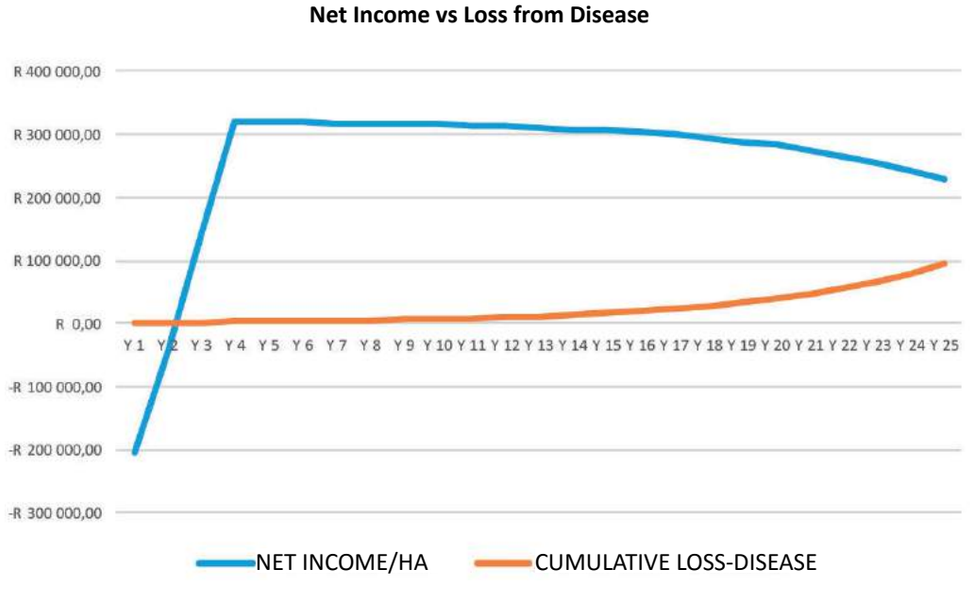
FIGURE 1. Modelled results for table grapes where rate of spread of virus is 20% per annum.

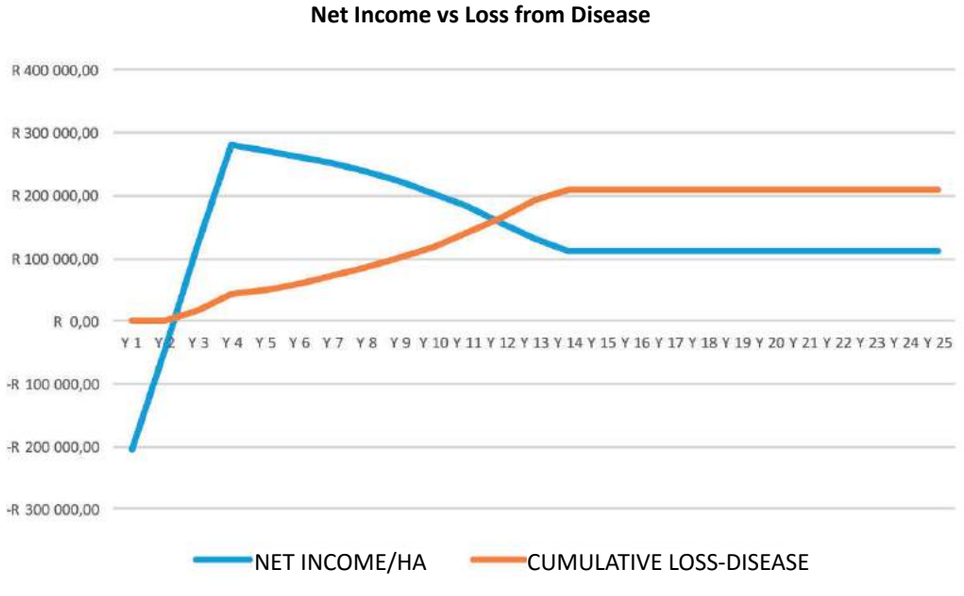
Top: Modelled annual net income (blue) and loss from disease (red) as affected by initial incidence of virus in planting material.

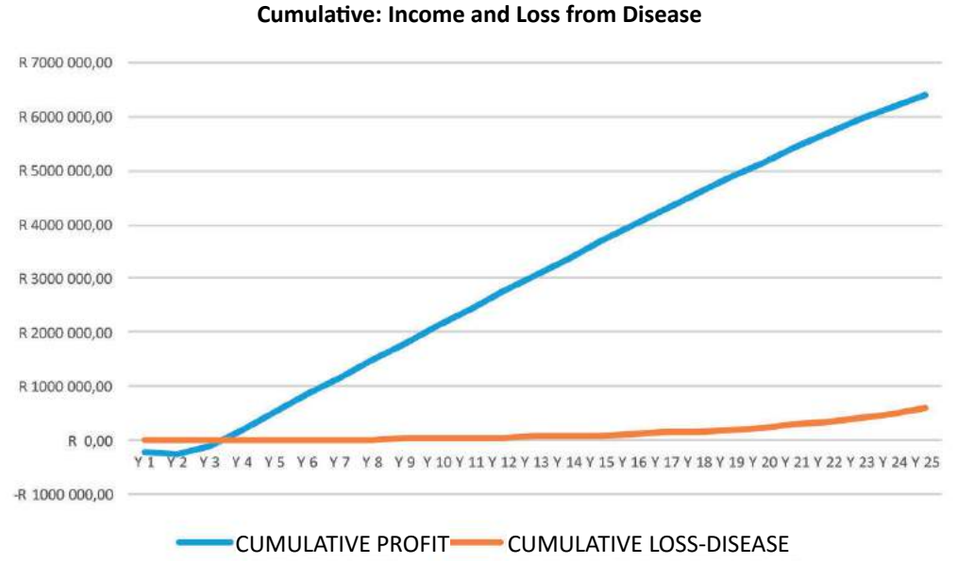
Bottom: Modelled cumulative net income (blue) and loss from disease (red) as affected by initial incidence of virus in planting material.

Left column: Certified virus-tested planting material used (0.5% incidence).

Right column: Uncertified planting material used (10% incidence).







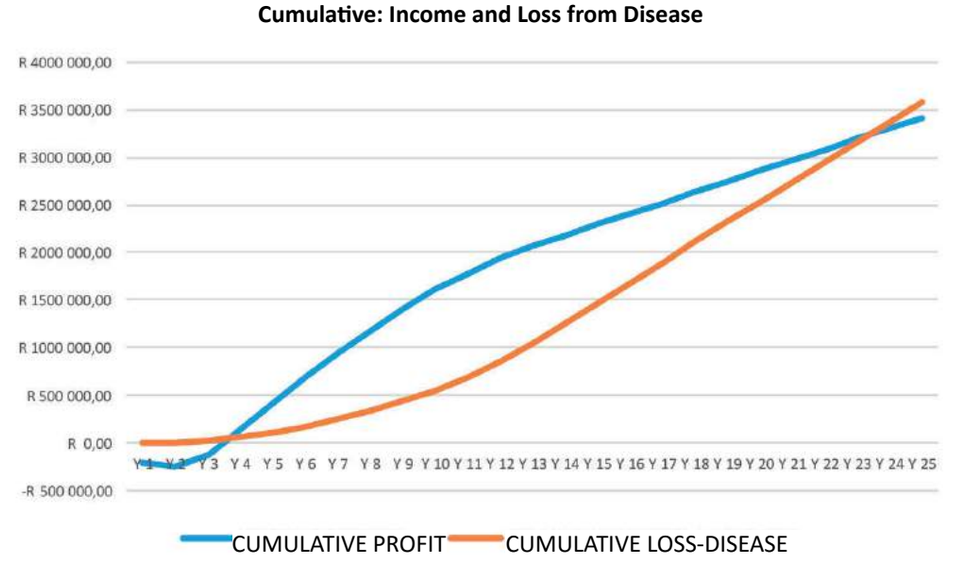


FIGURE 2. Modelled results for raisin grapes where rate of virus spread is 20%.

Top: Modelled annual net income (blue) and loss from disease (red) as affected by initial incidence of virus in planting material.

Bottom: Modelled cumulative net income (blue) and loss from disease (red) as affected by initial incidence of virus in planting material.

Left column: Certified virus-tested planting material used (0.5% incidence).

Right column: Uncertified planting material used (10% incidence).

vineyard stress and at the same time ensure that virus levels are as close to zero as possible at the outset.

CONCLUSION

At an industry level, all producers together with the nurseries and the plant improvement organisations are collectively responsible for the genetic and phytosanitary status of plant material, and the maintenance of high plant quality over a long time period. Since viruses cannot be treated and cured, and the process involved in supplying clean plant material at the start of the vineyard lifetime is long and costly, a lack of attention to this will eventually set the industry back many decades as the quality of vines gradually degenerates. It has been shown over decades of research globally that this leads to reductions in the quality and size of the fruit, the yield, the longevity of the vines, the sensitivity to stress and other adverse environmental factors. Ultimately the producers will pay the cost.

Since the main viruses of grapevines are easily transmitted between farms, there is an added imperative for every producer to do everything possible to minimise infections, thereby benefiting not only his own financial success, but also that of his neighbours and the production region. Otherwise the efforts of some producers and the plant improvement organisations will become ineffective.

Although the main focus in this study was on viruses, it is important that the economic influences of other organisms such as bacteria (for example fireblight in grapes) are also investigated in future.

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