SHORT COMMUNICATION

First Report of Potato Stolbur Phytoplasma in Hemipterans in Southern Moravia

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Abstract

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In 2005, the first screening tests to confirm the presence of potato stolbur phytoplasma in hemipterans by the PCR method were carried out to determine the spectrum of possible vector species for further analyses. Potato stolbur phytoplasma was confirmed in two out of five tested individuals of *Hyalesthes obsoletus* and in one mixed sample (10 individuals) from 17 tested samples of *Lygus* spp. As far as we know this is the first occurrence of potato stolbur phytoplasma in *Hyalesthes obsoletus* and *Lygus* spp. confirmed by the PCR method in the Czech Republic.

Keywords: stolbur; *Hyalesthes obsoletus*; vector; PCR

In European and Mediterranean countries, stolbur phytoplasma causes dangerous diseases of economically important crops, especially solanaceous plants such as tomato, pepper, potato and aubergine, and also grapevine.

The planthopper *Hyalesthes obsoletus* Signoret 1865, is considered to be the most important vector of potato stolbur phytoplasma (Valenta 1953; Sforza *et al.* 1998) (Figure 1). This planthopper is a circummediterranean species of the middle Asia region, its most northern occurrence has been found in Germany and Poland. The Czech Republic presents the boundary of the spread of this planthopper. *Hyalesthes obsoletus* (Figure 2) is a monovoltine species with five larval instars. Larvae of the 3th to 4th instars are overwintering stages. Adults are polyphagous, but they prefer plants from the family Solanaceae. Larvae suck on the roots of weeds, especially on bindweed (*Convolvulus* sp.), *Cardaria* sp., common nettle (*Urtica dioica*) and also on lavender (*Lavendula* sp.). All the plants mentioned above are the natural reservoirs of stolbur.

Phytoplasmas occur in the phloem of host plants and are transferred in persistent manner by insects with stylate mouth-parts. The vector often stays inoculative for the rest of its life. Another possible transfer is via parasitic dodder (*Cuscuta* sp.).

In addition to *H. obsoletus*, other reported stolbur phytoplasma vectors occurring in the Czech Republic are the planthoppers *Reptalus panzeri*, *Euscelis incisus*, *Macrosteles laevis, M. quadripunctulatus, M. cristatus, M. viridigriseus, Speudotettix subfusculus, Anoscopus albifrons* and *Aphrodes bicinctus,* and true bugs of the genus *Lygus* (Neklyudova & Dikit 1973; Palermo *et al.* 2004).

Figure 1. Symptoms of stolbur on tomato

In 2005, the first screening tests to confirm the presence of stolbur phytoplasma in hemipterans by the PCR method were carried out to determine the spectrum of possible vector species for further analyses.

Insects were sampled at two localities, Lednice and Podivín (southern Moravia, Czech Republic) on July 21st and September 15th, 2005. One field each of tomato and celeriac infected by potato stolbur phytoplasma were chosen on the basis of a previous confirmation by PCR of infection by stolbur phytoplasma in host plants at these localities. Insects were collected by sweeping and immediately afterwards stored in 100% ethanol in labelled plastic bottles, transported to the laboratory and stored at –80°C.

Figure 2. Adult of the planthopper *Hyalesthes obsoletus*

Nucleic acids were isolated from species listed in Table 1, except of *Eupteryx atropunctata* which does not belong to phloem-feeding species. Two different isolation methods were used in our study, a modified CTAB (cetyltrimethylammonium bromide) method and a commercial isolation kit. Because of the large number of samples, DNAs from leafhoppers of the genus *Empoasca* (not considered as stolbur phytoplasma vectors) were isolated using the modified CTAB extraction method (Ahrens & Seemüller 1992). Mixed samples, divided into groups of two to six individuals according to locality, sampling date and sex, were ground using a micropestle in a sterile 1.5 ml Eppendorf tube containing 600 µl of preheated CTAB extraction buffer (2.5% CTAB, 1.4M NaCl, 20mM EDTA, 100mM Tris-HCl, pH 8, 0.2% 2-mercaptoethanol) and incubated for 20 min at 60°C; 600 µl of precooled chloroform-isoamyalcohol (24:1) was added to the cool mixture and the aquaeous layer (300 µl) was, after centrifugation, precipitated overnight at –20°C with 600 µl of isopropanol. The DNA pellet was washed with 600 µl of precooled 70% ethanol, vacuum-dried for 15 min and resuspended in 25 µl TE buffer.

DNAs from other leafhoppers and bugs were extracted by DNeasy Plant Mini Kit (Quiagen) according to the manufacturer's instructions. Individuals of *H. obsoletus* and species with only one captured individual (Table 1) were tested separately. Representatives of other taxa were

Table 1. Summary of hemipterans collected at two localities on two sampling dates in 2005

Taxon	Podivín (celeriac)			Lednice (tomato)						Total
	21st July			21st July			15th September			
	female	male	total	female	male	total	female	male	total	
Hyalesthes obsoletus	2	–	2	2	1	3	–	–	–	5
Eupteryx atropunctata	3	3	6	–	–	–	–	–	–	6
Laodelphax striatella	–	–	–	–	1	1	–	–	–	1
Psammotettix alienus	–	–	–	3	1	4	1	–	1	5
Macrosteles sp. *laevis*?	–	–	–	1	–	1	2	–	2	3
Eupteryx atropunctata	–	–	–	3	3	6	3	3	6	12
Empoasca decipiens	9	2	11	–	7	7	–	1	1	19
Empoasca affinis	–	–	–	–	8	8	–	14	14	22
Empoasca pteridis	–	–	–	–	6	6	–	66	66	72
Empoasca spp.	–	–	–	38	–	38	120	–	120	158
Emelyanoviana mollicula	–	–	–	–	1	1	4	4	8	9
Cicadella viridis	–	–	–	–	–	–	–	1	1	1
Mocydia crocea	–	–	–	–	–	–	1	–	1	1
Zyginidia pullula	–	–	–	–	–	–	–	1	1	1
Lygus spp.	1	3	4	4	6	10	7	8	15	29
Lygus spp.	–	–	–	–	–	–	not distinguished		5	5
Lygus spp. – larvae	–	–	–	–	–	–			24	24
Total										368

Lygus spp. = *Lygus rugulipennis* and *Lygus pragensis*

tested in groups of 2–10 individuals according to the locality, sampling date and sex.

Direct polymerase chain reaction (PCR) using stolbur-specific primer pair fSTOL/rSTOL (Maixner *et al.* 1995) was used for stolbur phytoplasma detection in the insects. Positive samples in previous direct PCR with stolbur-specific primers were re-tested by direct PCR with universal primer pair P1/P7 (Deng & Hiruki 1991; Schneider *et al.* 1995) and following restriction fragment length polymorphism (RFLP) analysis of amplified products using *Alu*I, *Rsa*I, *Mse*I and *Sfe*I restriction enzymes according to Marcone *et al.* (1997). Potato stolbur phytoplasma isolated from infected tomato was used as positive control. All insects that were positive in PCR with a stolbur-specific primer pair were also positive in PCR with the universal primer pair. RFLP analyses confirmed stolbur phytoplasma in insect bodies.

Althogether 368 individual hemipterans were caught during three samplings (Table 1). Representatives of *Empoasca* spp. (*E. decipiens*, *E. affinis*, *E. pteridis*; 271 individuals = 73% of the total catch) were the most abundant. Members of this genus, despite the fact that they feed on both mesophyll and phloem, have not been confirmed as vectors of stolbur phytoplasma so far. They were not found to be infected with stolbur phytoplasma in our study. Representatives of the taxon *Lygus* spp. belonged to eudominant species (53 individuals, 14%). One mixed sample from September 15th, 2005, consisting of 10 larvae of *Lygus* spp. (*L. pratensis* and *L. rugulipennis*) gave positive results in PCR. Five individuals of *H. obsoletus* were caught at both localities on the first sampling date (July 21st, 2005). Two of these five individuals were found to be infected with stolbur phytoplasma. One positive individual was a female caught at Podivín (host

plant celeriac), the second was a male captured at Lednice (host plant tomato).

According to our knowledge, this is the first occurrence of potato stolbur phytoplasma in *Hyalesthes obsoletus* and *Lygus* spp. confirmed by the PCR method in the Czech Republic.

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