

Aster yellows phytoplasma in grapevines: identification of vectors in South Africa

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Abstract

Since the discovery of aster yellows phytoplasma on grapevine in South Africa in 2006, a concerted effort by various research groups to identify the vector(s) was co-ordinated by Winetech. This included qualitative and quantitative surveys for two years of leaf- and planthoppers occurring in vineyards in affected areas, testing specimens of leaf- and planthopper species for the presence of aster yellows phytoplasma with PCR, transmission experiments, and relating presence of leaf- and planthoppers in the field to the time of disease transmission. Twenty-eight leaf- and planthopper species/species groups have been recorded from vineyards in aster yellows-infected regions. The four most abundant species/species groups were *Acia lineatifrons* (Naudé), *Austroagallia* spp., *Cicadulina* spp. and *Mgenia fuscovaria* (Stål) (Hemiptera: Cicadellidae). *Austroagallia* spp. and *M. fuscovaria* repeatedly tested positive for the presence of AY. Results of transmission experiments and a field trial to determine leaf-/planthopper abundance in vineyards along with time of transmission in the field suggest that *M. fuscovaria* is a vector of aster yellows phytoplasma in South Africa. The concerted effort between industry and researchers allowed for fast identification of a potential vector of aster yellows phytoplasma in South Africa.

Key words: Cicadellidae, Coelidiinae, leafhopper, *Mgenia fuscovaria*, phytoplasma.

Introduction

A phytoplasma was recorded for the first time from grapevine, *Vitis vinifera* L. (Vitaceae), in South Africa in 2006 (Engelbrecht *et al.*, 2010). Aster yellows phytoplasma (AY), 16SrI group (Engelbrecht *et al.*, 2010), until recently had been reported to occur in grapevine in two regions but lately was also found in a third region, also in the Western Cape Province. Aster yellows disease (AY) has been recorded in other countries from a broad host range (Hogenhout *et al.*, 2008). Economic losses caused by infection of cultivated plants can be severe. Phytoplasmas can be transmitted through phloem-feeding insect vectors (Auchenorrhyncha (Cicadellidae, Fulgomorpha, Psyllidae)) (Weintraub and Beanland, 2006). Identification of vectors is essential if disease spread is to be managed.

In general, identification of vectors of phytoplasmas of grapevine (grapevine yellows) has proved difficult, and in several instances these have not been identified (Constable, 2010). Due to the urgency of identifying vector(s) in order to manage the disease effectively, whilst recognizing the challenges this entails, Winetech (Wine Industry Network for Expertise and Technology) initiated a multidisciplinary programme in 2008 involving researchers from several organizations for rapid identification of the vector(s) of AY in South Africa.

Materials and methods

To identify potential vectors, the programme commenced in 2008 with qualitative and quantitative surveys to identify leaf-/planthopper and other potential vector species present in vineyards and to determine their abundance. Insects in two grapevine growing areas in four vineyards each where AY occurs were monitored with vacuum sampling (DVAC) for two years. Where possible, specimens of potential vectors were identified to species level. Subsamples of potential vector species collected were tested for the presence of phytoplasma with nested PCR (Engelbrecht *et al.*, 2010). To confirm that species that tested positive are vectors of AY, transmission experiments using field-collected insects from highly AY-infected vineyards were conducted. Field-collected insects, rather than laboratory-reared insects, were used because of the difficulties experienced in establishing cultures. Individual phytoplasma-free grapevine (cv. Chardonnay) or *Nicotiana benthamiana* L. (Solanaceae) plants served as recipient plants. To confirm that insects and plants from collection sites for transmission experiments were infected with AY, leaves from subsamples of plants and subsamples of leafhopper specimens were tested for AY with real-time PCR (Angelini *et al.*, 2007). Recipient plants were confirmed AY-free in the same manner prior to transmission experiments. Plants were tested starting five weeks post-AY transmission.

In addition, identification of vector(s) was done by monitoring leaf-/planthopper populations for 12 months and correlating this to the time of AY infection of grapevines in the field. To this end, 10 AY-free grapevine plants (cv. Cabernet franc, cv. Chardonnay, cv. Chenin blanc) together with 10 yellow sticky traps and, when available, periwinkle (*Catharanthus roseus* (L.) G. Don (Apocynaceae)) plants, were placed in two AY-infected vineyards, the rationale being that plants would only become infected when AY-infected vectors are present and feed on them. Bait plants were replaced weekly for a year, treated with a systemic insecticide and maintained in insect-free enclosures and were subsequently tested for AY with real-time PCR (Angelini *et al.*, 2007) starting seven weeks after exposure in the field. All leaf-/planthopper species collected on the yellow sticky traps were identified to species level where possible.

Results

Since 2008, 28 leaf-/planthopper species/species groups have been recorded from grapevine and vegetation within vineyards in regions where AY occurs. The most abundant species/species groups were *Acia lineatifrons* (Naudé), *Austroagallia* spp., *Cicadulina* spp. and *Mgenia fuscovaria* (Stål) (Hemiptera: Cicadellidae). Amongst these, *Austroagallia* spp. and *M. fuscovaria* tested positive for phytoplasma. Furthermore, *M. fuscovaria*, was collected in the largest number from grapevines, with very few collected from weeds. In subsequent transmission experiments with field-collected *M. fuscovaria*, and based on real-time PCR tests, AY was transmitted to three out of 20 grapevine plants. None of the control plants, not exposed to *M. fuscovaria* and kept under the same conditions as recipient plants, tested positive for AY. Thus far, AY-transmission by field-collected *Austroagallia* spp. to individual *N. benthamiana* plants has not been demonstrated.

A comparison of the time of AY infection in vineyards with leaf-/planthopper abundance over a period of 28 weeks also suggests that AY transmission was due to *M. fuscovaria*, which is the most prevalent species in the vineyard where AY-infected bait plants were recorded. The only other species whose presence coincided with the time of exposure of bait plants and the latter becoming infected with AY, were single individuals belonging to three species collected in the course of four different weeks, whereas *M. fuscovaria* occurred in large numbers on these occasions.

Discussion

Because *M. fuscovaria* was the dominant species and in some instances the only species found on the traps in the weeks when AY-infected bait plants were obtained it is likely that AY-transmission was due to *M. fuscovaria* feeding. This, together with the transmission of AY to

healthy grapevine plants maintained in insect-free facilities using field-collected *M. fuscovaria*, suggests that this species is a vector of AY in South Africa. Planned work includes transmission experiments with AY from grapevine to grapevine with specimens of the *Austroagallia* species group to determine if they, too, are vectors.

The qualitative and quantitative surveys together with direct tests of species for AY allowed us to focus on specific species for transmission experiments to assess whether they serve as vectors. Monitoring leaf-/planthopper populations together with determining the time of AY transmission not only facilitated the identification of potential vectors, but will also assist with the development and timing of control measures. The concerted effort by a multi-disciplinary team co-ordinated by Winetech has resulted in a rapid identification of a potential vector of AY in South Africa.

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