Industry allocated project number

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FINAL REPORT
(2018)

1. PROGRAMME AND PROJECT LEADER INFORMATION

	Research Organisation Programme leader	Project leader
Title, initials, surname	Dr A. Meyer	Dr. E. Allsopp
Present position	Acting Research Team Manager	Senior Researcher
Organisation, department	ARC Infruitec-Nietvoorbij Private Bag X5026 Stellenbosch 7599	ARC Infruitec-Nietvoorbij Private Bag X5026 Stellenbosch 7599
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2. PROJECT INFORMATION

Research Organisation Project number	WW 05/23 (P04000075)
Project title	Biology of the Aster Yellows vector *Mgenia fuscovaria*
Short title	Biology of *Mgenia*

Fruit kind(s)	Wine grapes		
Start date (mm/yyyy)	04/2014	**End date** (mm/yyyy)	03/2018

Key words	Biology, leafhopper, aster yellows, vector, *Mgenia*

3. TOTAL PROJECT COST

	CFPA	DFTS	SAAPPA SASPA	SATI	Winetech	THRIP	ARC
TOTALS					**499 824**		**520 227**
Total cost of project from start to date in real terms					**R 1 202 051**		

Approved by Research Organisation Programme leader (tick box) ✓

4. EXECUTIVE SUMMARY

Objectives & Rationale

The leafhopper *Mgenia fuscovaria* is the only confirmed vector of aster yellows (AY) phytoplasma of grapevines in South Africa to date, but its biology is still largely unknown. The objectives of this research were to identify its indigenous host plants, study its seasonal abundance and life cycle on these plants and determine whether any of these plants can serve as a reservoir for aster yellows phytoplasma.

Methods

To identify native host plants, vegetation in and around vineyards where *M. fuscovaria* were previously found, were sampled for the presence of the leafhopper, using a vacuum sampler and yellow sticky traps. Seasonal abundance of *Mgenia* on indigenous host plants was determined by means of yellow sticky traps. Adult leafhoppers were collected in the field with sweep nets and confined on potted host plants in the insectary to study the life cycle and shoots of wild host plants were inspected during the season to find leafhopper eggs. Wild hosts near vineyards infected with aster yellows phytoplasma were sampled to determine if they harbour the phytoplasma.

Key Results

- Two evergreen, indigenous rambling plants that grow near watercourses were identified as host plants for *M. fuscovaria,* namely wild bramble (*Rubus* sp.) and *Cliffortia odorata* (wilde wingerd).
- Seasonal occurrence on wild host plants: numbers peaked in spring (Sept/Oct) and again in May/June, but very few leafhoppers occurred in the adjacent vineyards. Numbers were much lower in 2016/17 than in 2015/16, probably reflecting the effect of the drought on host plant quality and leafhopper survival.
- Life cycle: adult leafhoppers survived on the wild bramble and *Cliffortia odorata* plants in the insectary, but did not to lay eggs, consequently the life cycle could not be studied. Eggs were found in shoots of wild hosts, but they turned out not to be eggs of *M. fuscovaria.*
- *Rubus* plants adjacent to the AY-infected vineyard tested negative for the phytoplasma, therefore AY transmission studies with *M. fuscovaria* and the indigenous host plants were not carried out.

Conclusion and Discussion / Recommendation

Two evergreen, indigenous rambling plants were identified as indigenous host plants for *M. fuscovaria,* namely wild bramble (*Rubus* sp.) and *Cliffortia odorata* (wilde wingerd). These plants grow near watercourses and if present in an area, it is likely that *M. fuscovaria,* the vector of Aster Yellows phytoplasma, would be present as well. This means that great care should be taken not to bring plant material infested with AY into the area, as it could be transmitted by the vector. To date it does not seem likely that wild brambles are reservoirs for AY phytoplasma. Data on seasonal occurrence concurred with studies on grapevines by other researchers and showed that leafhopper numbers peaked during spring and again in autumn. As found in previous studies, *M. fuscovaria* does not appear to be an abundant leafhopper in healthy vineyards, but research has shown that it is attracted to grapevines infected by AY.

The finding that *M. fuscovaria* is not a very abundant leafhopper in healthy vineyards and the absence of aster yellows phytoplasma in its wild bramble host lead to the conclusion that AY can be successfully contained and eradicated if infected vines are destroyed, weeds are properly managed and only certified disease-free plant material is used.

5. PROBLEM IDENTIFICATION AND MOTIVATION

The leafhopper *Mgenia fuscovaria* is the only confirmed vector of aster yellows (AY) phytoplasma of grapevines in South Africa to date. Although its seasonal occurrence has been studied on vines and weeds in vineyards, its biology is still largely unknown. This research aims to identify its host plants, study its life cycle and seasonal abundance on these plants and determine whether any of these plants can serve as a reservoir of AY phytoplasma.

6. OBJECTIVES

- Identify indigenous host plants of *Mgenia fuscovaria.*
- Study seasonal abundance on indigenous host plants.
- Study life cycle on indigenous host plants.
- Determine if indigenous host plants are reservoirs for AY phytoplasma.

7. PERFORMANCE CHART

Milestone	Target Date	Extension Date	Date completed
1. Identify indigenous host plants of *M. fuscovaria*	April 2015	July 2016	July 2016
2. Determine seasonal abundance on indigenous host plants	April 2017		April 2017
3. Determine life cycle in insectary	April 2016	April 2018	Not achieved
4. Determine if indigenous host plants are reservoirs for AY phytoplasma	April 2016	April 2018	March 2018
5. Transmission studies with leafhoppers infected with AY phytoplasma	April 2017		Abandoned
6. Publications	2017	2019	Pending

8. WORKPLAN (MATERIALS AND METHODS)

Milestone 1. Identify indigenous host plants of *M. fuscovaria*

Three trial sites were selected where the highest numbers of *M. fuscovaria* were found during Dr. de Klerk's survey (ADK 1), namely a vineyard in the Voor-Groenberg area north of Wellington, one south of Wellington on the Blouvlei road and one on a farm next to the river on the Wolseley side of Bain's Kloof.

Vegetation in and around vineyards were sampled for the presence of *M. fuscovaria*. Sweep net sampling was found to be impractical because many of the surrounding plants (like wild brambles) are thorny. Sampling continued by means of yellow sticky traps and a vacuum suction sampler. All plants identified as host plants were taken to the herbarium at the Stellenbosch University Botanical Garden for confirmation of plant identity.

Milestone 2. Seasonal abundance on indigenous hosts

The seasonal abundance of *Mgenia* on indigenous host plants was determined by means of sticky traps placed in the plant canopy in vineyards and in adjacent stands of indigenous

plants identified as host plants. Traps were collected every two weeks during the growing season and monthly during winter.

Milestone 3. Determine life cycle in insectary

Adult leafhoppers were collected from indigenous hosts by means of vacuum sampling and with sweep nets and placed on potted host plants in cages in an insectary at ±25 °C. Shoots of host plants were collected at various times during the year and inspected under a stereo microscope for the presence of leafhopper nymphs and eggs.

Milestone 4. Determine if indigenous host plants are reservoirs for aster yellows phytoplasma

Wild brambles (*Rubus* sp.) growing adjacent to a Colombar vineyard near Waboomsrivier winery where aster yellows had been confirmed, were tested for the presence of the phytoplasma. A number of vines in the vineyard showed AY symptoms and *Mgenia* was present on the wild bramble (collected with a vacuum sampler). The vineyards adjacent to stands of wild brambles (*Rubus* sp.) and *Cliffortia odorata* where the seasonal occurrence of *M. fuscovaria* was studied, did not exhibit any symptoms of AY, therefore these indigenous host plants were not tested for the presence of AY phytoplasma.

9. RESULTS AND DISCUSSION

Milestone 1. Identify indigenous host plants of *M. fuscovaria*

Two *M. fuscovaria* adults were collected on a sticky trap in the vegetation next to the vineyard in the Voor-Groenberg area in the first week of January 2015. Vegetation along the watercourse here consists mainly of reeds and the indigenous rambler *Cliffortia odorata* (wildewingerd), but no *Mgenia* was collected at this site for the rest of the season. Due to the absence of leafhoppers and spraying in the vineyard, this site was abandoned. By 27 January 2015 the sticky trap next to the vineyard at Bain's Kloof yielded 3 adult *M. fuscovaria* and 1 adult was found with vacuum sampling in the vineyard. Adjacent vegetation consists mainly of wild bramble (*Rubus* sp.) and taaibos (*Rhus* sp.). On the Blouvlei road farm 17 adult *M. fuscovaria* were found on the sticky trap in the vineyard and one on the sticky trap next to the wild brambles alongside the vineyard. Since both *Cliffortia odorata* and wild bramble (*Rubus* sp.) are evergreen plants and appeared to be likely host plants for *Mgenia*, they were selected for further sampling.

Cliffortia odorata (wildewingerd)

Wild bramble (*Rubus* sp.)

Sticky trap sampling in stands of wild bramble (*Rubus* sp.) and wildewingerd *(Cliffortia odorata)* during the 2015/16 season confirmed these plants as hosts for *M. fuscovaria.* During the 2017/18 season *M. fuscovaria* was collected on sticky traps placed in an isolated stand of wild bramble removed from vineyards on Nietvoorbij research farm at Stellenbosch and adult *Mgenia* were collected with sweep nets from a solid stand of *Cliffortia odorata* next to a watercourse in Somerset West with no vineyards in the vicinity. This is further confirmation of the host plant status of these plants.

Milestone 2. Study seasonal abundance and life cycle on indigenous hosts

Seasonal abundance of *Mgenia* on indigenous host plants and in vineyards was monitored over two seasons, namely 2015/16 and 2016/17.

Site 1: located at the Slanghoek end of Bain's Kloof Pass. During a survey conducted in the Waboomsrivier area by Dr de Klerk in 2014, the highest numbers of *M. fuscovaria* were found in this vineyard. Yellow sticky traps were hung in a stand of wild bramble next to the Chenin blanc vineyard, in the edge row of vines adjacent to the wild bramble and in the centre of the vineyard. The numbers of *M. fuscovaria* adults sampled during 2015/16 are presented in Fig. 1.

Figure 1. Occurrence of *Mgenia fuscovaria* in *Rubus* and vineyard near Bain's Kloof Pass during 2015/16.

No *Mgenia* was collected on traps in the vineyard, but a few were trapped in the edge row adjacent to the wild bramble. No insecticides were applied in the vineyard during the season, therefore the absence of *Mgenia* was not due to insecticide toxicity. Sampling at this site was discontinued in the 2016/17 season because the wild brambles adjacent to the vineyard were cleared by the farmer.

Site 2: located south of Wellington on the Blouvlei road. Yellow sticky traps were hung in a stand of wild *Rubus* next to a Cabernet Sauvignon vineyard, in the edge row of vines adjacent to the *Rubus*, in a stand of *Cliffortia odorata* on the other side of the vineyard and halfway up a vine row opposite to the *Cliffortia* patch. The numbers of *M. fuscovaria* adults sampled during 2015/16 are presented in Fig. 2 and numbers for 2016/17 in Fig. 3.

Figure 2. Occurrence of *Mgenia fuscovaria* in stands of *Rubus* sp*, Cliffortia odorata* and in a Cabernet Sauvignon vineyard on Blouvlei road during 2015/16.

Figure 3. Occurrence of *Mgenia fuscovaria* in stands of *Rubus* sp*, Cliffortia odorata* and in a Cabernet Sauvignon vineyard on Blouvlei road during 2016/17.

Very few *Mgenia* leafhoppers were captured on the traps in the vineyard. No insecticides were applied, therefore the apparent absence of *Mgenia* in the vineyards is not due to insecticides. During the 2015/16 season significant numbers of *Mgenia* were captured in

the *Rubus* stand during the early part of the growing season (September to November 2015), low numbers were captured during the height of summer and numbers again increased in June and July. Fewer *Mgenia* were captured in the *Cliffortia* stand in spring, but higher numbers occurred here during summer than in the *Rubus* stand. These fluctuations probably reflect changes in attractiveness of the host plants due to water stress as the season progressed.

The numbers of *Mgenia* caught on the sticky traps during spring of the 2016/17 season (Fig. 3) were very low compared to the numbers caught during the same time of the year in 2015/16 (Fig. 2). This is ascribed to the drought, which probably affected the quality of the indigenous host plants and leafhopper survival negatively. During both seasons it was noticeable that the number of *Mgenia* caught on the sticky traps was mostly lower than the numbers of various other leafhoppers, both on the indigenous hosts and in the vineyard. It would appear that *M. fuscovaria* is not a very abundant leafhopper. Results from a survey conducted by Dr de Klerk in the Wabooms River area during 2008/09 and 2009/10 and the Robertson area during 2011/12 and 2012/13 (Final Report ADK1) indicate the same trend. Sticky trap monitoring of leafhoppers in a vineyard with moderate AY infection by Prof. K. Krüger in Vredendal (Final Report UPKK01) also showed that *M. fuscovaria* was not the most abundant of the 27 species of leafhoppers sampled over 59 weeks.

The continued presence of *Mgenia* on *Rubus* sp,and *Cliffortia odorata* during the growing season indicated that *Mgenia* did not migrate in large numbers from the indigenous hosts to the grapevines at both sites. These vines showed no symptoms of Aster Yellows. However, in Prof. Krüger's study (Final Report UPKK01) *M. fuscovaria* was by far the most abundant leafhopper over 59 weeks in another vineyard with severe symptoms of AY infection. Prof. Krüger also showed that *M. fuscovaria* is highly attracted to plants infected with AY and will choose them over healthy grapevines. This may explain the leafhopper's abundance in the AY-infected vineyard and its scarcity in the vineyards in this study where AY was not present.

Milestone 3. Determine life cycle in insectary

Adult *M. fuscovaria* collected from the field with a vacuum sampler did not survive when placed on host plants in cages. Leafhoppers collected with sweep nets survived on the wild bramble and *Cliffortia odorata* in the insectary. The insectary room was maintained at a temperature of 25±1 °C and the north-facing glass wall allowed for a natural light regime. Adults were collected at various times throughout the season, but we were unable to get them to lay eggs.

Shoots of brambles and *Cliffortia odorata* were inspected for the presence of eggs or oviposition scars. Although some eggs were found in the shoots (see below), they turned out to be eggs of beetles and Lepidoptera.

Milestone 4. Determine if indigenous host plants are reservoirs for aster yellows phytoplasma

Samples of *Rubus* plants growing adjacent to a vineyard near Waboomsrivier winery where some vines are infected by AY tested negative for the presence of Aster Yellows phytoplasma, even though the leafhopper was present in the vineyard (very few) and on the wild brambles. The vineyards at the two trial sites, De Brug and Ernita, did not exhibit any symptoms of AY, therefore the *Rubus* and *Cliffortia odorata* next to these vineyards were not tested for the presence of AY phytoplasma.

Since the *Rubus* plants adjacent to the AY-infected vineyard tested negative for the phytoplasma, transmission studies with *M. fuscovaria* and the indigenous host plants were not carried out.

10. CONCLUSIONS AND RECOMMENDATIONS

Two evergreen, indigenous rambling plants were identified as indigenous host plants for *M. fuscovaria,* namely wild bramble (*Rubus* sp.) and *Cliffortia odorata* (wilde wingerd). These plants grow near watercourses and if present in an area, one would expect that *M. fuscovaria,* the vector of Aster Yellows phytoplasma, to be present as well. This means that great care should be taken not to bring plant material infested with AY into the area, as it could be transmitted by the vector. Grapevines infected with AY should preferably be removed and destroyed to prevent spreading of the disease. At present it does not seem likely that wild brambles are reservoirs for AY phytoplasma. Data on seasonal occurrence concurred with studies on grapevines by other researchers and showed that leafhopper numbers peaked during spring and again in autumn. It was also evident that population levels of *M. fuscovaria* are significantly impacted by climate and host plant condition. As found in previous studies, *M. fuscovaria* does not appear to be an abundant leafhopper in healthy vineyards, but research has shown that it is attracted to grapevines infected by AY. Although *M. fuscovaria* could not be reared successfully in the insectary during this project, efforts to achieve this will continue.

The findings in this study and surveys conducted by Dr de Klerk that *M. fuscovaria* is not a very abundant leafhopper in vineyards and the absence of aster yellows phytoplasma in its wild bramble host indicate that AY can be successfully contained and eradicated if infected vines (which are more attractive to the leafhopper than healthy vines) are destroyed and only certified disease-free plant material is used.

11. ACCUMULATED OUTPUTS

a) TECHNOLOGY DEVELOPED, PRODUCTS AND PATENTS

None.

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

Popular article in Winetech tegnies to inform growers of the natural host plants that harbour *M. fuscovaria.*

Talk at information day about *Mgenia,* its role in spreading AY and the importance of leafhopper monitoring in areas where the disease occurs.

c) HUMAN RESOURCES DEVELOPMENT/TRAINING

None.

d) PUBLICATIONS (POPULAR, PRESS RELEASES, SEMI-SCIENTIFIC, SCIENTIFIC)

Popular article in Winetech tegnies to inform growers of the natural host plants that harbour *M. fuscovaria.*

e) PRESENTATIONS/PAPERS DELIVERED

None.

12. PERSONS PARTICIPATING IN THE PROJECT

Initials & Surname	Highest Qualification	Degree/ Diploma registered for	Race (1)	Gender (2)	Institution & Department	Position (3)	** Cost to Project R
E. Allsopp	PhD		W	F	ARC Infruitec-Nietvoorbij Plant Protection	PL	
L. Williams	BA		B	M	ARC Infruitec-Nietvoorbij Plant Protection	TA	

*** (Only applicable to persons who participate as Consultants or on Contract)*

(1)Race
B = African, Coloured or Indian
W = White

(2)Gender
F = Female
M = Male

(3)Position
Co = Co-worker (other researcher at your institution)
Coll = Collaborator (participating researcher that does not receive funding for this project from industry)
PF = Post-doctoral fellow
PL = Project leader
RA = Research assistant
TA = Technical assistant/ technician

13. BUDGET

TOTAL COST SUMMARY OF THE PROJECT

YEAR	CFPA	DFTS	Deciduous	SATI	Winetech	ARC	TOTAL
2014/15					159 999	166 529	326 528
2015/16					171 233	178 222	349 455
2016/17					110 229	114 729	224 958
2017/18					58 363	60 747	119 110
Total					**499 824**	**520 227**	**1 202 051**

EVALUATION BY INDUSTRY

This section is for office use only

Project number	WW 05/23 (P04000075)

Project name	Biology of the Aster Yellows vector *Mgenia fuscovaria*

Name of Sub-Committee*	Grapevine Protection: Insects & Pests

Comments on project

Committee's recommendation

- Accepted. ☐
- Accepted provisionally if the sub-committee's comments are also addressed. Resubmit this Final Report by_____ ☐
- Unacceptable. Must resubmit Final Report. ☐

Chairperson_____ Date_____

*SUB-COMMITTEES

Winetech

Viticulture: Cultivation; Soil Science; Plant Biotechnology; Plant Protection; Plant Improvement;
Oenology: Vinification Technology; Bottling, Packaging and Distribution; Environmental Impact; Brandy and Distilling; Microbiology

Deciduous Fruit

Technical Advisory Committees: Post-Harvest; Crop Production; Crop Protection; Technology Transfer
Peer Work Groups: Post-Harvest; Horticulture; Soil Science; Breeding and Evaluation; Pathology; Entomology

SATI

Technical Committees
SATI Research and Development Committee