Industry allocated project number

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Indicate (X) client(s) to whom this final report is submitted. Replace any of these with other relevant clients if required.

FINAL REPORT
2014

Programme & Project Leader Information

	Research Organisation Programme leader	Project leader
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Project Information

Research Organisation Project number	WW 05-21
Project title	Determining reaction time of grapevine mealybug crawlers to systemically applied imidacloprid.

Fruit kind(s)	Wine grapes		
Start date (mm/yyyy)	10/2013	**End date** (mm/yyyy)	07/2014

Project keywords	Imidacloprid, mealybug, virus transmission

Approved by Research Organisation Programme leader (tick box) ✓

THIS REPORT MUST INCLUDE INFORMATION FROM THE **ENTIRE** PROJECT

Executive Summary

Give an executive summary of the total project.

The aim of this research was to determine whether systemically applied imidacloprid either kills or stops grapevine mealybugs, *Planococcus ficus* (Signoret), feeding before they can transmit leafroll virus (GLRaV-3).

Even under a stereo microscope it was not possible to determine whether a grapevine mealybug had stopped feeding, due to the positioning of the mealybug's mouthparts under the body and the insect's sensitivity to damage when manipulated under the microscope. Two bioassays were therefore conducted to test whether grapevine mealybug nymphs can transmit GLRaV-3 to grapevines treated with imidacloprid.

Bioassay 1: Thirty viruliferous mealybug crawlers were transferred to each of 10 potted Cabernet franc indicator vines treated with imidacloprid after bud burst and two untreated control plants. After 72 hours plants were sprayed with malathion to kill all mealybug nymphs and after 7 weeks in an insect-free glasshouse petioles of leaves on which mealybug nymphs were released, were collected for nested RT-PCR analysis to determine if GLRaV-3 was present. ELISA and nested RT-PCR showed that GLRaV-3 was transmitted to 7 out of 10 grapevines treated with imidacloprid and to one of the 2 untreated plants. Some mealybugs were still alive after 72 hours on treated vines, although no longer moving or feeding actively.

Bioassay 2: Thirty viruliferous mealybug crawlers were transferred to each of 20 potted Cabernet franc indicator vines treated with imidacloprid after bud burst. Ten untreated indicator plants were kept aside as controls. After 72 hours treated plants were sprayed with malathion to kill all mealybug nymphs. After 8 weeks in an insect-free glasshouse, petioles of leaves on which mealybug nymphs were released, were collected for PCR analysis to determine if GLRaV-3 was present. Petiole samples were also taken from the control plants not exposed to mealybugs and similarly analyzed. Nested RT-PCR showed that GLRaV-3 was transmitted to 3 out of 11 grapevines treated with imidacloprid, while all the control plants not exposed to viruliferous mealybugs tested negative.

Conclusion: These results showed that systemically applied imidacloprid does not take effect rapidly enough to prevent grapevine mealybug nymphs from transmitting GLRaV-3, therefore virus-free grapevines treated with imidacloprid will not be protected from virus transmission if viruliferous mealybugs should enter the vineyard. To maintain virus free grapevines in new plantings, leafroll-infected vines in nearby vineyards should still be removed as part of the leafroll control strategy.

Problem identification and objectives

State the problem being addressed and the ultimate aim of the project.

At the Winetech Grapevine Virus Workshop on 29 May 2012 the question was asked whether a mealybug crawler carrying leafroll virus can transmit it to a virus-free grapevine treated with imidacloprid before the insecticide takes effect and kills the mealybug. Research by dr. K. Krüger has shown that an infected crawler can transfer the virus within 15 min after it starts feeding. If systemically applied imidacloprid does not stop the insect from feeding and/or kill it in a shorter period than the inoculation access period (time required to transfer virus), such insecticide applications will not protect virus-free vineyards from becoming infected if viruliferous mealybug crawlers enter the vineyard. This has serious implications for the industry's grapevine leafroll control strategy.

The aim of this research was to determine how soon after a grapevine mealybug (*Planococcus ficus*) starts feeding on a treated grapevine the imidacloprid takes effect and stops the mealybug feeding before it dies. The ultimate aim is to determine whether systemically applied imidacloprid either kills or stops grapevine mealybug feeding before it can transmit leafroll virus (GLRaV-3).

Workplan (materials and methods)

List trial sites, treatments, experimental layout and statistical detail, sampling detail, cold storage and examination stages and parameters.

1. Determine how soon systemically applied imidacloprid kills or stops grapevine mealybug feeding.

Grapevine mealybug egg sacs were obtained from the insectary at Nietvoorbij and placed on leafroll-infected grapevines (virus status confirmed with ELISA). Twenty-four hours after hatching, single crawlers were transferred onto the leaves of a virus-free indicator grapevine treated with imidacloprid at the registered dosage and kept in an insectary room at 25 ˚C. Each of 25 crawlers were to be regarded as a replicate.

Individual crawlers were observed under a stereo microscope in an attempt to determine the time from when it first begins to feed until it stops feeding and/or dies.

2. Determine if systemically applied imidacloprid prevents transmission of GLRaV-3 by mealybugs.

Bioassay1:

- Grapevine mealybug egg sacks from the colony maintained at Nietvoorbij were placed on leaves of vine shoots collected from a leafroll-infested grapevine (virus status confirmed with ELISA by Vititec) at Nietvoorbij research farm. Hatched crawlers were allowed to feed on these leaves for 48 hours, after which first and second instar nymphs were transferred to potted Cabernet franc indicator vines treated with imidacloprid (Confidor® 350 SC) three weeks after bud burst, according to recommendations by the registration holder.
- Groups of five nymphs were placed on six leaves of each of the 10 experimental vines and two untreated control vines (30 nymphs per plant). The number of replicates was limited by the number of indicator vines (Cabernet franc) that Vititec had available at short notice for this trial.
- After 72 hours all plants were sprayed with malathion to kill any remaining mealybugs and moved to a glasshouse.
- After seven weeks petioles of leaves on which mealybug nymphs were released, were collected and sent to the ARC-PPRI Virology Unit for nested RT-PCR analysis to determine if GLRaV-3 was present.
- Remaining leaves of the experimental vines were sent to an independent laboratory to determine if imidacloprid was still present in the leaves.

Bioassay 2:

- Twenty potted Cabernet franc indicator vines, obtained from Vititec, were treated with imidacloprid (Confidor® 70WG) three weeks after bud burst, according to recommendations by the registration holder.
- Ten Cabernet franc indicator vines, not treated with imidacloprid, were kept aside as untreated controls.
- In February 2014 petiole samples of all indicator vines were sent to the ARC-PPRI Virology Unit to be tested for the presence of GLRaV-3. Samples were tested using ELISA and those with borderline results were re-tested using PCR.
- Grapevine mealybug egg sacks from the colony maintained at Nietvoorbij were placed on leaves of vine shoots collected from a leafroll-infested grapevine (virus status confirmed with ELISA) at Nietvoorbij research farm. Hatched crawlers were allowed to feed on these leaves for 48 hours, after which first and second instar nymphs were transferred to the twenty indictor plants treated with imidacloprid. The other 10 control plants were not exposed to mealybugs.
- Groups of five nymphs were placed on six leaves of each of the 20 treated vines (30 nymphs per plant).

- After 72 hours all plants were sprayed with malathion to kill any remaining mealybugs. Plants were kept in a glasshouse for eight weeks to allow sufficient time for virus multiplication.
- After eight weeks petioles of leaves on which mealybug nymphs were released and petioles from the 10 untreated control vines were collected and sent to the ARC-PPRI Virology Unit for nested RT-PCR analysis to determine if GLRaV-3 was present.

Results and discussion

State results obtained and list any industry benefits. If applicable, include a short discussion covering ALL accumulated results from the start of the project. Limit it to essential information only.

1. Determine how soon systemically applied imidacloprid kills or stops grapevine mealybug feeding.

Even under a stereo microscope it was not possible to determine whether a grapevine mealybug had stopped feeding, due to the positioning of the mealybug's mouthparts under the body and the insect's sensitivity to damage when manipulated under the microscope. Visual observations showed that some mealybug nymphs still reacted when prodded with a fine brush 72 hours after transference to leaves of imidacloprid-treated vines, even though these insects were clearly not actively moving or feeding anymore.

2. Determine if systemically applied imidacloprid prevents transmission of GLRaV-3 by mealybugs.

Bioassay1:

The presence of GLRaV-3 in the virus source (Cabernet Sauvignon) at Nietvoorbij was confirmed by ELISA done by Vititec. Chemical anaylsis by an independent laboratory (Hearshaw & Kinnes) confirmed that 5 ppm imidacloprid was present in the leaves of treated grapevines when the mealybugs were transferred onto them. According to information supplied by Bayer scientists, this is well above the lethal concentration for this product.

Both ELISA and nested RT-PCR-analysis showed that seven of the 10 grapevines treated with imidacloprid and one of the two untreated plants tested positive for GLRaV-3 (Table 1). These results indicate that systemically applied imidacloprid did not prevent transmission of GLRaV-3 by first and second instar nymphs of the grapevine mealybug.

Table 1. Results for Bioassay 1

Replicate	Result of ELISA	Result of PCR
T1	**positive**	**positive**
T2	**positive**	**positive**
T3	**positive**	**positive**
T4	negative	negative
T5	**positive**	**positive**
T6	**positive**	**positive**
T7	**positive**	**positive**
T8	**positive**	**positive**
T9	negative	negative
T10	negative	negative
C1	**positive**	**positive**
C2	negative	negative

Bioassay 2:

All indicator plants tested negative for the presence of GLRaV-3 in February 2014 before plants were exposed to mealybugs.

By the time the final petiole samples were taken (eight weeks after exposure to the mealybugs), the leaves exposed to mealybugs of four of the imidacloprid-treated plants had already senesced to such an extent that the petioles were no longer viable for virus testing. Five more samples had to be discarded due to problems encountered in sending the samples to Roodeplaat.

Nested RT-PCR-analysis showed that three of the eleven imidacloprid-treated plants tested positive for GLRaV-3, while all of the untreated control plants tested negative (Table 2). This serves to confirm that virus transmission by the mealybugs are responsible for the plants testing positive for GLRaV-3.

Table 2. Results for Bioassay 2

Treatment (Imidacloprid)	Result of PCR	Control (no treatment)	Result of PCR
T1	negative	C1	negative
T2	**positive**	C2	negative
T3	negative	C3	negative
T4	**positive**	C4	negative
T5	**positive**	C5	negative
T6	negative	C6	negative
T7	negative	C7	negative
T8	negative	C8	negative
T9	negative	C9	negative
T10	negative	C10	negative
T11	negative		

Conclusions

1. Two bioassays have shown that grapevine mealybug nymphs can transmit GLRaV-3 to indicator vines treated with imidacloprid.
2. This means that systemic applications of imidacloprid to newly planted, virus-free vineyards will not protect the vines from transmission of GLRaV-3 if they become infested by viruliferous grapevine mealybugs, although the insecticide will control mealybug populations effectively and prevent outbreaks in these vineyards.
3. Implications for Leafroll control strategy: to keep new plantings virus free, leafroll-infected vines in nearby vineyards should still be removed to prevent mealybugs from acquiring the virus.

Complete the following table

Milestone	Target Date	Extension Date	Date Completed	Achievement
1. Determine how soon systemically applied imidacloprid kills or stops grapevine mealybug feeding.	Feb. 2013		Feb. 2013	Unable to see when mealybugs actually stop feeding under the stereo microscope. Many nymphs still active after 24 h. After 72 h some nymphs still reacted when prodded with a fine brush, but clearly no longer active or feeding.
2. Determine if systemically applied imidacloprid prevents transmission of GLRaV-3 by mealybugs.	Apr. 2013	Apr. 2014	Apr. 2014	A bioassay was conducted in 2013 and another in 2014. Both showed that viruliferous mealybug crawlers can transmit GLRaV-3 to virus-free indicator grapevines treated with imidacloprid (7 out of 10 and 3 out of 11 plants tested positive).
3. Poster at ESSA Congress	July 2013		July 2013	Poster presented at 18th ESSA congress, Potchefstroom.
4. Scientific article for SAJEV	Oct. 2013	Oct. 2014		Manuscript in preparation.
5. Presentation at SASEV Congress	Nov. 2013	Nov. 2014		Abstract submitted.
6. Popular article for Wynboer	Oct. 2013	Dec. 2014		Manuscript in preparation.

Accumulated outputs

List ALL the outputs from the start of the project. The year of each output must also be indicated.

2013

Poster at XVIII Entomological Society of Southern Africa Congress, 30 June to 3 July 2013 in Potchefstroom entitled *Can grapevine mealybug transmit grapevine leafroll-associated virus 3 to grapevines treated with imidacloprid?*

2014

SAJEV: Manuscript in preparation.

Wynland/Wyboer: Manuscript in preparation. To be submitted when scientific paper is accepted for publication.

SASEV Congress: oral presentation

Conclusions

1. Two bioassays have shown that grapevine mealybug nymphs can transmit GLRaV-3 to indicator vines treated with imidacloprid.
2. This means that systemic applications of imidacloprid to newly planted, virus-free vineyards will not protect the vines from transmission of GLRaV-3 if they become infested by viruliferous grapevine mealybugs, although the insecticide will control mealybug populations effectively and prevent outbreaks in these vineyards.
3. Implications for Leafroll control strategy: to keep new plantings virus free, leafroll-infected vines in nearby vineyards should still be removed to prevent mealybugs from acquiring the virus.

Technology development, products and patents

Indicate the commercial potential of this project, eg. Intellectual property rights or commercial product(s)

None.

Suggestions for technology transfer

List any suggestions you may have for technology transfer

Talk at Vinpro/Winetech information day/s
Popular article will be written for Wynboer.

Human resources development/training

Indicate the number and level (eg. MSc, PhD, post doc) of students/support personnel that were trained as well as their cost to industry through this project. Add in more lines if necessary.

None.

Publications (popular, press releases, semi-scientific, scientific)

- SAJEV: Manuscript in preparation.
- Wineland/Wynboer: Manuscript in preparation. To be submitted when scientific paper is accepted for publication.

Presentations/papers delivered

- Poster at XVIII Entomological Society of Southern Africa Congress, 30 June to 3 July 2013 in Potchefstroom entitled *Can grapevine mealybug transmit grapevine leafroll-associated virus 3 to grapevines treated with imidacloprid?*
- Presentation at 2014 SASEV Congress.
- Talk to be devlivered at Winetech/Vinpro information day.

Total cost summary of the project

TOTAL COST IN REAL TERMS	COST
YEAR 1	142 857
YEAR 2	163 088
YEAR 3	
YEAR 4	
YEAR 5	
TOTAL	305 945

CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
				70 000		72 857	142 857
				79 913		83 175	163 088
				149 913		156 032	**305 945**