Bulletin of Insectology **60** (2): 397-398, 2007
ISSN 1721-8861

Maize redness in Serbia caused by stolbur phytoplasma is transmitted by *Reptalus panzeri*

Jelena Jović[1], Tatjana Cvrković[1], Milana Mitrović[1], Slobodan Krnjanjić[1], Andjeljko Petrović[1], Margaret G. Redinbaugh[2], Richard C. Pratt[3], Saskia A. Hogenhout[4], Ivo Toševski[5]

[1]*Institute for Plant Protection and Environment, Department of Plant Pests, Zemun, Serbia*
[2]*USDA, ARS Corn and Soybean Research and Department of Plant Pathology, The Ohio State University/Ohio Agriculture Research and Development Center, Wooster, USA*
[3]*Department of Plant Pathology, The Ohio State University/Ohio Agriculture Research and Development Center, Wooster, USA*
[4]*Department of Disease and Stress Biology, The John Innes Centre, Norwich Research Park, Colney, Norwich, UK*
[5]*CABI Bioscience Centre Switzerland, Delémont, Switzerland*

Abstract

Maize redness (MR) causes midrib, leaf and stalk reddening and abnormal ear development in maize in Serbia, Romania and Bulgaria. High populations of the ciixid *Reptalus panzeri* (Löw) were found in MR affected maize fields in the southern Banat region of Serbia in 2005 and 2006, and stolbur phytoplasma was detected in 20% of the insects. Stolbur phytoplasma was detected in 85% of symptomatic maize from these fields. Typical MR symptoms developed in healthy maize plants exposed to stolbur phytoplasma infected *R. panzeri*, and these plants were positive for the phytoplasma.

Key words: *Mollicutes*, Banat region, stolbur, vector, *Reptalus panzeri*.

Introduction

Maize redness (MR) has occurred intermittently in Serbia, Romania and Bulgaria since 1960 (Šutić *et al*., 2002). MR affected plants have reddening of the leaf midrib, leaves and stalks. Although seed set and yield are greatly reduced, dwarfing and phyllody are not commonly associated with MR. Because insecticide treated plots were less affected by MR, it was proposed that MR was caused by an insect transmitted pathogen. Recently, Duduk and Bertaccini (2006) demonstrated the presence of stolbur phytoplasma (subgroup 16SrXII-A) in MR affected plants from southern Banat in 2005. Insecticide treated plots had reduced MR incidence (Šutić *et al*., 2002). Phytoplasmas are prokaryotic, phloem-limited plant pathogens that, as yet, cannot be cultured (Lee *et al*., 2000).

Generally, phytoplasmas are transmitted by insects (Weintraub and Beanland, 2006). Stolbur phytoplasma is transmitted by two cixiids (Fos *et al*., 1992; Gatineau *et al*., 2001), and it was previously detected in *Reptalus panzeri* (Löw) from infected vineyards in Hungary (Palermo *et al*., 2004). Identification of MR vectors in Serbia is required to understand disease aetiology and epidemiology, and to develop effective approaches for disease management in maize.

Materials and methods

Potential Hemipteran vectors (leafhoppers, planthoppers and cixiids) were collected between 15 May and 1 October in 2005 and 2006 from three MR affected fields in southern Banat about 15 km ENE of Belgrade. Two fields about 16 km W and 6 km WNW of Belgrade that did not have MR were used as controls. Insects were collected from marked plots (10 x 10 m each) along a transect in each field with sweep nets, and stored in 80% ethanol at 8 °C. *R. panzeri* was identified as described by Holzinger *et al*., (2003).

DNA was isolated from individual insects or maize plants and amplified using a modification of the stolbur phytoplasma-specific nested PCR protocol (Daire *et al*., 1997; Clair *et al*., 2003).

To determine whether stolbur phytoplasma infected *R. panzeri* could transmit MR to maize, sweet corn ('ZP 231 su') pretreated with bifenthrin and deltamethrin was planted on 26 May into 11 mesh cages (2.2 x 2.2 x 2.5 m) at the Plant Protection Institute in Zemun, Serbia. Six weeks later, 120 *R. panzeri* collected from MR affected fields were released into each of seven cages. Symptoms were evaluated weekly on the plants. Five to nine weeks after exposure to *R. panzeri*, tissue was harvested and tested for stolbur phytoplasma using nested PCR.

Results

R. panzeri populations were much higher in the MR affected fields, with an average of 260 insects (n=20) collected per plot over two seasons. Populations were low in the control plots with an average of less than 25 *R. panzeri* per plot (n=20) over the same period. More than 17% of the *R. panzeri* were positive for the stolbur phytoplasma using the nested PCR assay (table 1). Less than 2% of insects from control fields were infected. Most (85%) symptomatic maize from MR affected fields was stolbur phytoplasma positive, but the phytoplasma was not detected in asymptomatic maize (table 1).

MR symptoms appeared on maize four weeks after release of *R. panzeri* from the affected area into cages of asymptomatic maize. Ultimately 57% of the plants became symptomatic, and 81% of these were positive for stolbur phytoplasma. No symptoms appeared on maize plants from cages without insects and none were positive for the phytoplasma.

Discussion

In addition to the typical MR symtpoms (Jović *et al*., 2007), some maize plants can also show red streaks along the midrib (figure 1a). Yield losses of 40-90% (Šutić *et al*., 2002) occur due to dramatically reduced seed set (figure 1b).

R. panzeri was a likely MR vector because it was abundant in the MR affected region and a significant portion of the insects were phytoplasma positive. The appearance of MR symptoms and presence of stolbur phytoplasma in maize after exposure to *R. panzeri* collected in affected fields stongly implicates the insect in transmission of this pathogen.

Table 1. The occurrence of stolbur phytoplasma in *R. panzeri* and maize**.**

Sample	Locale	# Indiv.	# Symp.	# PCR pos.
R. panzeri	MR field	404	na	17
	Control field	167	na	2
Zea mays	MR field	30	30	26
	MR field	20	0	0
	Test cages + *R. panzeri*	132	75	61
	Test cages no *R. panzeri*	83	0	0

R. panzeri or maize samples were collected from the indicated location (Locale). The total (# Indiv.) and number of symptomatic (# Symp.) samples are indicated, as is the number of samples that were positive in the nested PCR assay (# PCR pos.).

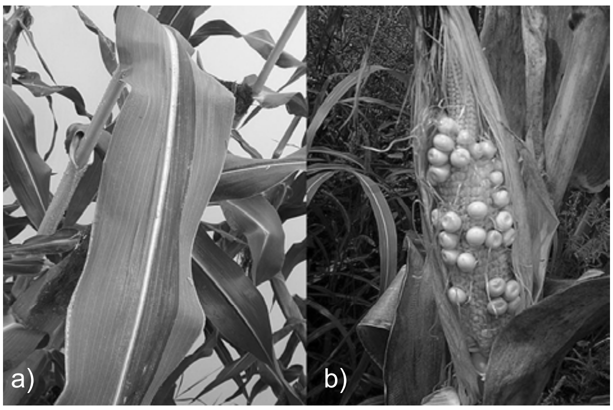

Figure 1. MR symptoms in maize. A maize leaf with a dark red streak adjacent to the midrib (a) and an ear with poor seed set (b) are shown.

Acknowledgements

We thank Kristen Willie for expert technical assistance. This study was supported by USDA Foreign Agriculture Service grant 58-3148-4-086 to the Ohio State University Research Foundation and Ministry of Agriculture, Forestry and Water Management, Republic of Serbia grant 321-01-750/2004-11.

References

CLAIR D., LARRUE J., AUBERT G., GILLET J., CLOQUEMIN G., BOUDON-PADIEU E., 2003.- A multiplex nested-PCR assay for sensitive and simultaneous detection and direct identification of phytoplasma in the elm yellows group and stolbur group and its use in survey of grapevine yellows in France.- *Vitis*, 42: 151-157.

DAIRE X., CLAIR D., REINERT W., BOUDON-PADIEU E., 1997.- Detection and differentiation of grapevine yellows phytoplasmas belonging to the elm yellows group and to the stolbur subgroup by PCR amplification of non-ribosomal DNA.- *European Journal of Plant Pathology*, 103: 507-514.

DUDUK B., BERTACCINI A., 2006.- Corn with symptoms of reddening: new host of stolbur phytoplasma.- *Plant Disease*, 90: 1313-1319.

FOS A., DANET J. L., ZREIK L., GARNIER M., BOVÉ J. M., 1992.- Use of a monoclonal-antibody to detect the stolbur mycoplasma-like organism in plants and insects and to identify a vector in France.- *Plant Disease*, 76: 1092-1096.

GATINEAU F., LARRUE J., CLAIR D., LORTON F., RICHARD-MOLARD M., BOUDON-PADIEU E., 2001.- A new natural planthopper vector of stolbur in the genus *Pentastiridius* (Hemiptera: cixiidae).- *European Journal of Plant Pathology*, 107: 263-271.

HOLZINGER W. E., KAMMERLANDER I., NICKEL H., 2003.- *The Auchenorrhyncha of Central Europe, Fulgoromorpha, Cicadomorpha.* Excl. Cicadellidae.- Brill Academic Publishers, Leiden, the Netherlands.

JOVIĆ J., CVRKOVIĆ T., MITROVIĆ M, KRNJAJIĆ S., REDINBAUGH M. G., PRATT R. C., GINGERY R. E., HOGENHOUT S. A., TOŠEVSKI I., 2007.- Role of stolbur phytoplasma and *R. panzeri* in the epidemiology of maize redness in Serbia.- *European Journal of Plant Pathology*, 118: 85-89.

LEE I.-M., DAVIS R. E., GUNDERSEN-RINDAL D. E., 2000.- Phytoplasma: phytopathogenic mollicutes.- *Annual Review of Microbiology,* 54: 221-255.

PALERMO S., ELEKES M., BOTTI S., EMBER I., ALMA A., OROSZ A., BERTACCINI A., KOLBER M., 2004.- Presence of stolbur phytoplasma in Cixiidae in Hungarian vineyards.- *Vitis*, 43: 201-203.

ŠUTIĆ D., TOŠIĆ M., STAROVIĆ M., STANKOVIĆ R., TOMIĆ T., 2002.- Redness of maize.- *Plant Protection,* 53: 57-73.

WEINTRAUB P. G., BEANLAND L., 2006.- Insect vectors of phytoplasmas.- *Annual Review of Entomology*, 51: 91-111.

Corresponding author: Margaret G. REDINBAUGH (e-mail: meg.redinbaugh@ars.usda.gov), USDA, ARS Corn and Soybean Research, 1680 Madison Ave., Wooster, OH 44691, USA.