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Potential insect vectors and alternative host plants of phytoplasmas in the Fynbos and Succulent Karoo biomes in South Africa

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Abstract

Potential insect vectors and alternative host plants of the phytoplasmas associated with grapevine yellows (GY) were surveyed in the Fynbos and Succulent Karoo biomes in the Western Cape, South Africa. Aster yellows phytoplasma (16SrI-B), which has been reported infecting grapevine in three regions in the Western Cape, was identified in a plant species belonging to the Aizoaceae. Other phytoplasmas were detected from species belonging to the Brassicaceae, Montiniaceae, Proteaceae and Zygophyllaceae and in a few insect specimens. The information will be used to confirm the insect vector status and the role of the plant species identified as alternative host plants in controlled transmission experiments.

Keywords: aster yellows phytoplasma, grapevine yellows, natural vegetation, epidemiology

Introduction

Grapevine yellows (GY), *e.g.* "bois noir", "flavescence doreé" and aster yellows, are serious diseases of grapevine (*Vitis vinifera*, Vitaceae) threatening production and international trade. Associated with these insect-transmitted diseases are a number of phloem-limited bacteria ('*Candidatus* Phytoplasma'). The grapevine production in South Africa is currently threatened by aster yellows (AY) phytoplasma, '*Candidatus* Phytoplasma asteris' subgroup 16SrI-B (Engelbrecht *et al*., 2010). AY has been reported in grapevine from countries in Africa, Europe, North and South America. Surveys were carried out in the Fynbos and Succulent Karoo biomes in the Western Cape in order to identify potential insect vectors and alternative host plants of these phytoplasmas.

Materials and Methods

Three surveys were carried out in September (spring) in 2017, and January (summer) and August (winter) in 2018 in the natural vegetation at nine sites (Figure 1). Insects were collected with vacuum sampling from 20 randomly selected plant samples per species at the specific sites. Branches with leaves from the same plant species were collected. Insects were preserved in 95% ethanol. Insects and plants were identified to species level when possible. Insects were identified based on morphological characteristics and plants based on morphological characteristics and sequencing. Both potential phloem-feeding insect vectors in the Hemiptera and plant samples were tested for the presence of phytoplasmas.

DNA from intact insects was extracted using a non-destructive TNES buffer method adapted from J. Peccoud and N. Sauvion (INRA Montpellier, France) based on Sambrook and Russell (2001). DNA from plant material was extracted using a 3% CTAB extraction buffer method (Doyle and Doyle, 1990). The extracted insect and plant DNA was used as template for nested PCR of a segment of the elongation factor Tu (*tuf*) gene using the primer pairs Tuf340a/Tuf890a and Tuf400a//Tuf835a (Makarova *et al*., 2012), and of a segment of the 16S rRNA gene with the primer pairs P1/P7 (Deng and Hiruki, 1991, Schneider *et al*., 1995), R16F2n/R2 (Gundersen and Lee, 1996) and R16(I)F1/R1 (Lee *et al*., 1994). RFLP analysis was performed with *Tru*I

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restriction enzyme on R16F2n/R2 amplicons, and with *Hha*I on R16(I)F1/R1 amplicons. Direct sequencing of the amplified products using the primers indicated above was also carried out. The psbA-trnH intergenic spacer region and the *rbcL* gene were used for barcoding for plant species identification as described (Roberts and Pietersen, 2017).

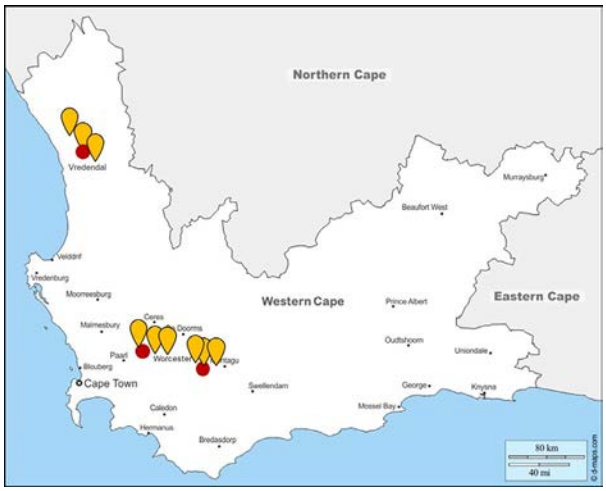

Figure 1. Sampling sites in the Western Cape in South Africa (red circle: areas where aster yellows phytoplasma has been detected; orange markers: sampling sites).

Results

The survey resulted in more than 1,200 insect specimens collected from 989 plant samples from 19 families and 42 species. Only two plant samples from *Mesembryanthemum crystallinum* (Aizoaceae), plant native to South Africa, tested positive for AY with both 16S rDNA RFLP analysis and sequencing analysis on the *tuf* gene amplicons placing the phytoplasma in the 16SrI-B subgroup. This plant species also occurs in other parts of Africa, Australasia, Asia, the Americas and Europe. Other phytoplasmas were detected in species belonging to the Brassicaceae, Montiniaceae, Proteaceae and Zygophyllaceae.

The majority of insects collected belong to the leafhopper family Cicadellidae, comprising more than 50 species in the Deltocephalinae (32 species), Agallinae, Coelidiinae, Typhlocybinae and Ulopinae. Common genera in Deltocephalinae included *Aconurella, Balcutha, Bonaspeia, Caffrolix, Cicadulina, Curculifier, Discolopeus, Exitianus, Hadroca, Maiestas, Orosius, Pravistylus* and *Renosteria*. Fulgoroidea, the planthopper group, included species in Cerocopidae, Cixiidae, Delphacidae, Issidae, Meenoplidae and Tropiduchidae. As yet unidentified phytoplasma-positive insect species were collected from the wild clove bush, *Montinia caryophyllacea* (Montiniaceae), and *Sorocephalu spinifolius* (Proteaceae). Five insect samples tested positive for phytoplasmas with primers for the *tuf* gene, but only two were positive using the 16S ribosomal gene primers. These latter phytoplasmas were identified as belonging to the 16SrII and 16SrIII groups.

Discussion

Phytoplasma infection of Proteaceae has been reported from South Africa (Wieczorek and Wright, 2003) and the 16SrII group phytoplasmas were sporadically detected in grapevine in this country (Botti and Bertaccini, 2006). The identification of phytoplasmas in potential insect vectors and plant species occurring in natural vegetation suggest that they could potentially serve as alternative vectors and reservoirs, respectively, in the vicinity of the vineyards. Further studies with controlled transmission experiments are required to confirm the insect vector status and the role of the plant species identified as alternative host plants.

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References

Botti S and Bertaccini A 2006. First report of phytoplasmas in grapevine in South Africa. *Plant Disease*, 90: 1360.

Deng S and Hiruki C 1991. Amplification of 16S rRNA genes from culturable and non-culturable mollicutes. *Journal of Microbiological Methods*, 14: 53-61.

Doyle JJ and Doyle JL 1990. Isolation of plant DNA from fresh tissue. *Focus*, 12: 13-15.

Engelbrecht M, Joubert J and Burger JT 2010. First report of aster yellows phytoplasma in grapevines in South Africa. *Plant Disease*, 94: 373.

Gundersen DE and Lee I-M 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathologia Mediterranea*, 35: 144-151.

Lee I-M, Gundersen DE, Hammond RD and Davis RE 1994. Use of mycoplasma-like organism (MLOs) group specific oligonucleotide primers for nested-PCR assays to detect mixed-MLO infections in a single host plant. *Phytopathology*, 84: 559-566.

Makarova OV, Contaldo N, Paltrinieri S, Kawube G, Bertaccini A and Nicolaisen M 2012. DNA barcoding for universal identification of '*Candidatus* Phytoplasmas' using a fragment of the elongation factor *Tu* gene. *Plos One*, 7(12): e52092.

Roberts R and Pietersen G 2017. A novel subspecies of '*Candidatus* Liberibacter africanus' found on native *Teclea gerrardii* (Family: Rutaceae) from South Africa. *Antonie van Leeuwenhoek*, 110: 437-444.

Sambrook J and Russell DW 2001. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York, USA.

Schneider B, Seemüller E, Smart CD and Kirkpatrick BC 1995. Phylogenetic classification of plant pathogenic mycoplasma like organisms or phytoplasmas. In: *Molecular and Diagnostic Procedures in Mycoplasmology*, pp 369-380. Eds S Razin and JG Tully, Academic Press, San Diego, CA, USA.

Wieczorek AM and Wright MG 2003. PCR detection of phytoplasma from witches' broom disease on *Protea* spp. (Proteaceae) and associated arthropods. *Acta Horticulturae*, 602: 161-166.