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O33: A potential new vector of aster yellows phytoplasma in vineyards in South Africa

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INTRODUCTION

Aster yellows phytoplasma (AY; 16SrI-B group) is associated with a severe disease in grapevine (*Vitis vinifera*) and is of phytosanitary concern in South Africa. It was recorded for the first time from vineyards in the Western Cape in 2006 (Engelbrecht et al., 2010). The phytoplasma has a broad host range (Hogenhout et al., 2008) and has been recorded from various wild and crop plants growing in and around vineyards in South Africa (Krüger et al., 2015). Several leafhopper species (Hemiptera: Auchenorrhyncha: Cicadellidae) are known to transmit AY (Weintraub and Beanland, 2006). The leafhopper *Mgenia fuscovaria* (Stål) has been identified as a vector of AY in South Africa (Krüger et al., 2011). However, the demonstrated transmissibility of AY to *Catharanthus roseus* (periwinkle) in the field is in contrast to the inability of *M. fuscovaria* to transmit the pathogen to this host (Krüger et al., 2015). This, together with the presence of AY in other leafhopper species, suggests that further taxa might be involved in the AY pathosystem in South African vineyards. In order to assist with the development of AY management strategies, leaf- and planthoppers were screened for the presence of AY, and transmission assays were carried out to identify vectors other than *M. fuscovaria.*

MATERIALS AND METHODS

Experiments were carried out in Vredendal in the Western Cape, South Africa, during different times of the year with leaf- and planthoppers collected in AY-infected vineyards. Transmission assays with field-collected insects were performed by giving one to six adults of the same species or species group access to an artificial sucrose feeding medium through Parafilm® for 24 to 48 hours. The media were screened after completion of feeding for the presence of AY phytoplasma following Bosco and Tedeschi (2013). In addition, transmission assays were carried out with *Nicotiana benthamiana, Triticum aestivum* (wheat, cultivar Duzi), and periwinkle as recipient plants. Groups of 3 to 20 adult leafhoppers per species were given inoculation access periods (IAPs) of 24 to 72 hours. Group size was determined by the number of insects collected in the field. In order to confirm that insects were collected from AY-infected vineyards and that recipient plants were AY-free prior to transmission assays, leaves from grapevine plants in vineyards and recipient plants were tested for the presence of AY. Nucleic acid extraction from leaf veins was done using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany). Nucleic acids from single intact leafhoppers were obtained 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) to preserve specimens for morphological identification. Field-collected insects, feeding media and plant samples were tested for the presence of AY with real-time PCR (Angelini et al., 2007).

Results and discussion

A total of 136 adult leaf- and planthopper samples were tested for the presence of AY, 80 of which were used in the artificial feeding assays. The leafhopper *Aconurella prolixa* (Lethierry) tested positive for AY and successfully transmitted the pathogen to the artificial feeding medium (Table 1). *A. prolixa* is a grass-feeding species, and controlled transmission assays were carried out with wheat. Eight wheat plant samples out of 51 used in transmission assays with this species tested positive for AY. One *Exitianus* sp. adult tested positive for AY but did not transmit the phytoplasma to the artificial feeding medium nor to *N. benthamiana* or periwinkle (*N. benthamiana*, n = 11; periwinkle, n = 2). Although *Cicadulina* spp. and *Toya* sp. tested positive for AY in an earlier study (A. de Klerk, pers. comm.) they did not transmit AY to the feeding medium nor to *N. benthamiana* (*Cicadulina* spp: n = 5, *Toya* sp.: n = 17). None of the other species tested positive for AY or transmitted the phytoplasma to the artificial feeding medium (Table 1).

Table 1. Presence of aster yellows phytoplasma (AY) in field-collected leaf- and planthoppers and transmission of AY to an artificial sucrose feeding medium used for screening for potential insect vectors.

Insect species	Insects tested/AY-positive insects	Feeding media tested/AY-positive media
Cicadellidae (leafhoppers)		
Aconurella prolixa	12/1	3/1
Cicadulina spp.	39/0	25/0
Exitianus sp.	45/2	19/0
Delphacidae (planthoppers)		
Toya sp.	3/0	2/0
Unidentified spp.	37/0	31/0

Previously, several plant species, including Poaceae, were identified as reservoir plants of AY in South African vineyards, and the leafhopper vector *M. fuscovaria* was able to transmit AY experimentally to several poaceous plant species (maize, wheat, triticale) (Krüger et al., 2015). It is not known whether *M. fuscovaria* or *A. prolixa* can transmit AY from Poaceae to grapevine. However, the identification of the grass-feeding *A. prolixa* as a potential vector suggests complex interactions between the phytoplasma, host plants and potentially more than one leafhopper vector species.

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