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The syndrome "basses richesses" of sugar beet in France is associated with different pathogen types and insect vectors

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Abstract

The syndrome "basses richesses" (SBR) of sugar beet in France is associated with two phloem-restricted uncultivable bacteria: a stolbur phytoplasma and a γ-3 proteobacteria. The known vector of proteobacteria is a cixiid planthopper, *Pentastiridius leporinus* (Hemiptera Cixiidae), formerly shown to transmit both the prokaryotes. The role of *P. leporinus* and of two other planthopper species, *Cixius wagneri* and *Hyalesthes obsoletus*, in spreading the two pathogens to sugar beet were compared and quantified. Because of its abundance and high infection rates with proteobacterium, *P. leporinus* was confirmed to be the economic vector of SBR disease. *P. leporinus* and *C. wagneri* were infected by and transmitted proteobacterium, neither was infected by the phytoplasma. Neither of two populations of *H. obsoletus* living near sugar beet fields on bindweed and nettle respectively, carried proteobacteriumbut they were highly infected with two RFLP-differentiable stolbur types. Only the bindweed stolbur type was transmitted and pathogenic to sugar beets. Symptoms associated with the two prokaryotes were similar, but stolbur caused a stronger reduction on taproot biomass and sugar content than proteobacteria. This work underlines possible confusion in aetiology of diseases associated with phytoplasmas or phloem-restricted proteobacteria and the increasing importance of the latter plant pathogens.

Key words: syndrome "basses richesses", stolbur phytoplasma, phloem-restricted proteobacteria, planthoppers, cixiids, *Hyalesthes obsoletus*, *Cixius wagneri*, *Pentastiridius leporinus*.

Introduction

Hyalesthes obsoletus Signoet, *Reptalus panzeri* (Löw) *Reptalus quinquecostatus* (Dufour) and *Pentastiridius* sp., later identified as *Pentastiridius leporinus* (L.) (Nusillard, personal communication) are cixiid species known as vectors or suspected vectors of stolbur phytoplasmas (Stp) in Europe. Different Stp types have been associated with both different host plants and *H. obsoletus* populations (Langer and Maixner, 2004).

Other plant pathogenic phloem-restricted bacteria are proteobacteria. Marginal chlorosis of strawberry and syndrome "basses richesses" (SBR) of sugar beet are associated with two related γ-3 proteobacteria in the Arsenophonus clade, i.e '*Candidatus* Phlomobacter fragariae' and SBR proteobacterium (SBRpr), transmitted respectively by *Cixius wagneri* China and *P. leporinus* (Danet *et al.*, 2002; Gatineau *et al.*, 2002; Sémétey *et al*., 2007b). SBR can also be associated with a Stp, which is also transmitted by *P. leporinus* and causes no differentiable symptoms in affected sugar beets (Gatineau *et al*., 2001, 2002; Sémétey *et al*., 2007a). In the present work the complex of planthopper cixiids involved with transmission of both Stp and SBRpr to sugar beet were studied.

Materials and methods

Insect sampling was conducted in two sugar beet fields located in Jura department (France) during SBR epidemics, using transparent sticky traps maintained above the vegetation layer with stakes planted in the ground. Traps were posted at regular distances (10 meters) at nodes of a rectangular array in two sugar beet fields in 2005 (28 traps) and 2006 (72 traps). They were maintained in fields from the beginning of June until sugar beet harvesting, covering the flight time of most planthopper cixiids in temperate regions. Trapped cixiids were counted and removed every 2-3 days. Insects were preserved in 70% ethanol for later identification of uncertain species, or frozen at –80 °C for PCR detection of Stp and SBRpr.

Transmission assays were conducted with *C. wagneri*, *H. obsoletus* and *P. leporinus* collected in 2005 and 2006 with a D-Vac aspirator in sugar beet fields, on weeds and in fallow fields. *H. obsoletus* collected from bindweed (b) or nettle (n) were separated. Planthoppers were caged in groups of 4-5 (*C. wagneri* or *P. leporinus*), 8-10 (*H. obsoletus* b), or 20 (*H. obsoletus* n) specimens per plant, on 35-50 day-old healthy sugar beet seedlings. Unexposed seedlings were kept as healthy controls.

Stp and SBRp were identified by PCR and RFLP analysis using total DNA extracted from insects and plants. Insects were tested separately or in groups of 3 (*H. obsoletus*). '*Ca*. Ph. fragariae' and SBRpr were identified by RFLP of a 16Sr DNA fragment amplified with primers Fra5 (Zreik *et al.*, 1998) and rA_PB_2 (A. Bressan, unpublished.) Primer pairs Alb1/Oliv1 (Sémétey *et al*., 2007a) and Pfr1/Pfr4 (Foissac *et al*., 2000) were used for specific detection in insects of SBRpr and '*Ca*. Ph. fragariae', respectively. Stp was detected with nested-PCR of Stol11 fragment (Clair *et al*., 2003). Stp in positive samples were typed according to Langer and Maixner (2004).

Parameters for virulence assessment of Stp and SBRpr were fresh weights of entire sugar beet and of tap root, and an estimation of sugar content by refractometric dried substance (RDS) of tap root pulp mesurement 5 months after planthopper pathogen transmission.

Table 1. Infection of individual insects with SBR bacterium and two types of stolbur phytoplasma and their transmission efficiency in batches (see text) to sugar beet seedlings.

Planthopper species	SBR bacterium		Stolbur phytoplasma (bindweed)		Stolbur phytoplasma (nettle)	
	infected/tested insects	diseased/inoc. sugar beets	infected/tested insects	diseased/inoc. sugar beets	infected/tested insects	diseased/inoc. sugar beets
P. leporinus	62/75	15/25	0/75	0/25	0/75	0/25
C. wagneri	38/83	12/25	0/83	0/25	0/83	0/25
H. obsoletus (b)	0/61*	0/19	38/61	13/19	0/61	0/19
H. obsoletus (n)	0/25*	0/36	0/25	0/36	19/25	0/36

**H. obsoletus* were tested in groups of three specimens.

Results

Insects - *P. leporinus* were much more abundant than *C. wagneri* or *H. obsoletus*. Of 4,033 and 10,047 cixiid specimens captured in 2005 and 2006 respectively, *P. leporinus* specimens represented 97.5% (3,933) and 98,7% (9,917), *C. wagneri* 1.9% (75) and 0.5% (51), and *H. obsoletus* 0.6% (25) and 0.8% (79), respectively. SBRpr was detected in *P. leporinus* and in *C. wagneri* but not in *H. obsoletus* (b) or (n) populations. None of 69 *C. wagneri* specimens were positive for '*Ca*. Ph. fragariae' (data not shown). Stp was not detected in either *P. leporinus* or *C. wagneri* whereas both (b) and (n) populations of *H. obsoletus* were phytoplasma positive (table 1). Stp types detected in *H. obsoletus* (b) or (n) were according to Langer and Maixner (2004).

Pathogens - *Taq*I restriction of Fra5/rA_PB_2 16S rDNA differentiate SBRpr from '*Ca*. Ph. fragariae' (not shown). SBRpr was transmitted by *P. leporinus* and *C. wagneri,* transmission of Stp was obtained only with *H. obsoletus* (b) (table 1) although survival on sugar beet of (b) and (n) populations was comparable (not shown).

Virulence on sugar beet - Similar symptoms and reduction of the biomass and sugar content were observed in plants inoculated with SBRpr or Stp(b). Data showed statistically significant differences from healthy controls, but the proportion of tap root weight to total plant weight was lower in Stp-infected than in SBRpr-infected sugar beets.

Discussion

The aboundant populations of *P. leporinus* in sugar beet fields with a very high proportion of SBRpr positive specimens, confirmed its major role in SBR disease spreading. Our data showed that virulence of Stp(b) to sugar beet was similar or higher than that of SBRpr. *C. wagneri* can also be a natural vector of SBRpr. We were not able to detect the Stp(n) type in sugar beets that remained symptomless although they were inoculated with phytoplasma positive *H. obsoletus* (n). In addition, it is possible that competition between Stp and SBRpr in plant tissues and insect organs may have progressively excluded the presence of Stp that was earlier detected in *P. leporinus* and sugar beets (Gatineau *et al*., 2001). This work underlines common traits between diseases associated with phytoplasmas and phloem-restricted proteobacteria and the new importance of latter diseases.

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