Survival of vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), on grapevine root remnants in soil in the Western Cape Province, South Africa

E. Allsopp*, J.C. Fourie

Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

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Grapevine leafroll is the most damaging grapevine virus disease in South Africa, and the primary vector of *Grapevine leafroll-associated virus 3* (GLRaV-3) is vine mealybug, *Planococcus ficus* (Signoret). Preventing re-infection of newly planted, virus-free grapevines is critical to control and prevent the spread of leafroll disease. Results from a previous survey raised concern that mealybugs surviving on leafroll-infected root remnants in the soil could transmit the virus to newly planted grapevines. This study aimed to determine if *P. ficus* commonly occurs on grapevine roots in the Western Cape Province of South Africa, if and for how long it can survive on remnant roots in soil, and if it can transmit GLRaV-3 to healthy grapevines after surviving on remnant roots. Surveys to determine the occurrence of mealybugs on grapevine roots were conducted at different times of the growing season in vineyards near Robertson, McGregor, Montagu, Somerset West and Malmesbury over three seasons. A field trial was conducted on a sand-clay-loam soil with 23% clay and a sandy-loam soil with 10% clay over 12 months to determine survival of different life stages of vine mealybug confined on root sections from leafroll-infected Pinotage/R110 grapevines. Results indicate that *P. ficus* does not readily occur on grapevine roots in the Western Cape, and that it does not survive well on root remnants of grapevines for any length of time. Implications for planting virus-free grapevines in soil where leafroll-infected vines were removed, are discussed.

INTRODUCTION

The detrimental effects of grapevine leafroll disease on grapevine performance and longevity are well documented worldwide (Naidu *et al*., 2014), and its negative effect on grape and wine quality in South Africa was recently demonstrated, as reported in the January 2018 edition of the Institute for Grape and Wine Science's Basket Press Newspaper (http://igws.co.za/article/in-the-news/the-basket-press-newspaper/the-basket-press--january-2018). According to Pietersen (2004) grapevine leafroll is the most damaging grapevine virus disease in South Africa, with *Grapevine leafroll-associated virus 3* (GLRaV-3) the most prevalent leafroll-associated virus. The most common and efficient vector of GLRaV-3 in South Africa is the vine mealybug, *Planoccus ficus* (Signoret) (Hemiptera: Pseudococcidae) (Walton & Pringle, 2004; Douglas & Krüger, 2008).

Preventing re-infection of newly planted, virus-free grapevines in soil where vines infested with grapevine mealybug and infected with leafroll virus were removed, is critical to the South African wine industry's strategy to control and prevent the spread of leafroll disease (Pietersen *et al*., 2013; Pietersen *et al*., 2017). This strategy entails a multi-pronged approach which includes treating young grapevines with a systemic insecticide shortly after planting; monitoring for leafroll symptoms in these vineyards over two seasons and removing all leafroll infected grapevines, known as roguing; monitoring and controlling mealybugs in adjacent leafroll-infected vineyards where roguing is not feasible; and ensuring that workers and equipment do not move from mealybug-infested vineyards to the virus-free vineyards.

In a once-off survey conducted by Walton & Pringle (2004), *P. ficus* was found on grapevine roots in one mealybug-infested vineyard in each of the following districts in the Western Cape during March 2000: Hex River Valley, Malmesbury, McGregor, Robertson, Stellenbosch and Vredendal. This raised concern that the movement of viruliferous mealybugs from remnant roots of infected grapevines to developing roots of virus-free vines may contribute to the spread of GLRaV-3 (Pietersen, 2004). Almeida *et al.* (2014) also expressed concern that mealybugs surviving on leafroll-infested root remnants pose a potentially serious replant problem.

*Corresponding author: E-mail address: allsoppe@arc.agric.za
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The survival of *P. ficus* on remnant roots of grapevines and the virus transmission ability of such surviving mealybugs have not been studied. The objectives of this study were to determine if *P. ficus* commonly occurs on grapevine roots in the Western Cape Province of South Africa, if and for how long it can survive on remnant roots in soil, and if it can transmit GLRaV-3 to healthy grapevines after surviving on remnant roots of leafroll-infected grapevines.

MATERIALS AND METHODS

Occurrence of *P. ficus* on grapevine roots

Commercial vineyards with high mealybug infestations were identified in consultation with industry consultants and willing farmers, and were selected to include the Malmesbury, McGregor, Robertson and Stellenbosch districts where Walton & Pringle (2004) found vine mealybugs on grapevine roots in March 2000. The leaves, bunches, cordon arms and vine stems of five mealybug-infested grapevines in each vineyard were inspected to record the location of mealybugs on the plant surfaces and under the loose bark, following the protocol of Walton & Pringle (2004). The base of the grapevine stem (trunk) and the attached roots of each of the five plants were carefully exposed to record any mealybugs that occurred there. After inspection, the soil was returned to cover the exposed roots. In vineyards where weeds were present, these were also inspected for the presence of mealybugs.

In 2015, five vineyards infested with vine mealybugs were selected for the survey: two in the McGregor district, one in the Robertson district, one near Stellenbosch and one near Somerset West. Grapevines were inspected in January when mealybug populations began to peak, and again after harvest in March, as well as in April when leaf senescence began. In the next season, inspections were conducted during February and March 2016, when mealybug populations peaked on two farms in the Robertson district, one near McGregor, three in the Montagu district and one near Malmesbury. In 2017, a final survey was conducted on two farms in the McGregor district and one in the Robertson district during the month of May in order to determine if the grapevine mealybugs moved down to the vine roots as it became cooler and as the leaves began to drop.

Survival of *P. ficus* on grapevine root remnants

In April 2016, field trials were laid out on the ARC Bien Donné research farm near Simondium (-33.841938°, 18.976619°) and the ARC Nietvoorbij research farm near Stellenbosch (-33.914098°, 18.863943°). These sites were selected based on soil analysis by an independent laboratory, to provide one trial site (Bien Donné) with a lighter, sand-loam soil and one site (Nietvoorbij) with a heavier soil (Table 1).

Sections of grapevine roots, varying between 1 mm and 7 mm in diameter, were collected from symptomatic grapevines in a Pinotage/R110 vineyard on Nietvoorbij research farm where vines have tested positive for the presence of GLRaV-3. Three to four root sections of varying diameters were placed in each of 100 Petri dishes lined with damp filter paper. At least 25 virus-free grapevine mealybugs, obtained from the Nietvoorbij culture, were transferred onto the root sections in each dish and were allowed to settle on the root sections for 24 hours. Each dish contained mealybugs of all life stages, including females with egg sacks.

Fifty PVC cylinders, measuring 40 cm in length and with a diameter of 11 cm, were constructed as shown in Figure 1. Openings cut into the sides of the cylinders were covered with 50 mesh nylon netting. One end of the cylinder was also covered by nylon netting. A soil core was removed from the 0-20 cm soil layer in the designated trial site, using a custom-made soil auger, and placed in a plastic container. Thereafter, a core was removed from the 20-40 cm soil layer and placed in a separate container. The filter paper with vine roots and mealybugs from one Petri dish was placed in the bottom of the cylinder, and soil from the 20-40 cm soil layer was placed on top. A nylon netting disc plus a piece of filter paper with vine roots and mealybugs from another Petri dish were placed at the 20 cm mark, followed by the soil from the 0-20 cm soil layer. Each cylinder thus contained a total of at least 50 mealybugs. The whole cylinder was then lowered into the hole made by the auger and covered by the last of the soil removed from the top layer.

The trial at each site consisted of 25 cylinders buried in the soil in a 5 x 5 grid. Treatment times (3, 6, 9, 12 or 18 months) were assigned randomly to the five cylinders in each row by using random number tables. Five cylinders were removed at each site after each time interval. In October 2016, the five cylinders removed in April (after three months) were prepared as described above and re-buried at each site to gauge mealybug survival on root remnants placed in the soil during summer. Two of these were lifted at each site in January 2017 (after 3 months), and the remaining three in April 2017 (after 6 months). After removal from the soil, the soil in each cylinder was removed carefully to expose the root remnants on the netting discs, and these were examined under a stereo-microscope to determine mealybug survival.

Transmission of GLRaV-3 from root remnants

In July 2016, root sections on which mealybugs were found to have survived in the soil at Bien Donné research farm

TABLE 1
Soil classification for trial sites.

Location	% Clay	% Silt	% Sand	% Stone	Classification
Bien Donné -33.841938°, 18.976619°	10	2	88	none	Sand-Loam
Nietvoorbij -33.914098°, 18.863943°	23	19	58	none	Sand-Clay-Loam

FIGURE 1
Nylon netting and PVC cylinder constructed for field trials. Length 40 cm and diameter 11 cm.

were tested for the presence of GLRaV-3, using both ELISA and PCR analyses.

RESULTS

Occurrence of *P. ficus* on grapevine roots

Results of the surveys are presented in Table 2. Even though mealybugs sometimes occurred low down on the stems and close to the soil surface, they generally were not found under the soil surface, but for a few exceptions. On 29 January 2015, three mealybugs were found on the stem of a single vine just below the soil surface on a farm just outside McGregor, and a single crawler was also found on the stem of a vine just below the soil surface on a farm between McGregor and Bonnievale. On 17 March 2016, a single mealybug was found on a thick vine root just below the surface of a vineyard with sandy soil near Montagu. No mealybugs were found on any of the weeds or their roots. During inspections in April 2015 and May 2017, when leaf senescence started, mealybugs were clearly evident under the loose bark on grapevine cordon arms and stems, but their absence on roots indicated that there was no mass migration to the stem or roots below the ground. At times, what seemed like the white, waxy secretions of mealybugs were spotted on vine roots, but these turned out to be fungal growth, most probably *Sclerotinia* sp., according to ARC Infruitec-Nietvoorbij plant pathologists.

Survival of *P. ficus* on grapevine root remnants

Results of the field trials are presented in Table 3. In the Nietvoorbij trial, only a single mealybug survived for three months during the summer from October 2016 to January 2017, at 20 cm soil depth. This soil, with 23% clay and 19% loam content, became very wet and waterlogged during winter, and the sticky clay made removal of the root sections from the gauze cylinders very difficult. This may explain why the mealybugs did not survive during the winter in this trial.

At Bien Donné, where the soil consisted of 88% sand and only 10% clay and 2% loam, more mealybugs survived, although none survived for more than three months at 40 cm depth. Six mealybugs (2.4%) survived for three months (April to July), one (0.4%) survived for six months (April to October) and three (0.6%) survived for 12 months (Table 3).

After six months in the soil, the quality of the root sections had deteriorated noticeably, particularly in the waterlogged soil at Nietvoorbij. The roots placed in the soil in October 2016 also showed early signs of root decay after only three months, and after six months white fungal growth covered most of the root sections in the lighter soil of the Bien Donné trial site.

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TABLE 2
Occurrence of vine mealybug, *Planococcus ficus* (Signoret), on grapevines during surveys conducted in various wine grape growing districts in the Western Cape Province of South Africa during 2015, 2016 and 2017.

District	Cultivar	Sampling dates (mm/dd)	Mealybug occurrence
2015			
McGregor: farm 1 33° 56' 50.91" S 19° 50' 26.30" E	Colombar	01-06, 01-29 03-03, 04-16	On 29 January, three mealybugs were found on the main stem (crown) of one grapevine just below the soil surface. No mealybugs were found on roots on any sampling date.
McGregor: farm 2 34° 0' 21.87" S 19° 53' 32.12" E	Colombar	01-06, 01-29 03-03, 04-16	One crawler was found on the stem below the soil surface on 6 January. No mealybugs were found on roots on any sampling date.
Robertson: farm 1 33° 54' 15.03" S 19° 52' 19.94" E	Chenin blanc	01-06, 01-29 03-03, 04-16	No mealybugs found on grapevine stems under the soil surface or on roots on any sampling date.
Stellenbosch 34° 0' 4.64" S 18° 52' 18.58" E	Shiraz	01-23, 03-06	No mealybugs found on grapevine stems under the soil surface or on roots on any sampling date.
Somerset West 34° 6' 33.30" S 18° 54' 47.59" E	Cabernet Franc	02-04, 02-13 02-20, 03-06 03-27	No mealybugs found on grapevine stems under the soil surface or on roots on any sampling date.
2016			
Robertson: farm 1 33° 54' 15.03" S 19° 52' 19.94" E	Chenin blanc	02-16	No mealybugs found on weeds, grapevine stems under the soil surface or on roots.
Robertson: farm 2 33° 49" 24.21" S 19° 52' 31.27" E	Pinotage	02-16	No mealybugs found on weeds, grapevine stems under the soil surface or on roots.
	Sauvignon blanc	02-16	No mealybugs found on weeds, grapevine stems under the soil surface or on roots.
	Ruby Cabernet	02-16	No mealybugs found on grapevine stems under the soil surface or on roots.
McGregor: farm 3 34° 02' 11.04" S 19° 57' 51.82" E	Chenin blanc	02-25	No mealybugs found on weeds, grapevine stems under the soil surface or on roots.
	Chardonnay	02-25	No mealybugs found on weeds, grapevine stems under the soil surface or on roots.
	Chardonnay	02-25	No mealybugs found on weeds, grapevine stems under the soil surface or on roots.

TABLE 2 (CONTINUED)

District	Cultivar	Sampling dates (mm/dd)	Mealybug occurrence
Malmesbury 33° 26' 3.28" S 18° 43' 14.04" E	Pinotage	03-16	No mealybugs found on grapevine stems under the soil surface or on roots.
Montagu: farm 1 33° 41' 43.80" S 20° 02' 58.97" E	Colombar	03-17	No mealybugs found on grapevine stems under the soil surface or on roots.
Montagu: farm 2 33° 42' 33.89" S 20° 02' 41.62" E	Chardonnay	03-17	No mealybugs found on grapevine stems under the soil surface or on roots.
Montagu: farm 3 33° 48' 09.21" S 20° 11' 23.36" E	Colombar (1)	03-17	No mealybugs found on grapevine stems under the soil surface or on roots.
	Colombar (2)	03-17	A single mealybug was found on a thick root of one grapevine, about 1.5 cm under the surface of the sandy soil.
2017			
McGregor: farm 2 34° 0' 21.87" S 19° 53' 32.12" E	Pinotage	05-11	Mealybug under bark on arms and stems, but none below ground on stems or roots of vines.
McGregor: farm 3 34° 02' 11.04" S 19° 57' 51.82" E	Chenin blanc	05-11	Mealybug under bark on arms and stems, but none below ground on stems or roots of vines.
Robertson: farm 2 33° 49" 24.21" S 19° 52' 31.27" E	Pinotage	05-11	Mealybug under bark on arms and stems, but none below ground on stems or roots of vines.

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TABLE 3
Survival of vine mealybug, *Planococcus ficus* (Signoret), confined on grapevine roots at 20 cm and 40 cm soil depth in a sand-clay-loam soil at Nietvoorbij research farm and a sand-loam soil at Bien Donné research farm between April 2016 and April 2017.

Location	Duration (months)	No. replicates	No. live mealybugs		% Survival overall
			20 cm	40 cm	
Nietvoorbij	3 (Apr - July)	*n* = 5	0	0	0
	6 (Apr - Oct)	*n* = 5	0	0	0
	9 (Apr – Jan)	*n* = 5	0	0	0
	12 (Apr – Apr)	*n* = 10*	0	0	0
	3 (Oct - Jan)	*n* = 2	1	0	1
	6 (Oct - Apr)	*n* = 3	0	0	0
Bien Donné	3 (Apr - July)	*n* = 5	2	4	2.4
	6 (Apr - Oct)	*n* = 5	1	0	0.4
	9 (Apr – Jan)	*n* = 5	0	0	0
	12 (Apr – Apr)	*n* = 10*	3	0	0.6
	3 (Oct - Jan)	*n* = 2	0	0	0
	6 (Oct - Apr)	*n* = 3	0	0	0

***** Due to low mealybug survival, the trial was terminated after 12 months and the five cylinders that were to be removed after 18 months, were also removed, hence the 10 replicates.

Transmission of GLRaV-3 from root remnants

The ELISA and PCR analyses confirmed that the root sections on which the mealybugs had survived in the soil, were all negative for GLRaV-3 (data not shown), therefore the virus transmission trial was terminated.

DISCUSSION

The current industry strategy to prevent the spread of leafroll disease is highly dependent on preventing re-infection of newly planted, virus-free grapevines (Pietersen *et al*., 2017). The possibility that vine mealybugs surviving on root remnants could infect new plantings of grapevines with GLRaV-3, postulated by Walton & Pringle (2004), would compromise the success of this strategy significantly. Since these researchers only conducted a once-off survey during March 2000, additional surveys were undertaken in the wine grape growing districts that they surveyed, to assess whether *P. ficus* commonly occurs on grapevine roots in the Western Cape. The findings that mealybugs were not found on grapevine roots in summer or autumn over three seasons, and that mealybugs were only found below ground on the stem of a grapevine in each of the two vineyards in the McGregor district on one occasion, lead us to conclude that *P. ficus* does not commonly occur below ground level on grapevine stems or roots in the Western Cape. As winter approached, vine mealybugs moved from the leaves and shoots to the cordon arms and trunks of grapevines to overwinter under the loose bark. However, this study found no evidence of a large-scale migration to the roots during winter. This concurs with reports of *P. ficus* on wine grapes in other countries, where it was found to overwinter under loose bark on the trunk and cordon arms of grapevines, for example in Israel (Berlinger, 1977), Italy (Duso, 1989) and Sardinia (Lentini *et al*., 2008). In contrast, *Pseudococcus calceolariae* (Maskell), one of the three mealybugs that transmit GLRaV-3 in New Zealand, frequently occurs on roots of grapevine and numerous weed hosts (Bell *et al*., 2009).

The survival rate of *P. ficus* on root remnants of leafroll-infected grapevines in the field trials was very low in both the sandy soil and in the heavier sand-clay-loam soil, with the highest rate of survival, namely 2.4%, recorded from April to July in the sandy soil. Only a single mealybug survived for three months from October to January in the heavier soil. This indicates that *P. ficus* is not well adapted to survive on grapevine roots during winter under local conditions, when soils often become waterlogged. Subsequent transmission of GLRaV-3 to virus-free indicator vines could not be tested, because the grapevine roots used in the field trials tested negative for GLRaV-3, even though the Pinotage scion exhibited severe leafroll symptoms and tested positive for the virus. At present, it is not known how long GLRaV-3 can persist in root remnants under South African conditions.

These findings are in marked contrast to those of Bell *et al*. (2009) in their study of *Ps. calceolariae* in New Zealand. In one trial, they found eight adults and juveniles of *Ps. calceolariae* surviving on rootstock 3309 roots, 51 weeks after vines were cut and stumps were treated with a herbicide. The roots and two crawlers tested positive for GLRaV-3. In another trial, 20 juvenile and adult *Ps. calceolariae* were found surviving on roots of Gewürztraminer in two plots, six months after removal of the vines. The roots from both plots and five of the mealybugs from one plot tested positive for GLRaV-3. Virus transmission by mealybugs that survived on leafroll-infected roots to new grapevines was, however, not

demonstrated in this study.

It has been shown that viruliferous *P. ficus* lose GLRaV-3 and their infectivity within four days after virus acquisition, and when they moult (Krüger *et al*., 2006; Tsai *et al*., 2008). It is likely that mealybugs surviving on root remnants for several months will lose the ability to transmit GLRaV-3. According to Tsai *et al*. (2008), first instar crawlers of *P. ficus* are the most efficient vectors of GLRaV-3, while adult mealybugs are not very efficient vectors and also tend not to move actively. The few mealybugs that did survive for some time on roots in our field trials were all adults, and would therefore not be considered very efficient vectors of leafroll virus.

Bell *et al*. (2009) also postulated that the presence of *Ps. calceolariae* on roots of weed hosts could complicate efforts to contain the spread of leafroll disease even more. In their survey of mealybugs in vineyards in the Western Cape, Walton & Pringle (2004) found *Pseudococcus viburni* (Maskell) and several other mealybug species on the roots of various weed species, but *P. ficus* was not found on the roots of any weeds. In this study, *P. ficus* was also not found on weeds or their roots in any of the vineyards surveyed.

Results from the current study indicate that *P. ficus* does not commonly colonise grapevine or weed roots in the Western Cape Province of South Africa and that it does not survive well on root remnants in the soil, particularly not in soil with a higher loam and clay content. Therefore, the risk that GLRaV-3 will be transmitted to the roots of newly-planted grapevines by viruliferous mealybugs surviving on grapevine root remnants or weed hosts, is considered to be very low to minimal.

CONCLUSIONS

This study showed that the grapevine mealybug, *P. ficus*, does not readily occur on grapevine roots under South African conditions, and that survival of mealybugs on grapevine root remnants for more than three months was rarely achieved. Therefore, the risk of mealybugs transmitting GLRaV-3 from infected root remnants to newly planted, virus free grapevines is considered to be very low. Planting a winter cover crop that is not a host for *P. ficus* or leafroll viruses after removal of leafroll-infested grapevines should be sufficient to render the risk of leafroll transmission from any remnant roots negligible, particularly in soils with a higher percentage of clay and loam. In more sandy soils, it would be advisable to cultivate a non-host cover or cash crop for at least a year before replanting grapevines, in line with the current industry protocol. Longer fallow periods are not deemed necessary to avoid virus transmission from remnant roots by *P. ficus*. It is still vital to follow the current industry protocol and to remove as many roots and other plant material as possible when removing a leafroll-infected vineyard, and to remove and destroy any volunteer vines, because these vines can serve as a reservoir for leafroll viruses from which the newly planted vines can be infected.

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