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Diversity of leafhopper and planthopper species in South African vineyards

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***Abstract* - The discovery of aster yellows phytoplasma ('*Candidatus* Phytoplasma asteris') in grapevine in the Western Cape in South Africa prompted surveying and monitoring of leafhopper and planthopper (Hemiptera: Auchenorrhyncha) species in order to determine species diversity, the abundance of the leafhopper vector *Mgenia fuscovaria* and to identify further potential vectors. Surveys were carried out in vineyards since 2008 using vacuum sampling, sweep netting and visual plant inspection. Weekly insect monitoring with yellow sticky traps commenced in 2009. Over a period of 10 years, 27 leafhopper (Cicadellidae) species, four planthopper (Delphacidae) species, one species of Cixiidae, and six species of other Auchenorrhyncha were identified.**

Key words* - *Aconurella prolixa*, '*Candidatus* Phytoplasma asteris', Cicadellidae, *Mgenia fuscovaria

I. Introduction

Phytoplasmas (class Mollicutes) are plant pathogenic phloem-limited, wall-less bacteria (Bertaccini *et al.* 2014). They are transmitted by hemipteran phloem-feeding insects of the suborders Auchenorrhyncha and Sternorrhyncha (Weintraub and Beanland 2006). Within the Auchenorrhyncha phytoplasma vectors have been reported from the leafhopper family Cicadellidae and the planthopper families Cixiidae, Delphacidae, Derbidae, and Flatidae (Wilson and Weintraub 2007).

Aster yellows phytoplasma (AY), caused by '*Candidatus* Phytoplasma asteris' (16SrI-B group), was first recorded in grapevine (*Vitis vinifera* L., Vitaceae) in South Africa in 2006 (Engelbrecht *et al.* 2010). The disease is of phytosanitary concern in the country. The leafhopper species *Mgenia fuscovaria* (Stål) (Hemiptera: Cicadellidae) was identified as a vector (Krüger *et al.* 2011).

The aims of this study were to determine the diversity of leafhopper (Cicadellidae) and planthopper (e.g. Cixiidae, Delphacidae, Derbidae, and Flatidae) species in vineyards in the Western Cape in order to identify potential vectors of AY and to monitor the abundance of *M. fuscovaria*.

II. Material and Methods

Insect sampling

Leafhoppers and planthoppers were sampled in Vredendal (30°40′S, 18°30′E) and in Waboomsrivier (33°40′S, 19°15′E) in the Western Cape province of South Africa where AY has been recorded. Insects in grapevines, weeds and cover crops in commercial vineyards were sampled during different times of the year with vacuum sampling (DVac), sweep netting and hand searches since 2008. Insects were preserved in 95% ethanol. In addition, insects have been monitored weekly in a commercial vineyard infected with AY with yellow sticky traps in Vredendal since 2009. Adult insects were identified morphologically. Voucher specimens were deposited in the National Collection of Insects, ARC-Plant Protection, Pretoria.

DNA extraction and PCR

Subsamples of insect species preserved in 95% ethanol were tested for the presence of AY phytoplasma in order to identify potential insect vectors. Nucleic acids were extracted from single leafhoppers using a non-destructive TNES buffer (1 M Tris-HCl, pH 7.4, 5 M NaCl, 0.5 M EDTA, 10 % SDS) extraction method adapted from a protocol provided by J. Peccoud and N. Sauvion (INRA Montpellier, France) based on Sambrook and Russell (2001). Samples were tested for the presence of AY with real-time PCR following Angelini *et al.* (2007).

III. Results

The majority of the species collected belong to Cicadellidae (27 species; 71%), followed by Delphacidae (4 species; 11%), Cixiidae (1 species; 3%) and other Auchenorrhyncha (16%) (Table 1).

Some leafhopper species, such as *Aconurella prolixa* and the planthopper species *Toya* sp., were exclusively collected from grasses, whereas others are known to feed on grasses and shrubs, e.g. *Austroagallia.* During the growth period *M. fuscovaria* was predominately observed on grapevine and during winter on weeds in vineyards.

Mgenia fuscovaria adults and nymphs have been recorded throughout the year. Adults were abundant on yellow sticky

traps. They tended to peak in late summer/early autumn (February/March), winter (June/July) and spring (September/October), suggesting that this species has three generations per year.

Austroagallia spp., *Cicadulina* spp., *M. fuscovaria*, *A. prolixa* and one delphacid species, *Toya* sp., tested positive for the presence of aster yellows phytoplasma.

Table 1 Cicadellidae, Delphacidae, Cixiidae and other Auchenorrhyncha recorded in vineyards in the Western Cape. Species that tested positive for aster yellows phytoplasma (AY) with PCR are indicated with an asterisk.

Family	Subfamily	Species	AY
Cicadellidae (leafhoppers)	Agalliinae	*Austroagallia cuneata* (Cogan)	*
		Austroagallia nigrasterna (Cogan)	
		Peragallia caboverdensis (Lindberg)	
	Coelidiinae	*Mgenia angusta* Theron	
		Mgenia fuscovaria (Stål)	*
	Deltocephalinae	*Aconurella prolixa* Lethierry	*
		Afrosteles distans (Linnavuori)	
		Balclutha incisa (Matsumura)	
		Balclutha rosea (Scott)	
		Cicadulina anestae Van Rensburg	*
		Cicadulina mbila (Naudé)	
		Circulifer struthiola (Cogan)	
		Circulifer tenellus (Baker)	
		Deltocephalus cotulus (Cogan)	
		Exitianus taeniaticeps (Kirschbaum)	
		Goniagniathus agenor Linnavuori	
		Maiestas sp.	
		Nesoclutha erythrocephala (Ferrari)	
		Orosius albicinctus (Distant)	
		Penthimiola bella (Stål)	
		Tetartostylus sp.	
		Thaumatopoides ochracea (Naudé)	
	Iassinae	*Batracomorphus cedaranus* (Naudé)	
	Typhlocybinae	*Acia lineatifrons* (Naudé)	
		Empoasca spp.	
		Molopopterus sp.	
	Ulopinae	*Coloborrhis corticina* (Germar)	
Delphacidae (planthoppers)		*Embolophora britmusei* Asche	
		Sogatella sp.	*
		Toya sp.	
		Tropidocephala sp.	
Cixiidae (primitive snout bugs)		Unidentified	
Aphrophoridae		*Cordia* sp.	
Cercopidae		*Rhinaulax sp.*	
Flatidae		*Calauria suliceps* Stål	
Membracidae		*Anchon dukei* Capener	
Ricaniidae		*Mulvia albizona* Spinola	
Tettigometridae		*Hilda patruelis* (Stål)	

IV. Discussion

Most vectors of phytoplasmas have been reported from the Cicadellidae (Wilson and Weintraub 2007). In accordance, four of the five species that tested positive for AY in the current study belong to the family Cicadellidae.

Mgenia fuscovaria tested positive several times and was identified as a vector in controlled transmission experiments (Krüger *et al.* 2011). *Aconurella prolixa* was identified as an experimental vector of AY (Krüger *et al.* 2018). Transmission of AY by the other three species/species groups that tested positive for the presence of AY has not been demonstrated (Krüger, pers. obs.). However, the list of species recorded together with the list of alternative host plants of AY in South Africa (Krüger *et al.* 2015) suggest that further species may be vectors of AY in this country.

The systematics of common species in the Western Cape, which includes the Fynbos Biome, is reasonably well understood, but our knowledge concerning species diversity and the biology of most species is scanty and needs further investigation.

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