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A new natural planthopper vector of stolbur phytoplasma in the genus *Pentastiridius* (Hemiptera: Cixiidae)

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Abstract

A new disease of sugar beet called Syndrome des Basses Richesses, which appeared in Burgundy and Franche-Comté, France, in 1991, is of uncertain aetiology. However, evidence for aerial transmission of the disease, symptom similarity with yellow wilt and preliminary results of phytoplasma detection, support the hypothesis of a phytoplasma being associated to the disease. A search for a natural phytoplasma vector, was conducted in Franche-Comté in 1997 and 1998, in an area where sugar beet crops had been affected since 1996. A cixiid, tentatively identified as *Pentastiridius beieri*, not described in the preceding years and not formerly reported as a phytoplasma vector, was present in sugar beet plots in high populations from June to August in 1997 and 1998. Individuals were captured and used for transmission experiments to periwinkle and sugar beet seedlings. They were further tested for the presence of a phytoplasma in their body, using PCR amplification of 16S rDNA of phytoplasmas. In 1997 and 1998, from 2% to 13.3% of the individuals carried a stolbur phytoplasma and insects which tested positive, appeared to have transmitted, through feeding, a stolbur phytoplasma to periwinkles and to sugar beets. This cixiid, whose vectoring capacity of stolbur phytoplasma to plants, is now clearly demonstrated, is available for experimental inoculations, in order to examine the role of phytoplasmas in the Syndrome des Basses Richesses, through the observation of symptom expression in phytoplasma-inoculated plants.

Introduction

A new disease of sugar beet called Syndrome des Basses Richesses (SBR), was first observed in Burgundy and Franche-Comté in 1991 and has occurred with variable severity on successive years in different areas of the same region. SBR causes economic loss on sugar beet crops, due to the poor sugar content of affected roots. Early symptoms are yellowing and incurvation of old leaves and a new growth of central leaves which appear chlorotic, lanceolated and asymmetrical. Roots have a normal size but they contain brown vascular bundles and their sugar content suddenly decreases in the beginning of September.

Early observations (Richard-Molard et al., 1995) ruled out the role of soil and cultivation practice, as well as that of known viruses, in the aetiology of SBR. However, the latter observations supported an aerial transmission of the disease. Leaf symptoms showed some similarity with yellow wilt (Bennet, 1967; Urbina-Vidal and Hirumi, 1974) and suggested the possible involvment of phytoplasmas in the aetiology of SBR. Stolbur phytoplasmas were eventually detected in diseased plants using PCR amplification of 16S rDNA of phytoplasmas (Richard-Molard et al., 1995; Boudon-Padieu et al., unpubl.). Nevertheless, the presence of detectable phytoplasma DNA in leaves and roots could not be reliably related to the expression of

SBR symptoms. It was felt that a better understanding and analysis of the successive events in the syndrome development were necessary for progress in the understanding of its aetiology.

In order to examine the possibility of a phytoplasma aetiology of SBR, a first step was to search for potential phytoplasma vectors in the area affected by SBR. Surveys of *fulgoromorpha* and *cicadomorpha* species present in sugar beet crops and preliminary tests to detect phytoplasma on trapped specimens, were set up. About 24 genera of *fulgoroidea*, *cercopidea* and *cicadelloidea* were trapped in sugar beet plots from June to September. A few specimens of several species eventually tested positive for phytoplasmas in their body, but no stolbur-type phytoplasma was detected (Gatineau et al., unpubl.), except for one cixiid species never described in preceding surveys (Richard-Molard et al., 1995). The latter species was present in high numbers in sugar beet plots. In addition, a stolbur phytoplasma, similar to the agent previously detected in a few sugar beets, was detected in a number of specimens. The species was tentatively identified as *Pentastiridius beieri* Wagner, 1970 (Gatineau et al., 1998). The present study describes experiments conducted with this species trapped in sugar beet plots, to check the ability of naturally phytoplasma-infected individuals to transmit phytoplasma to test plants under controlled conditions.

Materials and methods

Insects trapping

Weekly trappings of the cixiid of interest were made in 1997 and 1998 in sugar beet (*Beta vulgaris*) plots of Franche-Comté, France, using a D-Vac apparatus. All captures were done between 11 and 16 h. In 1997, trappings were made in a sugar beet plot from the end of June to the end of September. In 1998, trappings were made from the end of May to the end of August in two adjacent sugar beet plots. Occasional trappings were also made in fallow land next to sugar beet crops.

Transmission experiments

In 1997, batches of 10–50 insects of the above species, were caged on individual 2-month-old healthy seedlings of sugar beet or periwinkle (*Catharanthus roseus*) in a controlled-environment insectarium (22 °C, photoperiod 16/8 h, humidity 80%). Sugar beet were sown every two weeks. Insects were left on the plants until they died (for 15–21 days). They were collected immediately and stored at −20 °C in separate tubes. The plants were observed during the following weeks for symptom expression. Four periwinkle and 13 sugar beet seedlings were inoculated, using insects captured in the period from the 27th to the 32nd week of 1997. In addition, insect specimens collected on the 26th week, were directly stored at −20 °C after capture.

In 1998, all the sugar beet test-plants to be used during summer, were sown on mid-April, according to the sowing date of field crops and they were grown in containers in outdoor conditions under an insect-proof tunnel, thus ensuring that field plants and test plants were of the same age and were grown in similar environmental conditions during the potential period of inoculation. Insect trapping was made during the period of presence of the species from the 24th to the 32nd week. Batches of 10–40 insects of the species were caged for 3 weeks on individual sugar beet plants. The remaining insects still alive in some of the batches, were transferred for an additional 2-week period to a caged healthy periwinkle seedling. Along with the duration of feeding transmission on both receptor plant species, dead insects were quickly collected and stored at −20 °C. Subsequently, sugar beets and periwinkles exposed to insects were observed for symptom expression and tested for phytoplasma infection. Ten periwinkles and 67 sugar beets were used for transmission experiments with insects trapped in sugar beet plots; eleven periwinkles and 43 sugar beets were used for experiments with insects from the fallow land.

Plant tissues and insects used for phytoplasma detection

Vein and petiole tissues of sugar beet and periwinkle which had been exposed to feeding by insects trapped in the field, were processed for phytoplasma detection assays. Negative controls were taken from similar tissues of non-insect-exposed healthy seedlings of both plant species. Positive controls were taken from periwinkle which had been graft-inoculated with periwinkle-maintained reference phytoplasma strains, i.e. VAC, the agent of Vaccinium witches' broom, STOL C and Phi (Caudwell et al., 1971), which have been classified in the Western X group, the stolbur group (Seemüller et al., 1994) and the clover phyllody group (Boudon-Padieu, unpubl.), respectively.

Insects that had been deep-frozen after trapping in the fields or after feeding on test plants, were individually submitted to phytoplasma detection assays. Negative controls were taken from healthy colonies of the leafhopper species *Euscelidius variegatus* Kbm, hatched and reared in the insectarium (Caudwell et al., 1971). Positive controls were taken from phytoplasma-infected colonies of *E. variegatus*, obtained by feeding healthy leafhoppers on Phi-infected broadbean plants (Caudwell et al., 1971).

PCR assays for amplification of phytoplasma DNA

DNA extraction

Total DNA was extracted from plants according to the procedure described by Daire et al. (1997a) and from insects, according to the procedure described by Sforza et al. (1998), with slight modifications. One and a half gram of fresh plant tissue was ground, using a ball bearing grinding apparatus (Tecam for Bioreba), in 7.5 ml of extraction buffer (30 g/l cethyltrimethylammonium bromide, 100 mM Tris–HCl, 10 mM EDTA, pH 8, 1.4 M NaCl, 2 ml/l 2-mercaptoethanol). One ml of the resulting brei was transferred to a 1.5 ml centrifugation tube and incubated for 20 min in a water bath at 65 °C. After an extraction with 1 ml of chloroform and a 10 min, 10,000*g* centrifugation, the aqueous phase (800 µl) was collected. The nucleic acids were precipitated with an equal volume of *iso*propanol and collected by a 15 min, 10,000*g* centrifugation. The supernatant was discarded and the pellet was washed with 1 ml of 70% ethanol, dried in vacuum and dissolved in 150 µl of 10 mM Tris, 1 mM EDTA, pH 8.

Individual deep-frozen insects were crushed in 400 µl of extraction buffer (20 g/l cethyltrimethylammonium bromide, 100 mM Tris–HCl, 10 mM EDTA, pH 8, 1.4 M NaCl, 1 ml/l 2-mercaptoethanol). The brei was heated for 5 min at 65 °C in a water bath. Extraction was done as above with an equal volume of chloroform, followed by centrifugation and 400 µl of the aqueous phase was collected. The nucleic acid pellet obtained after *iso*propanol precipitation and centrifugation, was similarly washed with 70% ethanol, dried in vacuum and dissolved in 50 µl of 10 mM Tris, 1 mM EDTA, pH 8.

PCR conditions

A nested amplification in PCR with two successive primer pairs, was used. P1 (Deng and Hiruki, 1991) and P7 (Smart et al., 1996) was the first pair; fU5/rU3 (Lorenz et al., 1995) was the second pair. These primer pairs specifically amplify rDNA in all known phytoplasmas. The 20 µl amplification mixture contained 0.375 µM of each primer (P1 and P7 for the first series of amplification or fU5 and rU3 for the nested amplification), 0.150 µM of each DNTPs, 1 mM of $MgCl_2$, Taq buffer (Appligene), 1 unit/100 µl of Taq DNA polymerase (Appligene). One microliter of total extracted DNA was added for the first step. The mixture was overlaid with mineral oil. A first denaturation step at 92 °C for 90 s, was performed. After 30 cycles of P1/P7 amplification realised as follows: denaturation at 92 °C for 45 s, annealing at 57 °C for 45 s and elongation at 72 °C for 105 s, the amplification product was diluted to 1/1000 and 1 µl of the diluted product was submitted to 35 cycles of amplification with fU5/rU3: first denaturation at 92 °C for 75 s, denaturation at 92 °C for 30 s, annealing at 57 °C for 30 s and elongation at 72 °C for 50 s.

Analysis of PCR products

Seven microliter of the second amplification product were analysed by electrophoresis in 1.2% agarose gel, stained with ethidium bromide and visualized under U.V. light. Characterization of phytoplasmas was done on the basis of the RFLP of fU5/rU3 fragment after digestion with the restriction enzyme *Tru9*I. This method allows the molecular characterization of the main groups of phytoplasma, including stolbur, AY, EY and Western X groups (Daire et al., 1997b) but also Clover phyllody group (data unpubl.). For RFLP analyses, 10 µl of the fU5/rU3 amplification product were digested with 1 unit of *Tru9*I restriction enzyme at 37 °C overnight. The digested DNA was analysed by electrophoresis in 10% polyacrylamide gel, stained with ethidium bromide and visualised under U.V. light.

Results

Insect trapping

Cixiid specimens trapped in 1997 were compared to the *Pentastiridius* collection in the Museum National d'Histoire Naturelle, Paris, France, and the species name *P. beieri* was subsequently proposed (Gatineau et al., 1998). However, the definitive systematic position of these *Pentastiridius* specimens is still to be fully determined. Nevertheless, the name *P. beieri* will be used in this manuscript.

Table 1. Results of trappings of *P. beieri* in sugar beet crops in 1997 and 1998

Week of capture		1997			1998		
		No. captured	No. of stolbur phytoplasma-infected insects/no. tested	%	No. captured	No. of stolbur phytoplasma-infected insects/no. tested	%
June	24	—			21	0/18	0
	25	—			338	32/274	11.8
	26	21	0/21	0	1442	60/1188	5
	27	32	0/31	0	41	1/41	2.4
July	28	215	6/147	4.1	24	1/22	4.5
	29	96	12/90	13.3	2	0/2	0
	30	49	3/49	6.1	0		
August	31	13	0/13	0	1	NT	
	32	9	0/9	0	1	NT	
	33	1	0/1	0	—		
Total		436	21/361	5.8	1861	94/1545	6.1

—, no capture; NT, not tested.

P. beieri was the most abundant hemiptera species trapped in sugar beet crops. The species was more successfully trapped with D-Vac than with yellow-sticky traps. Table 1 (column 1 and 4) shows results of captures of *P. beieri* in sugar beet plots, during summer 1997 and summer 1998. Specimens were much more numerous in 1998. First adults were captured on the first week of experiments (26th week) in 1997 and during the 24th week in 1998. Unfortunately, insecticide treatments have been applied by sugar beet growers from the 27th week in 1998 and a sudden drop was observed in the number of insects captured on the following weeks.

Transmission experiments

1997 experiments

Among the 4 periwinkle seedlings used in transmission experiments with *P. beieri*, one plant exposed to insects captured on the 28th week, showed typical symptoms of phytoplasma infection 20 days after exposure. Symptoms were yellowing of the leaves and reduction of flower organs.

Among the 13 sugar beet seedlings used, one plant exposed to insects captured on the same 28th week, showed a yellowing and asymmetrical growth of the lamina of young leaves, about one month after exposure to insects. Three other sugar beets exposed to insects captured on the 28th and the 29th week, showed vegetative disorders six months after their exposure to *Pentastiridius* specimens. Subsequently, all sugar beet test and control plants were submitted to repeated attempts of phytoplasma detection with PCR.

1998 experiments

Among the 10 periwinkles used in transmission trials with insects trapped in the sugar beet plot on the 25th and 26th week, 3 plants showed symptoms of phytoplasma infection 3–4 weeks after their exposure to *P. beieri*. Among the 67 sugar beets used, no clear symptoms could be observed on any plant, however a number of them showed vegetative disorders in the winter, i.e. 5–6 months after their exposure to insects.

Similarly, among the 11 periwinkles exposed to insects from the fallow land, 3 plants showed early symptoms of phytoplasma infection. Among the 43 sugar beets used, a few showed vegetative disorders in the winter. As in 1997, all the sugar beet test plants and control plants were then repeatedly blind-tested with PCR for phytoplasma infection.

PCR-RFLP detection and characterization of phytoplasma in insects and plants

Insects

An amplification product of the expected size (about 0.9 kb) was consistently obtained in PCR using specific primers for ribosomal phytoplasma DNA, with DNA prepared from a number of *P. beieri* individuals (Figure 1A, lane b). Other insect specimens tested negative (Figure 1A, lane a). All the PCR-positive insects

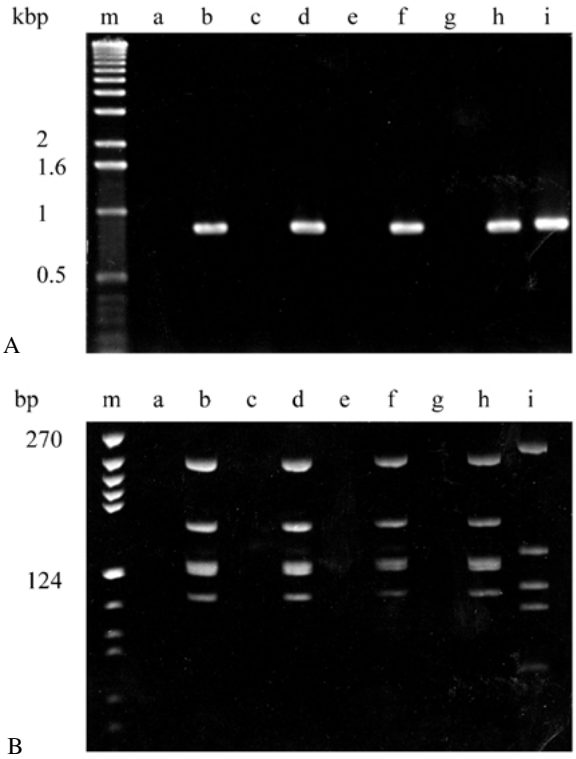

Figure 1. Gel electrophoresis of the PCR products (0.86 Kb) obtained with primer pairs P1/P7 and fU5/rU3 from DNA of insects and plants. A: native amplification products (1.2% agarose gel). B: *Tru9*I restriction profile of the same products (10% polyacrilamide gel). M, molecular weight marker: A, 1 Kb ladder. B, pBR322/*Hae*III (Appligene). Lane a and b, two wild specimens of *P. beieri*. Lane c–f, plants exposed to *P. beieri*; lane c and d, non-symptom-bearing and symptom-bearing sugar beet, respectively; lane e and f, non-symptom-bearing and symptom-bearing periwinkle, respectively. Lane g, healthy sugar beet control not exposed to insects. Lane h and i, reference phytoplasma strains maintained in periwinkle; lane h, STOL C; lane i, VAC.

captured in 1997 and 1998, showed a RFLP profile of the amplification product, similar to the profile of the STOL C reference phytoplasma maintained in periwinkle (Figure 1B, lane b & h, respectively).

Plants. In the plants submitted to transmission experiments in 1997, positive amplification signals were obtained from sugar beets showing early or late vegetative disorders and periwinkle with typical symptoms of phytoplasma infection (Figure 1A, lane d and f, respectively) and from all periwinkles infected with phytoplasma reference strains used as positive control (Figure 1A, lane h and i). No product was obtained from healthy-looking sugar beets or periwinkles with no phytoplasma symptoms, which had been caged with insects (Figure 1A, lane c and e, respectively). A similar result was obtained from healthy sugar beet seedlings cultivated in the greenhouse and used as negative control (Figure 1A, lane g). The RFLP profile obtained after *Tru9*I digestion of the DNA fragments amplified in fed-inoculated periwinkle or sugar beet plants, was similar to the STOL C profile (stolbur phytoplasma) (Figure 1B, lane d, f and h, respectively).

The profiles obtained as control from the reference phytoplasmas VAC and STOL C in periwinkle, were similar to the profiles expected for the groups to which they have been assigned, i.e. Western X and stolbur groups, respectively (Figure 1B, lane h and i).

Same results were obtained in 1998 with periwinkles that had been caged with insects captured either in the sugar beet plot or in the nearby fallow land. All periwinkles showing symptoms of phytoplasma infection, tested positive to phytoplasma detection and presented a stolbur-type RFLP profile (not shown). In sugar beet plants exposed to insects, first positive detection of stolbur phytoplasma was achieved in 6 plants, 5 months after exposure. Another attempt 2 months later, confirmed the presence of stolbur phytoplasma into the latter 6 plants and revealed 5 additional stolbur-positive plants.

Importance of phytoplasma-infected specimen in insects, according to the date of capture

Table 1 (column 2 and 5) shows the number of phytoplasma-infected insects trapped in a sugar beet plot in 1997 and 1998. In 1997, though the species could be captured from the 26th to the 33rd week, positive individuals carrying a stolbur phytoplasma, were detected only from the 28th to the 30th week, with a maximum ratio of 13.3% on the 29th week (column 3). In 1998, the species was captured from the 24th to the 32nd week with phytoplasma-infected individuals from the 25th to the 28th week. Maximum ratio of 11.8% of stolbur-infected individuals was observed on the 25th week (column 6).

Table 2 (column 2) shows the number of phytoplasma-positive insect batches in the insects trapped in the sugar beet plot and used for transmission to test-plants in 1997. All the batches of insects trapped and used on the 29th week contained at least one PCR-positive insect. Six batches of the 7 trapped

Table 2. Results of transmission trials in 1997 on periwinkle (PW) and sugar beet (SB) seedlings with *P. beieri* trapped in sugar beet plot

Week of capture	No. of stolbur phytoplasma infected batches/ no. of batches used	Plants used for transmission trials	No. of plants exposed to infected batches/ no. of plants used	Phytoplasma detection in plants (no. infected/ no. exposed to infected batches)
27	0/2	PW	0/1	0/0
		SB	0/1	0/0
28	6/7	SB	3/4	$1^b/3$
		PW	3/3	$1^a/3$
29	3/3	SB	3/3	$1^a + 1^b/3$
30	1/3	SB	1/3	$1^b/1$
31	0/1	SB	0/1	0/0
32	0/1	SB	0/1	0/0
Total	10/17	4 PW	3/4	$1^a/3$ (33.3%)
		13 SB	7/13	$1^a + 3^b/7$ (57.1%)

Detection a = early, b = late; NT, no transmission trial.

and used on the 28th week, contained infected insects. Only 1 of 3 batches used on the 30th week contained at least one PCR-positive insect. However, all the insects were not found again in the cages and consequently were not tested. As a whole, at least 3 periwinkles out of 4 and 7 sugar beets out of 13 had been caged with infected insects (Total column 4).

Table 3 Part A (column 2) shows similar results obtained in 1998 with insects from the sugar beet plot. As many as 11 out of the 21 batches of insects trapped and caged on sugar beet on the 25th week and 23 out of the 40 batches of insects caged on sugar beet on the 26th week, contained stolbur-infected insects. In addition, the only batch from the 27th week, contained infected insects. Insects captured in the fallow land were divided into 43 batches and 25 of these were subsequently shown to contain at least one phytoplasma-infected insect (Table 3 Part B column 2). However, as in 1997, all the insects were not found again in the cages. As a whole, out of the 21 periwinkles and the 110 sugar beets that had been caged with insects in 1998, 10 periwinkle and 60 sugar beet plants at least, had been caged with phytoplasma-infected insects (Total A + B, column 4).

Transmission efficiency of insect batches to periwinkle and sugar beet

In 1997 and 1998, all transmissions occurred with insects captured in a limited period of time, i.e. from the 28th to the 30th week in 1997 (Table 2), and on the 25th and 26th week in 1998 (Table 3). Comparison of transmission data with data on the presence of stolbur-positive insects in batches used to feed on test plants, showed that all stolbur-positive plants, either periwinkles or sugar beets, had been caged with stolbur-positive insects (data not shown). Transmissions to periwinkle and sugar beet were successful with batches containing only one PCR positive insect. Conversely, in some cases where several insects in a batch tested positive, no transmission to periwinkle or sugar beet could be evidenced (data not shown). In 1997 (Table 2, column 5), 1 periwinkle of 3 plants exposed to infected insects and 4 sugar beets of 7 plants exposed to infected insects (57.1%), tested stolbur positive. In 1998 (Table 3: Total A + B, columns 5), 6 of the 10 periwinkles caged with infected insects (60%) were shown to contain a stolbur phytoplasma. Out of the 60 sugar beets caged with stolbur-positive insects, 6 plants (10%) tested stolbur-positive after 5 months and 11 (18.3%) after 7 months.

Discussion

For the first time, the present data bring together molecular and epidemiological evidence that another Fulgoromorpha species, presently assumed to be *Pentastiridius beieri*, can be a significant vector of stolbur phytoplasma. In addition to the high ratio of phytoplasma-infected insects in wild populations, we

Table 3. Results of transmission trials in 1998 on periwinkle (PW) and sugar beet (SB) seedlings with *P. beieri* trapped in sugar beet and fallow plots

Place of trapping	Week of capture	No. of stolbur phytoplasma infected batches/ no. of batches used	Plants used for transmission trials	No. of plants exposed to infected batches/ no. of plants used	Phytoplasma detection in plants (no. infected/ no. exposed to infected batches)
A Sugar beet plot	24	0/3	SB	0/3	0/0
	25	11/21	SB	11/21	$2^a + 1^b$/11
			PW	2/4	1^a/2
	26	23/40	SB	23/40	$2^a + 2^b$/23
			PW	2/6	2^a/2
	27	1/1	SB	1/1	0/1
	28	/	NT	/	/
	29	0/2	SB	0/2	0/0
	Total A	35/67	10 PW	4/10	3^a/4 (75%)
			67 SB	35/67	$4^a + 3^b$/35 (20%)
B Fallow plot	Total B	25/43	11 PW	6/11	3^a/6 (50%)
			43 SB	25/43	$2^a + 2^b$/25 (16%)
Total A + B		60/110	21 PW	10/21	6^a/10 (60%)
			110 SB	60/110	$6^a + 5^b$/60 (18.3%)

Detection a = early, b = late; NT, no transmission trial.

experimentally demonstrated not only that the species can transmit stolbur phytoplasma to an experimental plant such as periwinkle, but also that it can be a vector of stolbur phytoplasma to a natural host plant like sugar beet on which it lives during summer. Only one planthopper species, *Hyalesthes obsoletus* Sign, had been demonstrated as a vector of stolbur phytoplasma (Fos et al., 1992; Maixner, 1994; Sforza et al., 1998). In 1969, however, Leclant and Lacote (1969) had reported observations on the possible role of *Oliarius* sp. as a vector of stolbur. It is known that stolbur phytoplasma is associated with a number of diseases of plants belonging to very different species (Sforza et al., 1998). The polyphagous behaviour of *H. obsoletus* had been the only explanation for the ubiquity of stolbur phytoplasma. The discovery of a second vector in the same cixiid family identifies a new research area for the epidemiology of stolbur-associated diseases of plants. In addition, though very little is known about the mechanism of the specificity between phytoplasma and their vector, it is remarkable that the two vector species now demonstrated for stolbur phytoplasma, belong to the same family. Moreover, first observations on larval development and biology of *P. beieri*, show that they are very similar to development and biology of *H. obsoletus* (Sforza et al., 1999) and of other cixiids.

Vectoring efficiency of *P. beieri* was probably high since transmission to either periwinkle or sugar beet was obtained with only one infected insect in a single batch. Cases where no transmission was obtained in spite of the presence of several infected insects, might have been due to the actual infective state of these insects. The route of phytoplasmas in the body of cixiid vectors is not known. Studies on leafhopper vectors have shown a 4–5-week latency period between acquisition and infection of salivary glands, a prerequisite to the infective state of infected insects (Boudon-Padieu et al., 1989; Lherminier et al., 1989; Lefol et al., 1994). If a similar route is assumed for phytoplasmas in cixiid vectors, it could therefore be possible that some of the infected insects were not infective at the time of trapping. The actual time of acquisition by individual insects of phytoplasma from reservoir plants was not known. They might have overcome latency either during the 3-week feeding transmission period on sugar beet plants or even later, when they were already caged on periwinkle plants. Such a delay before becoming infective, could account for the observed discrepancy in the vectoring efficiency to sugar beet (18.3%) and periwinkle (60%) in 1998.

The two-month old sugar beet seedlings which were used in 1997, appeared to be quite sensitive to phytoplasma, since one plant developed very early symptoms and died rapidly and two others showed very conspicuous symptoms in the winter. In the 1998 experimental design, sugar beet test-plants of the same age as the

plants in field crops, grown outdoor in containers, were used. In the latter conditions, symptom development was very slow in sugar beet, as it required 5–7 months and most of the plants declined afterwards. In addition, PCR detection of phytoplasma in sugar beet tissues was difficult. These problems in detection of phytoplasma in sugar beet, might be related to the particular architecture and the biannual physiology of the plant. It is known that phytoplasma move after inoculation, first downwards to the root system, then upwards to growing shoots (Lherminier et al., 1994); in perennial woody plants, they are unevenly distributed and their titre may not be related to the severity of symptoms. In addition, plant contaminants of the DNA sample might inhibit some steps of the PCR process and might affect the sensitivity of detection (unpublished data). As a whole, the transmission efficiency of 18.3% obtained in 1998 by PCR detection, may be an underestimation of the actual transmission ratio achieved in sugar beet.

The present discovery of a natural vector of stolbur phytoplasma, which spends at least an important part of its life cycle in sugar beet crops and was found in very high numbers, will provide a valuable lead and experimental tool for further investigation on the association of phytoplasma to sugar beet. In particular, the possible role of stolbur phytoplasma in SBR can be evaluated, thanks to the observation of symptom expression in experimentally stolbur-inoculated sugar beets grown in field conditions. Surveys of the presence of the insect and occurrence of SBR in fields as well as experimental plots of controlled exposed-inoculated plants, are underway to provide reliable information on the aetiology of the disease.

More data are needed on this new cixiid vector of stolbur phytoplasma. A complete systematic description is underway in order to delineate a formal systematic position. Its biology, its ethology and its occurrence in Burgundy and Franche-Comté but also in other regions, might bring new information on its eventual role in the epidemiology of plant diseases associated with the ubiquitous stolbur phytoplasma.

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