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Hosts of stolbur phytoplasmas in maize redness affected fields

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Abstract

The plant host range of a phytoplasma is strongly dependent on the host range of its insect vector. Maize redness in Serbia is caused by stolbur phytoplasma (subgroup 16SrXII-A) and is transmitted by the cixiid planthoper, *Reptalus panzeri* (Löw). *R. panzeri* was the only potential vector found to be infected with stolbur phytoplasm in and around maize redness affected fields, and the phytoplasma was only found in monocotyledonous plants including maize, Johnsongrass, and wheat. Other known stolbur phytoplasma vectors and weedy plant hosts tested were not infected. These results are discussed with respect to potential differentiation of the pathogen in different host-vector systems.

Key words: Class *Mollicutes*; South Banat region; stolbur phytoplasma; vector.

Introduction

All phytoplasmas have a dual host infection cycle, multiplying in both their plant host and insect vector (Gasparich, 2010). The plant host ranges of phytoplasmas are generally considered to be controlled by the host range of their specific insect vector. Stolbur phytoplasma (subgroup 16SrXII-A) has a broad plant host range that includes important solanaceous crops, grapevine, celery, sugarbeet, strawberry, lavender and maize (Garnier *et al.*, 2000; Jović *et al.*, 2007). The phytoplasma also infects a number of dicotyledonous weeds, the most important of which are bindweeds (*Convolvulus arvense* L. and *Calystegia sepium* (L.) (R. Br.) and nettle (*Urtica dioica* L.). Known vectors of stolbur phytoplasma include the cixiid planthoppers *Hyalesthes obsoletus* Signoret, *Pentastiridius leporinus* (L.) and *Reptalus panzeri* (Löw). Bindweeds and nettle are the primary hosts of *H. obsoletus*. *P. leporinus* and *R. panzeri* are polyphagous, with early stages of development occuring on grasses.

Maize redness (MR) in the Banat region of Serbia is associated with stolbur phytoplasma transmitted by *R. panzeri* (Duduk and Bertaccini, 2006; Jović *et al.* 2007; 2009). The disease can cause catastrophic losses in maize (Bekavac *et al.*, 2008). The initial disease symptom, reddening of the leaf midrib, appears in late July. Reddening of leaves and stalks intensifies with time, and plants become dry by early September. Critically, ear development and seed set are adversely affected by the disease.

MR transmission in South Banat was associated only with *R. panzeri*. Very few *H. obsoletus* individuals were found in MR affected fields, and no *Pentastiridius* species were present (Jović *et al.,* 2009). In contrast, 'bois noir' of grapevine associated with stolbur phytoplasmas is transmitted only with *H. obsoletus.* The association of stolbur phytoplasma diseases with different vectors, suggests key differences in disease etiology and epidemiology between monocot and dicot hosts.

Materials and methods

Experiments were carried out as described in Jović *et al*. (2009). Briefly, insects were collected in July from MR affected maize fields. Insects were stored in 80% ethanol at -20°C prior to analysis. The DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) was used to isolate DNA from individual insects. Stolbur phytoplasma was detected in DNA samples using nested PCR (Clair *et al.*, 2003). Weedy plants were selected randomly from the borders of MR affected maize fields. Genomic DNA was extracted from the roots of individual plants (Angelini *et al.,* 2001). Winter wheat was collected in the spring from a field that had been planted after maize in an MR affected field in Samoš. DNA was isolated from individual wheat roots using the DNeasy Plant Mini Kit (Qiagen). Samples were assayed for the presence of stolbur phytoplasma by nested PCR.

Results

Phytoplasmas were detected in three of 13 Auchenorrhyncha species collected from MR affected fields (table 1). *R. panzeri* individuals were infected with stolbur phytoplasma, while *Mocydia crocea* (Herrich-Schaffer) and *Psammotettix alienus* (Dahlbom) were positive for phytoplasmas from the aster yellows group (16SrI-C and I-B). No phytoplasmas were detected in any of 139 *Zyginidia pullula* (Boheman) and 42 *Laodelphax striatella* (Fallen) individuals tested. Fewer than ten individuals were collected for eight other species, and phytoplasmas were not detected in these insects.

Table 1. Phytoplasmas in insect species collected from maize redness affected fields in Serbia (adapted from Jovic *et al*. 2007, 2009).

Species	# Positive/# Tested[a]	16Sr Group[b]
Reptalus panzeri	70/404	XII-A
Mocydia crocea	1/6	I-C
Psammotettix alienus	13/187	I-C (12) I-B (1)

[a] Number of phytoplasma positive specimens/# specimens tested.

[b] Classification of phytoplasmas into 16Sr groups by digestion with *Tru*I (Lee *et al*., 1998).

Roots of weeds were sampled from in and around MR affected fields. Of the seven species tested, 7 of 33 Johnsongrass (*Sorghum halepense* (L.) Pers.) samples were positive for stolbur phytoplasma. Phytoplasma was not detected in any of the broadleaf weeds, including *Medicago sativa* L., *Datura stramonium* L., bindweed (*Convolvolus arvense*), *Solanum nigrum* L. and *Bilderdykia convolvulus* (L.) Dumort and *Sinapis arvensis* L.. *R. panzeri* transmitted stolbur phytoplasma to 5.5% of wheat seedlings under laboratory conditions, and the phytoplasma was detected in about 2.5% of roots sampled from wheat planted into an MR affected maize field.

Discussion

Of the hemipteran species identified in MR affected fields in South Banat Serbia, only *R. panzeri* was found to be infected with stolbur phytoplasma (table 1, Jović, *et al.*, 2009). In particular, very few *H. obsoletus* individuals and no *Pentastridius* species were found. Populations of vectors of other phytoplasmas were similarly low. Insects previously shown to harbor stolbur phytoplasma such as *M. crocea* and *P. alienus*, which can harbor several phytoplasmas including stolbur (Tothova, 2004), were present, but these did not carry stolbur phytoplasma. While it is possible that some other species are capable to transmitting stolbur phytoplasma to maize, *R. panzeri* is likely to be the major vector of MR in South Banat.

Two monocots, Johnsongrass and wheat were identified as hosts of stolbur phytoplasma in MR affected areas. In contrast, the phytoplasma was not detected in dicotylendonous weeds previously shown to be hosts, including *C. arvensis*, *M. sativa*, *D. stramonium* and *S. nigrum* (Garnier, 2000). These results, with distinctly different plant and insect vector hosts for stolbur phytoplasma associated with MR raises the possibility of pathogen diversification. Strains of stolbur from different hosts can be distinguished on the basis of *tuf* and *vmp* genes, and genome size (Cimerman *et al*., 2009; Marcone *et al.*, 1999; Riolo *et al*., 2007). We are working to identify genomic characteristics that distinguish stolbur phytoplasma from MR affected plants from strains infecting other vectors and plant hosts.

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