Technical Brief

Control of Grapevine Leafroll Disease Spread at a Commercial Wine Estate in South Africa: A Case Study

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Abstract: Grapevine leafroll disease (LR) is a serious disease of grapevine worldwide. *Grapevine leafroll-associated virus 3* (GLRaV-3) is the most prevalent virus associated with this disease in South Africa and, despite a successful virus-elimination strategy within a certification scheme, spreads rapidly in local commercial vineyards. Since 2002 an integrated control strategy was used at a commercial wine estate to control LR and serve as a case study for the local and international wine industries to show that control in a commercial setting is possible. The strategy included planting of certified material tested free of detectable viruses, use of herbicide and subsequent removal of infected vine material, fallow periods during which time volunteer hosts were removed, and use of systemic and contact insecticides, sanitation, and horticultural practices to minimize spread of viruliferous mealybugs. Leafroll was reduced from a 100% infection in 2002 on 41.26 ha (111,431 vines) planted mainly from 1989 to 1992, to only 58 LR infected vines detected in 2012 on 77.84 ha (209,626 vines), an incidence of 0.027%. This reduction was achieved by replacing the fully infected vineyards and roguing 3105 infected vines within young and replaced new vineyards. The control strategies were successful in curtailing the spread of LR disease and have resulted in the removal of the disease from the majority of individual vineyards. Leafroll currently occurs at sufficiently low levels in the remaining vineyards that local eradication may be possible in these, in contrast to the general situation in the South African industry where the majority of producers do not apply LR control strategies and leafroll is widespread.

Key words: grapevine leafroll disease, mealybugs, *Planococus ficus*, *Vitis vinifera*

Grapevine leafroll disease (LR), a serious disease of grapevines, has a number of associated viruses (Fuchs et al. 2009). *Grapevine leafroll-associated virus 3* (GLRaV-3) is the most prevalent LR-associated virus in South Africa (Pietersen 2004, 2006), where it is transmitted by a very effective vector, the vine mealybug *Planococcus ficus,* which is the predominant mealybug in South African vineyards (Walton and Pringle 2004), as well as the more restricted *Pseudococcus longispinus* and a number of scale insect species (Douglas and Krüger 2008, Walton et al. 2009). Within the South African Certification Scheme for Wine Grapes (SACSWG), the LR-associated viruses are generally successfully eliminated from planting material by heat treatment and meristem tip culture to create nuclear planting material. This planting material, although tested free of a number of viruses including GLRaV-3, remains susceptible to viruses and becomes reinfected when planted in South Africa in vineyards in traditional wine-producing areas where LR is widespread. Over time LR infection negates the advances achieved by planting certified material and results in the forced replacement of vineyards after 12 to 15 years because of losses in yield and quality. A spatio-temporal study of spread of LR in 53 relatively young vineyards throughout the Western Cape wine production area between 2001 and 2005 (Pietersen 2006) revealed that, when left unchecked, LR infection levels increased exponentially ($y = 898.16e^{0.655x}$, $R^2 = 0.9983$) with an average year-on-year increase of 1.94 times (Pietersen, author's unpublished data, 2006). Secondary spread, primarily from a LR-infected vine to adjacent vines in a row, was the major cause of new LR infections and therefore roguing, combined with mealybug control, already demonstrated on an experimental scale would be a feasible method of LR control (Pietersen, author's unpublished data, 2003).

In the current study, we demonstrate commercial-scale LR control, achieved by an integrated strategy including: (1) reducing GLRaV-3 inoculum by planting certified *Vitis* material, (2) annual roguing of newly detected LR-infected *Vitis* material, (3) reduction in volunteer vines arising from previous LR-infected vineyards, (4) control of mealybug levels through the use of contact and systemic insecticide applications, and (5) prevention of dispersal of mealybug individuals by sanitary measures. While refinement and modification of these control interventions for optimal local implementation at other wine

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estates may be required, this study demonstrates that leafroll can be successfully controlled or even eradicated using the general principles assessed here. As leafroll infection rates appear to be very high in South Africa, this control strategy is likely to be even more effective in areas of slower leafroll spread such as New Zealand and the western United States.

Materials and Methods

Location and strategy. Studies were conducted on the historic Vergelegen Wine Estate established in 1700 and situated in Somerset West, South Africa (S: 34°04′816; E: 18°53′170). A planned expansion by the estate of new vineyards (primarily red winegrape cultivars) onto ~24 ha previously planted to citrus was an ideal opportunity to apply grapevine leafroll disease (LR) control strategies within a commercial environment. As the estate had also planned a later replacement of all older infected red cultivar vineyards because of low yield and berry quality, it was possible to replace these with new vineyards, once the new vineyards on virgin soil became productive. The area under grapevine monitored in this study increased from 41.26 ha at the start of the study in 2002 to 77.84 ha by 2012. Control of LR could therefore conveniently be divided into three phases, with phase 1 control focusing on young vineyards of five years, generally with LR incidences <2.5%, as well as on new vineyards established on ground not previously planted to *Vitis*. In phase 2, LR control was performed in vineyards where totally infected red cultivar vineyards were replanted to new vineyards, also of red cultivars. Phase 3, only recently initiated and not reported here, involves a phased replacement of older LR-infected white cultivar vineyards with new white cultivar vineyards.

The efficacy of control strategies was assessed in (1) new vineyards on virgin soils, (2) replacement of severely LR-infected vineyards with new vineyards, and (3) control of LR in existing infected vineyards. Vineyard sizes, number of vines, year of planting, and cultivar and rootstock information are shown in Table 1 and Table 2.

Disease detection and roguing. The incidence of LR in highly infected, older vineyards was determined by visual monitoring in autumn of a sample of 100 vines (10 rows by 10 vines) within the corner of the vineyard where coordinates for spatial mapping started. In the red cultivars, newly LR-infected plants were visually identified yearly in autumn (late April to May) by systematic row-by-row monitoring for symptoms. The location (row number, vine position) of infected vine was recorded. Any symptoms for which uncertainty existed were tested by enzyme-linked immunosorbent assay (ELISA). Vineyard blocks destined to serve as foundation or mother blocks (Van Rensburg 2004) were subjected to a second monitoring by inspectors of Vititec, the collaborating plant improvement company, who marked the stems of all LR-infected vines using emulsion polyvinyl acetate paint. Furthermore, infected vines were identified annually in May by ELISA in all plantings of foundation and mother-block status, from which planting material was required for the next season, according to the terms of SACSWG. White cultivars were tested annually by ELISA. Within two weeks of identifying infected vines, the stems were severed in two places aboveground to mark them for total removal. The stumps and roots were removed in winter after the rainy season had started, by manually digging out as much of the roots as possible.

ELISA. ELISA samples were prepared by collecting three lower leaf petioles from each vine in autumn, pooled in groups of 10 vines, and extracted in 5x (w/v) 0.1M Tris/HCl, pH 7.6 buffer with 0.01 M $MgSO_4$, 4% polyvinylpyrrolidone, and 2% Triton X-100 in filter-separated plastic bags, using a Homex 6 homogenizer (Bioreba AG, Reinach, Switzerland). The triple antibody sandwich type (TAS)-ELISA is capable of detecting *Grapevine leafroll-associated virus 1* (GLRaV-1), -2 (GLRaV-2), and GLRaV-3 separately or simultaneously (Goszczynski et al. 1995, 1996, 1997). Virus specific antibodies were developed at the Plant Protection Research Institute, Pretoria (PPRI) from electrophoretically separated coat proteins of the respective viruses (Goszczynski et al. 1996, 1997, 1998). Commercial goat-anti-rabbit antibodies conjugated with alkaline phosphatase (Sigma, St. Louis, MO) were used for sero-reaction detection. When a pooled group of vines yielded an absorbance value (405 nm) twice that of the healthy controls of a given microtiter plate, petioles from individual vines of that group were tested separately to identify the infected individuals (those with absorbance values greater than two times that of healthy controls).

Vine reset. Vines of the same cultivar and clone were reset in gaps produced by the removed LR-infected vines. This was only done in the first two seasons in vineyards of foundation or mother-block status. Resets were done in the growth season directly after the removal of vines, except for the third-to-last season where reset was delayed by a further season in vineyard 1 (Rooiland 2) to improve the control of LR-disease from this vineyard.

Mealybug monitoring. Monitoring of *Planococcus ficus*, the main vector of GLRaV-3 in South Africa (Walton and Pringle 2004), was done from the 2008–2009 growth season by *P. ficus*-specific pheromone capsules maintained in yellow delta traps with replaceable sticky pads (Chempac, Paarl, South Africa). Sticky traps were replaced every two weeks initially but in later seasons monthly when mealybug numbers were very low. *Planococcus ficus* male counts were made by the ARC-Infruitec-Nietvoorbij. Pheromone capsules were replaced every three months. A single trap was placed in the middle of each vineyard of less than 1 ha, while two were evenly spaced in vineyards greater than 1 ha.

Mealybug control. Dormant vines were treated annually in winter by two treatments of 96 g/100l chlorpyrifos (Dursban, Dow AgroSciences, Indianapolis, IN) two weeks apart using hand-gun high-volume sprays. Vines were drenched to ground level with a minimum of 4 liters of spray mixture per vine.

Systemic insecticide treatment was with imidacloprid (Confidor 350SC, Bayer, Leverkusen, Germany). In the initial four seasons, application was as per label recommendation of 1.5 mL product in 500 mL water per vine at budburst as a soil drench in a basin around the base of the stem immediately

Table 1 Vineyard history, name, cultivar and rootstock planted, year established, size, number of vines, and the annual number of leafroll infected vines observed or tested by ELISA on all phase 1 vineyards managed. Vineyard number corresponds to that depicted in Figure 1.

							Infected vines[b]										
Prior history	**Vineyd. Fig. 1**	**Vineyd. name**	**Cultivar clone/ rootstock[a]**	**Year estab.**	**Size (ha)**	**# Vines/ block**	**2001/ 2002**	**2002/ 2003**	**2003/ 2004**	**2004/ 2005**	**2005/ 2006**	**2006/ 2007**	**2007/ 2008**	**2008/ 2009**	**2009/ 2010**	**2010/ 2011**	**2011/ 2012**
Soft citrus	1	Rooiland 2	CS 46A x AA	1999	1.21	3246	nt	nt	37	49	7	18	24	32	17	3	2
Soft citrus	2	Rooiland 3	CS 46A x AA	1999	1.38	3710	17	47	10	5	5	17	1	4	1	0	0
Soft citrus	3	Rooiland 4	SH 99B x RQ	1999	0.98	2623	49	62	13	10	1	3	2	2	6	0	1
Soft citrus	4	Rooiland 5	CS 15M x AA	1999	1.17	3139	nt	nt	nt	nt	6	8	3	5	2	1	1
Pastures	5	Kopland 5a	SH 99B x RQ	2000	0.59	1591	nt	nt	nt	nt	15	4	4	4	5	3	2
Pastures	6	Kopland 5b	MO x RQ	2000	1.93	5176	nt	nt	nt	nt	277	138	22	16	14	5	3
Pastures	7	Kopland 5c	CS 17B x RQ	2000	2.64	7063	nt	nt	nt	nt	217	68	74	96	14	4	5
Pastures	8	Kopland 6a	CF 312 x RQ	2001	0.93	2500	nt	nt	nt	nt	4	10	5	14	8	1	1
Pastures	9	Kopland 6b	CF 1 x RQ	2001	1.87	5000	nt	nt	nt	nt	13	22	3	9	6	1	2
Soft citrus	10	Rooiland 6	SH 9 C(BO) x AA 219 A(N2)	2002	1.05	2826	na	nt	2	8	25	14	1	2	2	2	0
Soft citrus	11	Rooiland 7	CS 46 C(BO) x AA 219 A(F)	2002	1.67	4475	na	548	25	9	16	18	4	6	1	0	1
Soft citrus	12	Rooiland 8.1	CS 46 C(BO) x AA 219 A(F)	2002	1	2681	na	53	7	2	8	10	0	1	2	0	1
Soft citrus	13	Rooiland 8.2	CS 15 M(C1) x AA 219 F(P2)	2002	0.99	2665	na	4	3	0	0	9	0	0	0	0	0
Soft citrus	14	Rooiland 9.1	CS 15 M(C1) x AA 219 F(P2)	2002	1.05	2810	na	3	2	0	1	10	1	2	0	0	2
Soft citrus	15	Rooiland 9.2	SH 22 F(BO) x AA 219 A(N2)	2002	1.13	3020	na	1	0	0	0	9	0	0	0	0	0
Soft citrus	16	Rooiland 10.1	CS 46 C(BO) x AA 219 F(CO)	2002	1.53	4106	na	1	0	2	2	16	3	6	1	0	0
Soft citrus	17	Rooiland 10.2a	CS 1 E(A) x AA 219 F(P2)	2002	0.5	1353	na	1	0	2	1	8	2	3	1	0	0
Soft citrus	18	Rooiland 10.2b	CS 46 C(BO) x AA 219 F(CO)	2002	0.24	655	na	0	0	0	0	1	0	0	0	0	0
Soft citrus	19	Rooiland 11.1	CS 46 C(BO) x AA 219 F(CO)	2002	1.39	3719	na	0	0	0	1	14	3	3	3	0	2
Soft citrus	20	Rooiland 11.2	SH 22 F(BO) x AA 219 A(N2)	2002	1.59	4263	na	0	0	0	2	9	3	7	2	0	0
Soft citrus	21	Rooiland 12.1	MO 348 A(BO) x RQ 28 B(E)	2002	1.46	3900	na	1	0	1	0	3	0	0	0	0	0
Soft citrus	22	Rooiland 12.2a	CY 76 D(L2) x RQ 28 C(CO)	2002	0.51	1374	na	0	0	0	2	2	1	nt	0	0	0
Soft citrus	23	Rooiland 12.2b	CY 95 C(K3) x RQ 28 C(CO)	2002	0.38	1018	na	130	11	8	1	0	0	nt	0	0	0
Soft citrus	24	Rooiland 12.2c	CY 96 C(B) x RQ 28 C(CO)	2002	0.27	721	na	1	7	2	2	0	0	nt	0	0	0
Soft citrus	25	Rooiland 12.2d	CY 3 (L4) x RQ 28 C(N1)	2002	0.42	1116	na	112	11	8	11	2	7	nt	0	2	0
Soft citrus	26	Rooiland 13a	CY 548 B(L2) x RQ 28 C(CO)	2002	0.28	744	na	0	0	2	0	0	1	nt	0	0	0
Soft citrus	27	Rooiland 13b	CY 548 A(M3) x RQ C(CO)	2002	0.96	2561	na	0	0	0	0	0	0	nt	0	0	7
Soft citrus	28	Rocklands a	CY 9 D(GV) x RQ 28 C(GV)	2003	1.39	3726	na	na	nt	1	4	4	0	nt	0	1	2
Soft citrus	29	Rocklands b	CY 96 A(GV) x RY 3 A(GV)	2003	0.64	1707	na	na	nt	0	0	0	0	nt	0	0	0
Soft citrus	30	Rocklands c	CY 96 A(GV) x RY 41 B(GV)	2003	0.1	280	na	na	nt	0	0	0	0	nt	0	0	0
Soft citrus	31	Rocklands d	CS 37 C(GV) x AA 219 F(FC)	2003	1.87	4999	na	na	1	3	0	3	2	2	0	0	0
Soft citrus	32	Rocklands e	CS 46 C(GV) x AA 219 A(GV)	2003	0.39	1035	na	na	42	3	8	0	0	1	0	0	0
Soft citrus	33	Rooiland 14	CS 169 B(SW) x RQ 28 C(KD)	2005	1.15	3089	na	na	na	na	0	0	0	0	1	1	0
Soft citrus	34	Rooiland 15	CS 169 B(SW) x RQ 28 C(KD)	2005	1.96	5304	na	na	na	na	0	0	3	3	1	0	3
Totals					36.62	98195	66	964	171	115	629	420	169	218	87	24	37

[a]AA: 101-14 Mgt (*Vitis riparia* x *V. rupestris*); CF: Cabernet franc; CS: Cabernet Sauvignon; CY: Chardonnay; MO: Merlot; RQ: Richter 110 (*V. berlandieri* x *V. rupestris*); RY: Richter 99 (*V. berlandieri* x *V. rupestris*); SH: Shiraz. The cultivar is followed by the clone number and in parenthesis the local origin of the planting material.
[b]na: not applicable. nt: not tested.

Table 2 Vineyard history, name, cultivar and rootstock planted, year established, size, number of vines, and annual number of leafroll infected vines observed or tested by ELISA on phase 2 vineyards monitored. Vineyard number corresponds to that depicted in Figure 1.

							Infected vines[b]										
Prior history	**Vineyd. Fig. 1**	**Vineyd. name**	**Cultivar clone/ rootstock[a]**	**Year estab.**	**Size (ha)**	**# Vines/ block**	**2001/ 2002**	**2002/ 2003**	**2003/ 2004**	**2004/ 2005**	**2005/ 2006**	**2006/ 2007**	**2007/ 2008**	**2008/ 2009**	**2009/ 2010**	**2010/ 2011**	**2011/ 2012**
CS planted 1992	35	Nursery	MO 348 A(SW) x RQ 28 C(KN/DL/GV)	2005	2.14	5787	100%	Removed	Fallow	nt	0	0	2	2	1	2	2
CS planted 1991	36	Olive 1	CS 169 B(SW) x AA 219 A/F (GVE/FC)	2006	2.89	7790	100%	100%	Removed	Fallow	nt	nt	Poor growth due to location, not monitored				
CS planted 1992	37	Olive 2a	CS 1 C(SW) x RQ 28 C(KD)	2006	0.69	1852	100%	100%	Removed	Fallow	nt	nt	0	0	1	0	0
CS planted 1992	38	Olive 2b	CS 46 C(SW) x AA 219 F(KS)	2006	1.19	3228	100%	100%	Removed	Fallow	nt	nt	1	0	0	0	0
CS planted 1992	39	Saddle	MO 192(SW) x RQ 28 C(KD)	2006	1.14	3083	100%	100%	Removed	Fallow	nt	nt	0	0	0	1	0
Sangiovese planted 1994	40	Rooiland 1	MO 192(SW) x RQ 28 C(KD)	2006	0.76	2040	100%	100%	Removed	Fallow	nt	nt	1	1	1	0	0
MO planted 1991	41	Rondekop 1	CF 214 B(SW) x RQ 28 C(KD)	2007	0.67	1822	100%	100%	100%	Removed	Fallow	nt	nt	0	0	0	0
CS planted 1991	42	Rondekop 2a	CF 1 K(GV) x RQ 28 C(KD)	2007	1.08	2925	100%	100%	100%	Removed	Fallow	nt	nt	0	0	0	1
CS planted 1992	43	Rondekop 2b	CF 1 B(DL/GV) x RQ 28 C(KD)	2007	1.14	3077	100%	100%	100%	Removed	Fallow	nt	nt	1	0	0	0
MO planted 1991	44	Rondekop 3	MO 192(SW) x RQ 28 C(KD)	2007	2.16	5845	100%	100%	100%	Removed	Fallow	nt	nt	2	2	0	0
CF/CS planted 1991/92	45	Rondekop 4	CS 46 C(OHA/KJ) x RQ 28 C(KD)	2007	3.86	10431	100%	100%	100%	Removed	Fallow	nt	nt	0	1	1	1
CF planted 1991	46	Rondekop 5	PR 8719 B(SW) x RQ 28 C(DL)	2007	0.54	1468	100%	100%	100%	Removed	Fallow	nt	nt	14	2	3	14
MO planted 1992	47	Rondekop 6	PR 400 D(DL/HB) x AA 219 F(FC)	2007	0.21	556	100%	100%	100%	Removed	Fallow	nt	nt	2	0	0	0
MO planted 1992	48	Rondekop 7	CS 46 C(KJ) x UC 1 (GVP)	2007	0.53	1430	100%	100%	100%	Removed	Fallow	nt	nt	0	0	0	1
CF planted 1992	49	Rondekop 8	CS 338 C(SW) x AA 3(FC)	2007	1.11	2995	100%	100%	100%	Removed	Fallow	nt	nt	0	0	0	0
MO planted 1992	50	Rondekop 9	CS 169 B(SW) x RQ 28 C(KD)	2007	1.52	4101	100%	100%	100%	Removed	Fallow	nt	nt	2	1	1	0
CS planted 1991	51	Rondekop 10a	MO 348 A(SW) x RQ 28 C(KD)	2007	1.57	4252	100%	100%	100%	Removed	Fallow	nt	nt	6	2	1	1
MO planted 1990	52	Rondekop 10b	MO 348 A(SW) x RQ 28 C(KD)	2008	0.89	2413	100%	100%	100%	100%	Removed	Fallow	nt	31	4	4	2
CS planted 1989	53	Stonepine 1a	CS 1 E x RQ 28 C	2008	1.96	5287	100%	100%	100%	100%	Removed	Fallow	nt	0	0	0	1
CS planted 1989	54	Stonepine 1b	CS 46 C x AA 219 F	2008	2.23	6019	100%	100%	100%	100%	Removed	Fallow	nt	0	1	2	0
CS planted 1989	55	Stonepine 2	CF 1 B x RQ 28 C	2008	1.95	5269	100%	100%	100%	100%	Removed	Fallow	nt	12	4	0	2
MO planted 1990	56	Stonepine 3a	MO 128 D x RQ 28 C	2008	0.44	1184	100%	100%	100%	100%	Removed	Fallow	nt	0	0	0	0
MO planted 1990	57	Stonepine 3b	MO 192 x RQ 28 C	2008	1.29	3494	100%	100%	100%	100%	Removed	Fallow	nt	0	0	0	0
MO planted 1990	58	Stonepine 3c	MO 348 A x RQ 28 C	2008	0.56	1522	100%	100%	100%	100%	Removed	Fallow	nt	1	0	1	0
MO planted 1990	59	Stonepine 3d	MO 348 A x AA 219 F	2008	1.11	3006	100%	100%	100%	100%	Removed	Fallow	nt	28	37	8	3
CS planted 1990	60	Stonepine 4	CS 169 B x RQ 28 C	2008	1.73	4664	100%	100%	100%	100%	Removed	Fallow	nt	0	1	1	1
MO planted 1991	61	Stonepine 5	MC 71 B x AA 219 F	2008	1.73	4669	100%	100%	100%	100%	Removed	Fallow	nt	0	0	0	0
CS planted 1990	62	Stonepine 6	CS 338 C x RQ 28 C	2008	1.02	2761	100%	100%	100%	100%	Removed	Fallow	nt	0	0	0	0
CS planted 1989	63	Stonepine 7	CS 27 B x RQ 28 C	2008	3.13	8461	100%	100%	100%	100%	Removed	Fallow	nt	0	0	1	1
Totals					41.26	111431								102	58	26	30

[a]AA: 101-14 Mgt (*Vitis riparia* x *V. rupestris*); CF: Cabernet franc; CS: Cabernet Sauvignon; MC: Malbec; MO: Merlot; PR: Petit Verdot; RQ: Richter 110 (*V. berlandieri* x *V. rupestris*); UC: US 8-7 ([*V. aestivalis* x *V. cinerea* x *V. vinifera*] x Richter 99). The cultivar is followed by the clone number and in parenthesis the local origin of the planting material.
[b]When 100% infection indicated, it is based on visual evaluation of a selected 20 by 20 vine portion of the vineyard. nt: not tested.

followed up with a further minimum of 2 L clean water per vine. Beginning in 2006, imidacloprid was applied directly via the drip irrigation system (Daane et al. 2006) at flow rates of 2.3 L/ha to achieve doses of 1.5 mL imidacloprid per vine. Application was generally three weeks after budding but never later than mid-October. Whole vineyard imidacloprid application was performed every second season if more than 10 mealybugs were observed in pheromone traps during any two-week period of that season or every third season by default.

Implement and worker sanitation. Before 2006, the spatial configuration of the different phases allowed for the practical separation of vineyard workers and implements into two separate teams comprising those working in older highly infected vineyards and those working in healthy, new vineyards. After the older highly infected vineyards were removed, the separation of workers or implements was no longer used as a possible means of preventing dispersal of mealybugs between vineyards.

Vineyard replacement. All vines in vineyards destined for replacement were treated with 2 to 8% glyphosate (Roundup, Monsanto, St. Louis, MO) foliar application following the last harvest. In the subsequent winter following rains, herbicide-treated vines were mechanically removed by linking a chain around the stem and ripping out the stem and roots using a tractor. Sites were plowed and residual plant material removed. These sites were kept fallow for two growth seasons during which volunteer vines were removed by manual digging. In the season prior to establishing new vineyards on these sites, the soil was prepared for planting by deep ripping to a depth of 1.2 to 1.5 m based on soil profile analysis, followed by a 0.9 m deep plow and removal of any root material still present. Vines were established in new vineyards by planting grafted rooted plants and treated with imidacloprid as described above. The positions of volunteer vines in vineyards and feral vines growing in the proximity of new vineyards were recorded annually and were removed following rain in the winter by manual digging.

Windbreaks. Windbreaks of alder (*Alnus* sp.) and beefwood (*Casuarina* sp.) between the soft citrus groves and old vineyards were maintained and expanded where needed around new vineyards, as wind damage on this estate was a frequent occurrence. Individual trees were planted at 2 m spacing, with the beefwood generally between 6 and 8 m high and 4 m wide on average, while the alders were 4 to 6 m high and 3 m wide on average. No mealybug monitoring was done within the windbreaks, and the effects of mealybug spread by the windbreaks were not assessed.

Plant material. All new planting material was from the SACSWG and Vititec, Paarl, South Africa. Cultivars, clones, date of planting, size of vineyard, and number of vines present are shown in Table 1. At the initiation of this experiment, the origin of planting material was generally from existing foundation and mother-blocks typically maintained in grapevine production areas and subject to the practices and regulations of SACSWG at that time. All new vineyards planted after 2004 at Vergelegen, however, were planted using three-star certified planting material, a new category of planting material from SACSWG propagated in foundation and mother-blocks distant from commercial grape production (low-risk areas) and subjected to more stringent mealybug and virus tests and monitoring regimes (Van Rensburg 2004).

Results

Vineyard spatial position, history of vineyards prior to implementation of control strategies, year of planting of LR-controlled vineyard, size of vineyard, total number of vines, vine cultivar and scion planted, and annual number of LR-infected vines observed and/or tested by ELISA are shown (Figure 1, Table 1, Table 2). There is a clear reduction in the total number of infected vines year-on-year. For example, the percentage infection in phase 1 vineyards in 2003 was 1.71%. This was reduced after five years to 0.42% and to 0.039% by year 10.

Regressions applied to best describe the average annual decline in LR-infected vines following control of LR spread and roguing infected plants are shown (Figure 2). This analysis was for vineyards planted on virgin soil (phase 1 vineyards) that had an initial infection >1% (nine vineyards: 1, 3, 6, 7, 11, 12, 23, 25, and 32). As several of these vineyards did not have data for more than six seasons, only the first six seasons were included in the analysis.

Effective control of mealybug on this estate is evident from Table 3, which records the number of adult male *Planococcus ficus* males trapped in pheromone traps every two weeks. The average number of male mealybugs trapped every second week was 1.48, which compares favorably to an average of 136 males (minimum 4, maximum 1150) trapped every two weeks over the same time of the year in 2007–2008 on 11 vineyards of an immediately adjoining estate where mealybug control was just starting to be implemented (data not shown). The control on Vergelegen was obtained following annual dormant cane drenches and application of imidacloprid either two or three years apart. This has resulted in male *P. ficus* individuals not observed on sticky traps on most occasions in vineyards monitored. The use of herbicide to kill older vines down to their roots when performing vineyard replacements did not appear successful, with high numbers of live remnant roots observed on preparing soil for the new vineyards.

Discussion

At a commercial South African wine estate, leafroll was reduced from a 100% infection in 2002 on 41.26 ha (111,431 vines) planted mainly from 1989 to 1992, to only 58 LR infected vines detected in 2012 on 77.84 ha (209,626 vines), an incidence of 0.027%. This decline was achieved by replacing the fully infected vineyards and roguing 3105 infected vines within all the young and replaced new vineyards. Four of the vineyards were 13 years old in 2012 and in total had only four new infected vines in the 2011–2012 season. This level of control was achieved in a number of instances where vineyards had significant numbers of LR-infected vines on initiation of roguing (the highest at 12.2%, or 548 vines).

In seven of these vineyards (2, 3, 11, 12, 23, 25, and 32) for the final two successive years of this study, we found either zero or one infected vine, suggesting local eradication may be possible. Vineyards planted in 1998 containing LR at incidences of 2% or higher at the initiation of the study or on establishment would have been completely LR infected by 2012 had control methods not been applied. This assumption is based on a calculation of average (n = 4) year-on-year rate of increase of 1.94-fold as found within 53 vineyards throughout the Western Cape wine production area monitored for LR spread from 2001 to 2005 (Pietersen 2006; author's unpublished data, 2006). In addition, the spread of LR on the Vergelegen estate before applying LR-control interventions appeared similar to that of most commercial vineyards in South Africa, as vineyards established between 1989 and 1992 with certified planting material were totally infected within 10 to 13 years (when first incidences was recorded). By 2012 they were all 100% infected based on monitoring of 100 vines per vineyard.

The decline in LR-infected plants following the control of LR spread and roguing over six seasons on nine vineyards was best represented by a Power-law model regression ($y = 1353.5x^{-1.735}$; $R^2 = 0.96$) (Figure 2C). In these nine vineyards, 79% of the total number of vines that became LR infected over the six seasons were removed within the first two seasons. This effective control is probably due to LR being established in these new vineyards primarily by infected planting material, with little time for subsequent secondary spread. While the apparent diminishing return on control by roguing in subsequent years may suggest that it should only be performed for a limited number of years, the potential for eradication of LR at Vergelegen, demonstrated in specific vineyards in the current study, may make a sustained roguing program the option of choice. While the potential eradication of LR may hold true for Vergelegen, it is only likely to be feasible in estates relatively isolated from neighboring estates where LR and mealybug control are not necessarily applied and new LR infections are likely to occur annually through primary spread.

The effectiveness of reduction of LR infections by roguing in individual vineyards in this single estate differed. For example, in vineyards 5, 6, 7, and 11, all newly established on virgin soil, LR was probably introduced by infected planting material (based on the random distribution of infected plants shortly after establishment). In vineyard 11, roguing was applied directly after the first season of planting, as ELISA confirmed GLRaV-3 infection on 548 vines (12.2% of those established). After roguing the number of infected plants in the second season was considerably lower (25) and only a further 80 newly infected vines had to be removed in the

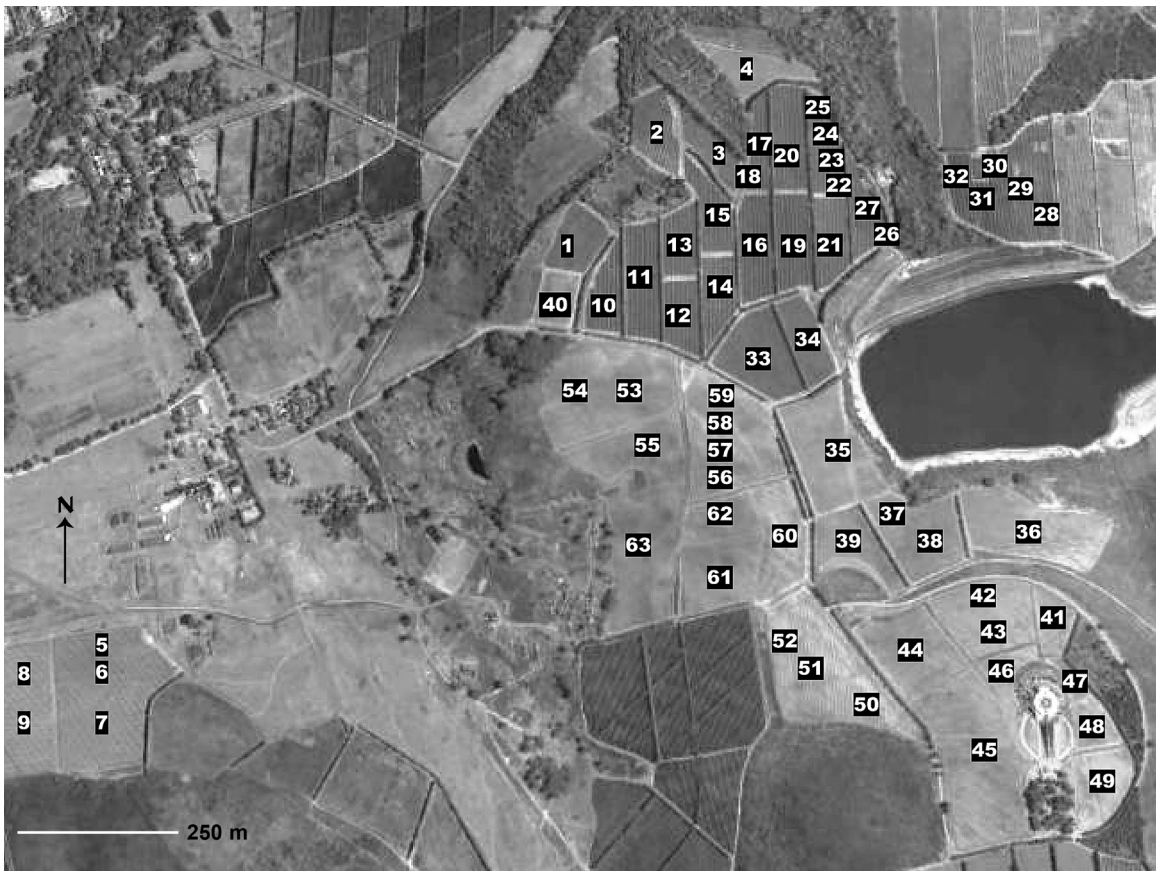

Figure 1 Aerial image showing location of vineyards 1 to 63 of Vergelegen Wine Estate, Somerset West, South Africa, on which leafroll disease control tactics were applied.

seasons leading up to 2012. In 2010, 2011, and 2012, one, zero, and one newly infected plants were observed, respectively, in this vineyard. In total, 14% more vines had to be removed than infected vines observed in the first season. In the second block (vineyard 5, 6, and 7), congruent vineyards established in 2000 and consisting of Shiraz 99B, Merlot, and Cabernet Sauvignon on Richter 110 rootstocks (5.16 ha, or 13,830 vines), LR monitoring was initiated only five years after establishment (2005) when 509 infected vines (3.6% of the total) were observed and removed. In the next season the number of infected vines was 110. Up to 2012, a further 481 newly infected vines, 94% more than initially observed, had been removed but LF was not yet eradicated (33, 12, and 10 newly infected vines were observed in 2010, 2011, and 2012, respectively). More vines were removed in the second vineyard, despite a lower initial incidence of disease than vineyard 11, and LR control has been less effective. Although differences in the two scenarios make strict comparison tenuous (different cultivars, sizes, date of establishment, mealybug numbers), the difference in rate of removal of LR is possibly due to more rapid spread of LR because of more mealybugs present in this vineyard than in vineyard 11 (Le Maguet et al. 2013), resulting in more vines infected due to secondary spread. This would result in recent infections in the vineyards that may not be expressing symptoms and may not be rogued annually.

Vergelegen Wine Estate represented an ideal opportunity to assess the effectiveness of an integrated approach to LR control, as the vineyards were relatively isolated from adjoining wine estates and hence control strategies could be assessed without coordination among different estate personnel or concern about noncompliance in adjoining vineyards. Furthermore, the estate had embarked on a expansion program by replacing citrus orchards with winegrapes five years prior to the initiation of this study; while already having established five vineyards on sites not previously planted to *Vitis* sp., a further 25 vineyards (~24 ha) were planned for further expansion at that stage. The estate had also planned a later replacement of all older infected red cultivar vineyards because of low yield and berry quality. In addition, the majority of red and white cultivar vineyards were spatially separated.

Each phase of control of LR on this estate represented the application of successively more control interventions. In phase 1, there was no danger of LR spread from volunteer hosts, viruliferous mealybugs, or remnant roots (Pietersen 1996), as vineyards were established on areas previously planted with citrus. Control in phase 1 therefore included three steps. First there was an annual roguing of infected

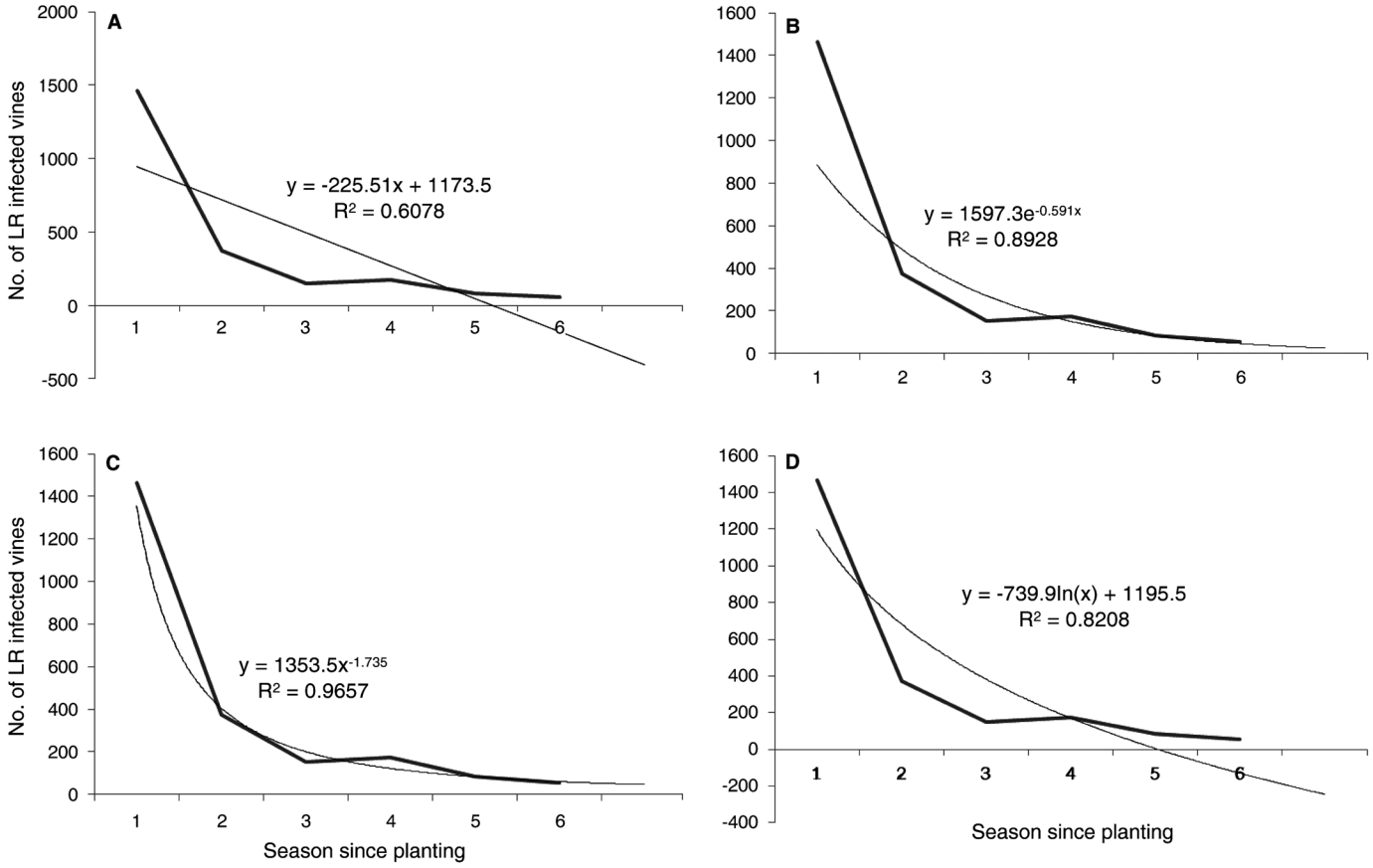

Figure 2 Plot of number of LR-infected vines observed in different seasons following roguing of infected plants. Various regressions applied to the curves to best describe the average annual decline in LR-infected vines following control of LR spread and roguing infected plants. Analysis is for the first six seasons of vineyards planted on virgin soil (phase 1 vineyards) that have an initial infection of >1%. (**A**) linear regression, (**B**) exponential regression, (**C**) power regression, and (**D**) logarithmic regression.

Table 3 Average numbers of male mealybugs per trap on vineyards of Phase 1 and 2 (for vineyard position refer to the vineyard number on Figure 1) (na = not applicable).

Vineyard Name	Vineyd. (Fig. 1)	# of traps	Average number of mealybugs per trap																				
			29 Oct 2008	14 Nov 2008	25 Nov 2008	10 Dec 2008	23 Dec 2008	07 Jan 2009	22 Jan 2009	26 Oct 2009	09 Nov 2009	23 Nov 2009	16 Dec 2009	30 Dec 2009	13 Jan 2010	26 Jan 2010	30 Oct 2010	29 Nov 2010	06 Dec 2010	18 Dec 2010	03 Jan 2011	10 Jan 2011	07 Feb 2011
Rooiland 2	1	1	0	0	2	0	0	12	4	1	0	3	0	0	0	0		7	4	na	5	na	3
Rooiland 3	2	1	0	2	1	0	5	3	1	1	0	0	0	2	6	0	na	2	11	na	8	na	2
Rooiland 4	3	1	0	1	0	0	1	1	2	0	0	0	0	2	8	0	na	2	2	na	4	na	2
Rooiland 5	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	na	8	3	na	2	na	1
Kopland 5	5, 6, 7	5	1	0.8	4.8	13.2	28	24	13	0.8	1.6	1.2	2.4	21	24	5	na	13	15	na	19	na	7.5
Kopland 6	8, 9	3	0	1	1.3	13	14	12	9.3	0	1	3	0.3	9.3	12	5.3	na	5.3	6	na	4	na	5.3
Rooiland 6	10	1	0	0	0	0	0	0	0	0	0	0	0	2	3	1	na	3	2	na	1	na	0
Rooiland 7	11	1	1	1	0	1	0	3	0	1	2	0	0	0	8	1	na	4	9	na	3	na	2
Rooiland 8.1	12	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	na	1	5	na	4	na	1
Rooiland 8.2	13	1	0	0	1	2	0	0	2	2	1	0	0	0	0	0	na	3	3	na	8	na	0
Rooiland 9.1	14	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	na	2	8	na	3	na	0
Rooiland 9.2	15	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	na	1	0	na	1	na	1
Rooiland 10.1	16	1	0	0	0	0	0	0	0	1	0	0	1	2	4	0	na	1	6	na	4	na	7
Rooiland 10.2	17, 18	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	na	1	0	na	0	na	1
Rooiland 11.1	19	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	na	1	3	na	6	na	0
Rooiland 11.2	20	1	0	0	0	0	0	0	0	0	0	0	0	2	0	3	na	3	1	na	4	na	1
Rooiland 12.1	21	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	na	2	0	na	3	na	2
Rooiland 12.2	22, 23, 24, 25	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	na	0	3	na	2	na	0
Rooiland 13	26, 27	1	0	0	0	0	1	0	0	0	0	0	0	1	5	1	na	1	2	na	1	na	2
Rocklands 1a	28, 29, 30	2	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	na	0	3	na	4	na	3
Rocklands 1b	31, 32	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	na	0	4.5	na	20	na	41
Rooiland 14	33	1	0	0	0	0	0	0	0	0	0	0	0	2	4	3	na	0	10	na	0	na	0
Rooiland 15	34	2	0	0	0	0	0	0.5	0	2.5	1.5	1.5	0	0	0	0.5	na	0	11	na	0	na	0
Nursery	35	3	0	0	0	0	0	0.3	0	1	0.3	2.3	0	0.3	2.6	2	na	0.3	1.6	na	1	na	1.3
Olive 1	36	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	na	0	4	na	4	na	4
Olive 2	37, 38	2	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	na	0.5	3	na	5	na	1.5
Saddle	39	1	0	0	0	1	0	0	0	0	0	0	0	1	3	0	na	0	5	na	9	na	3
Rooiland 1	40	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	na	2	1	na	2	na	1
Rondekop 1	41	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	na	1	1	na	15	na	2
Rondekop 2	42, 43	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	na	0	2	na	7	na	0
Rondekop 3	44	1	0	0	1	0	0	0	0	1	2	2	2	0	0	0	na	4	6	na	6	na	2
Rondekop 4	45	1	0	0	0	0	0	0	0	1	0	2	1	0	0	1	na	2	10	na	12	na	7
Rondekop 5	46	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	na	0	8	na	14	na	6
Rondekop 6	47	1	0	1	0	0	0	0	0	na	0	na	0	0	0	0	na	0	7	na	10	na	4
Rondekop 7	48	1	0	1	0	0	0	0	0	na	0	na	0	0	0	0	na	0	2	na	5	na	2
Rondekop 8	49	1	0	0	0	0	0	0	0	3	4	1	0	1	0	0	na	1	2	na	3	na	1
Rondekop 9	50	1	0	0	0	1	0	0	0	1	0	0	0	1	3	0	na	0	4	na	10	na	33
Rondekop 10	51, 52	1	0	0	0	1	0	0	0	0	0	0	0	1	6	0	na	0	21	na	13	na	24
Stonepine 1	53, 54	1	0	0	0	0	0	0	0	0	0	0	0	3	3	1	na	1	16	na	23	na	11
Stonepine 2	55	1	0	0	0	1	0	0	0	0	0	0	0	3	6	3	na	0	2	na	3	na	5
Stonepine 3	56, 57, 58, 59	1	0	0	0	0	0	0	0	2	2	0	0	5	8	0	na	0	29	na	19	na	10
Stonepine 4	60	1	0	0	0	0	0	0	0	1	3	0	0	0	0	0	na	0	0	na	11	na	47
Stonepine 5	61	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	na	1	5	na	9	na	22
Stonepine 6	62	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	na	0	4	na	2	na	28
Stonepine 7	63	1	0	0	1	1	0	0	0	0	0	0	1	1	0	0	na	0	15	na	14	na	10

vines (Pietersen, author's unpublished data, 2003), which had been generally introduced by infected planting material. This roguing could be performed by visual detection of the symptoms in the red cultivars (we had previously found a good correlation of late-season visual assessment of LR symptoms and the presence of GLRaV-3; Pietersen, author's unpublished data, 2006), but we needed ELISA in the two white cultivar vineyards to identify GLRaV-3. The second step was the control of mealybugs by application of chlorpyriphos on dormant canes and soil application of the systemic insecticide, imidacloprid. The third step was the prevention of viruliferous mealybug dispersal by isolation of the first-phase vineyards from the older LR-infected vineyards of phase 2 across the dividing road by a separation of work teams and implements in the new vineyards from those in the older vineyards. Windbreaks were required because of the windy location, and existing windbreaks around the previous citrus orchards were retained and expanded into those vineyards that lacked them. These are not part of the integrated control strategy, as it is unknown whether they reduced or actually enhanced wind dispersal of mealybugs (due to leeward deposition of mealybugs by wind backdrafts). Various factors affect the pattern of dispersal, including wind speed, angle of incidence of wind, permeability of the windbreak, turbulence, source of insects, insect behavior, insect species, and vegetative composition of windbreaks (Pasek 1988). Phase 2 involved replacing older infected vineyards with new vineyards of mainly red cultivars using an intervening fallow period, removal of volunteer hosts, and root remnant removal in addition to the strategies used in phase 1. Detection of infected vines for roguing during phase 2 was done by visual assessment of symptoms annually in autumn. LR disease symptoms on white cultivars are ambiguous or obscure and visual assessments are not reliable. Therefore, in addition to the strategies used in phases 1 and 2, phase 3 requires ELISA to detect GLRaV-3 infected plant before roguing can be done.

Numerous active remnant roots were observed and removed in the two seasons following herbicide treatment to kill the older infected vines and removal of the vine, and the herbicide treatment clearly was not effective. Any remaining remnant roots still present following the soil preparation of the new vineyards could potentially still serve as a sources for GLRaV-3 inoculum, as the persistence of this virus has been demonstrated in herbicide-treated roots (Bell et al. 2009).

The results of this study suggest that LR spread can be controlled using an integrated program. However, the relative effect of the individual interventions should be ascertained in specific, controlled trials, some of which are currently underway. The effective mealybug control achieved and the diligent annual roguing probably played major roles in the successful control of the disease. Furthermore, similar integrated control strategies are being applied within foundation blocks from the SACSWG, with concomitant improvements in the phytosanitary status of new planting material. The vineyards established on Vergelegen sites previously planted to citrus currently conform to SACSWG foundation block specifications. Following virus testing and mealybug control strategies described here, such material is being collected and used as foundation material in SACSWG. Planting material established on land previously planted to vineyards cannot be recognized as foundation block vineyards. However, as they comply with virus testing and other specifications, they can be recognized as a source of mother block propagation material. Distribution of planting material from this estate, now with a vastly improved phytosanitary status, will have a major impact on reducing LR incidence in other estates within the industry. It is anticipated that, should leafroll be eradicated (no infected plants observed in any of the estate vineyards for at least three seasons), the stringent chemical mealybug control used during this study may be replaced with biological control of *P. ficus* (Daane et al. 2006) through releases of commercially available predators (*Cryptolaemus montrouzieri*) and parasitoids (*Coccidoxenoides perminutus*).

Conclusion

Through the use of a rigorous application of several integrated methods to control the spread of leafroll, we have demonstrated that spread of this ubiquitous disease in South Africa can be controlled and that local eradication of LR disease on specific vineyards or estates is possible. The individual effectiveness of the separate control methods could not be ascertained in this case study, and controlled experiments to assess these individually are currently underway. In smaller estates with adjoining neighbors not controlling the disease, local eradication may not be possible, but the rate of spread could reduced and potentially confined to primary spread. This study serves as an example for both local and international industries of the use of an integrated control strategy for LR, heretofore a disease that is prevalent and generally uncontrolled in commercial situations in most grapevine production countries worldwide.

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