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FULL RESEARCH PAPER

Monitoring of proteobacteria and phytoplasma in sugar beets naturally or experimentally affected by the disease syndrome 'Basses richesses'

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Abstract The disease syndrome 'Basses richesses' (SBR) affects sugar beet (*Beta vulgaris*) crops and causes important economic damage in eastern France. Up to now two phloem-restricted prokaryotes which cannot be cultivated, a stolbur phytoplasma and a γ-3 proteobacterium (called SBR proteobacterium), have been associated with the disease. The SBR proteobacterium is closely related to endosymbionts of Hemiptera in the genus *Arsenophonus*. Both the phytoplasma and the proteobacterium are transmitted by the insect vector *Pentastiridius* sp. (Hemiptera: Cixiidae). In the present work, we developed sensitive PCR tools for routine detection of SBR proteobacteria in sugar beets. The monitoring with PCR since 1997, of both SBR pathogen agents, showed the predominant aetiological role of SBR proteobacteria in SBR disease. Detection of SBR proteobacteria in sugar beet was correlated with development of SBR symptoms and reduction of sugar content in the taproot. Severity of symptoms and sugar content in experimentally inoculated sugar beet plants were a function of the number of *Pentastiridius* sp. used for transmission or the length of inoculation access period (IAP), suggesting a direct relationship between importance or precocity of populations of inoculative insects in fields and low sugar yield of crops.

Keywords BLO · Cixiidae · *Pentastiridius* sp. · Phloem-restricted organisms · Plant pathogen · Stolbur phytoplasma

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Introduction

Since 1991, the disease syndrome 'Basses richesses' (SBR) has repeatedly affected sugar beet crops with fluctuating severity in Burgundy and Jura regions of Eastern France (Fig. 1). SBR symptoms appear in late summer. Affected sugar beets show proliferation of small leaves with narrow and twisted chlorotic laminae and yellowing and necrosis of older leaves (Muchembled, Garressus, Ecalle, Boudon-Padieu, & Gatineau, 1999). Tap roots have a normal size but a brownish discolouration of vascular tissues that can be observed after sectioning, is the most characteristic symptom of SBR (Muchembled et al., 1999). At the microscopic level, such discolouration corresponds to necrosis of some phloem tubes, lignification of phloem cell walls

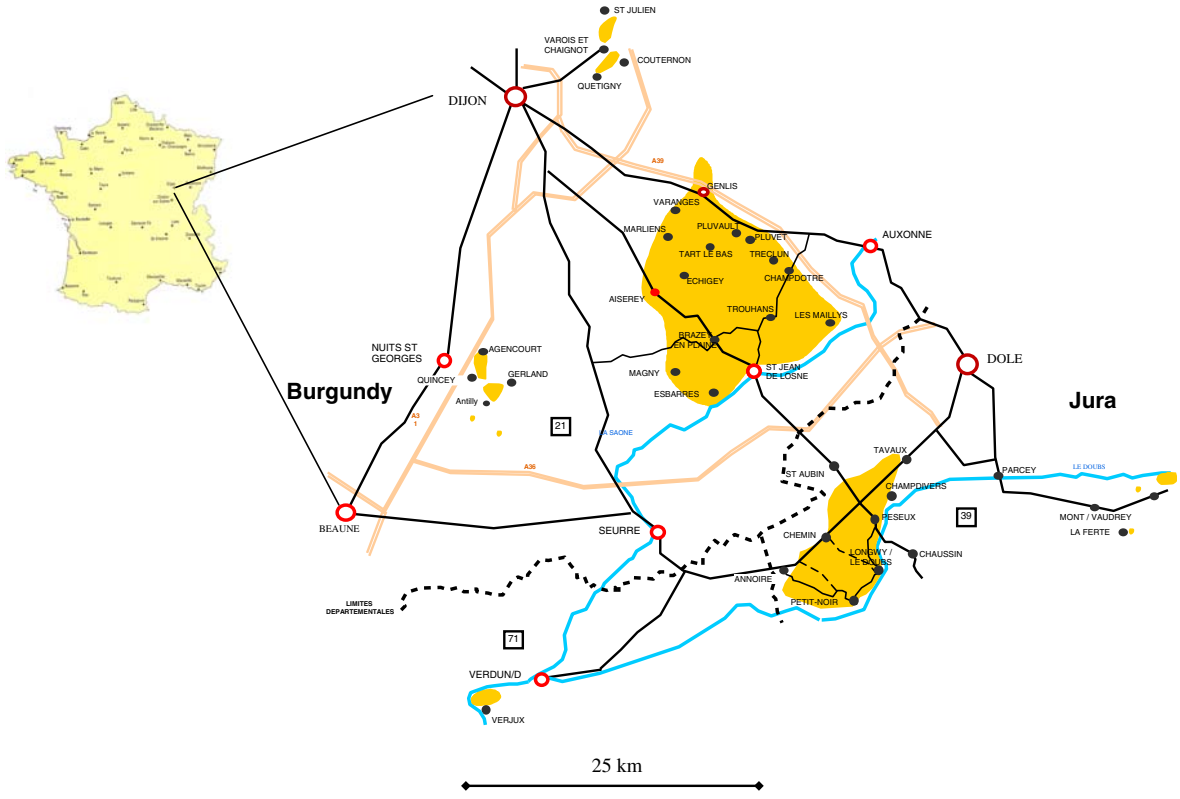

Fig. 1 Major areas affected by SBR in France

and deposit of phenolic compounds in the lumen of phloem cells (Gatineau et al., 2002). Overall, SBR causes a reduction of tap root sugar content which can have dramatic economic consequences for growers and the local sugar beet industry. In 1991, the loss of income was about 50% over 1,000 ha (Richard-Molard et al., 1995); in 2004, the rate of affected plants in plots ranged from 15 to 100% over 1,800 ha (S. Garessus, ITB, France, pers. comm.).

A planthopper of the genus *Pentastiridius* (Hemiptera, Cixiidae) (Gatineau et al., 2001), found abundant in sugar beet crops, has been shown to be the economic vector of the disease, since a large number of plants exposed to feeding by specimens of *Pentastiridius* sp. trapped in affected areas, exhibited symptoms similar to those of SBR. A bacterium-like organism (BLO), now referred as the SBR proteobacterium, and a stolbur phytoplasma, were visualized and detected in SBR-affected sugar beet plants, in *Pentastiridius* sp.—exposed symptomatic sugar beet seedlings and in *Pentastiridius* sp. specimens (Gatineau et al., 2002; Sémétey, Gatineau, Bressan, & Boudon-Padieu, 2007). These results supported the role in SBR of the two phloem-inhabiting prokaryotes and their transmissibility by *Pentastiridius* sp. However, stolbur phytoplasma was detected less frequently than SBR proteobacteria and double infection seemed to be very rare (Gatineau et al., 2002; Gatineau, 2002).

SBR proteobacteria were first observed by DAPI staining in some affected sugar beets free of phytoplasma infection. Using sequence analysis and phylogenetic study of partial 16S rDNA amplified with PCR, SBR proteobacteria have been identified as new plant pathogenic γ-3 proteobacteria, related to but different from '*Candidatus* Phlomobacter fragariae' associated with marginal chlorosis of strawberry (Zreik, Bové, & Garnier, 1998; Sémétey et al., 2007). However, sensitive assays for routine detection of SBR proteobacteria in affected sugar beet fields during the growing season were still lacking for

epidemiological studies and for studying the infection process and development of the syndrome in inoculated plants.

The aim of this study was to develop a sensitive PCR assay for detection of SBR proteobacteria and to use the assay to evaluate the role of both SBR pathogens in the disease. Since SBR proteobacteria appeared to be the major pathogen of SBR in France, we investigated the relationship between its detection and severity of SBR symptoms in field sugar beets, and the relationship between inoculation pressure (as number of insects and feeding duration) and severity of SBR symptoms in sugar beets experimentally inoculated with field specimens of *Pentastiridius* sp.

Materials and methods

Plant material

Detection and monitoring of pathogenic agents

Suspected SBR-affected sugar beets (*Beta vulgaris*) were selected on visual assessment of leaf symptoms. Selected plants were collected from fields during 5 years in 11 different plots grown in seven villages of Burgundy and Jura within an area of 60 × 50 km (Fig. 1). A total of 525 sugar beets were used for monitoring of pathogens. Controls were healthy sugar beet seedlings (variety Brigita) grown in an insect-proof greenhouse, naturally stolbur-infected sugar beet previously tested (Gatineau et al., 2001), reference stolbur phytoplasma maintained in periwinkle (*Catharantus roseus*) (STOLC isolate, obtained from MT Cousin, France), SBR proteobacteria maintained in periwinkle (Gatineau et al., 2002) and strawberry (*Fragaria vesca*) affected with Marginal chlorosis associated with *Candidatus* Phlomobacter fragariae (Zreik et al., 1998), kindly supplied by CIREF, Lanxade (France).

Comparison of SBR proteobacteria from different origins

DNA samples from 22 diseased sugar beets from different fields and years in France (Table 1) were used for molecular comparisons of isolates of SBR proteobacteria.

Rating of disease severity

One hundred and ninety-two sugar beets were collected in three plots of Burgundy affected by SBR in September 2004. From each plot all plants, whether showing symptoms or not, were sampled from one entire row chosen at random. For each sugar beet, SBR symptom severity were annotated, taking into account both tap root and foliar symptoms. For tap roots, a score of 2 was assigned when all vascular tissues in sections showed brownish discolouration, a score of 1 was assigned when vascular tissues were affected only on sectors of sections, and symptomless tap roots were scored 0. For foliar symptoms, a score of 2 was assigned when yellowing and necrosis of older leaves and a proliferation and deformation of central leaves occurred on the plant, a score of 1 was assigned for the occurrence of proliferation and deformation of central leaves but with no yellowing of older leaves, and plants with normal leaves were scored 0. Hence, each plant was assigned a score ranging from 0 (no symptoms) to 4 by the addition of scores for tap root and foliar symptoms. For each of the 192 annotated plants,

Table 1 Origin of sugar beet plants showing SBR symptoms and used for isolation and sequencing of 16SrDNA from SBR proteobacteria

Year	Burgundy					Jura	
	Eperney	Noiron	Savouges	Quincey	Agencourt	Chamblay	Longwy
1997	6	–	–	–	–	–	–
2000	–	–	–	–	–	2	2
2002	2	2	2	–	–	–	–
2003	1	–	–	1	–	–	–
2004	–	–	1	–	2	1	–

the sugar content of the tap root was measured by refractometry. Fifty grams of tissue of each tap root was roughly ground and centrifuged at low speed (5 mn at 2,900 × *g*). The supernatant was filtered through sintered glass and analysed in a refractometer. Brix degree values (corresponding to % of dry weight) were converted into sugar content (corresponding to % sugar in dry weight) by using corresponding tables (ICUMSA, 2000). DNA was extracted from tissues of each tap root according to the procedure described below.

Transmission assays of SBR

Pentastiridius sp. planthoppers were collected with vacuum aspiration using a D-Vac device in June during the flight period in sugar beet crops severely affected by SBR, in Longwy sur le Doubs (Jura) in 2002 and in Epernay sous Gevrey (Burgundy) in 2004, respectively (Fig. 1). In 2002 and 2004, 80 sugar beets (variety Brigita) were sown in March under insect-proof tunnels (6 m × 3 m) and confined with about 500 specimens of field-collected *Pentastiridius* sp. from the end of June until the insects died. Sugar beets were observed until expression of SBR symptoms, i.e. for 3–4 months, when 10 and 19 plants with symptoms were selected in October 2002 and 2004, respectively. Symptoms were annotated, sugar beet content was measured and DNA extracted as described. Other transmission assays with controlled inoculation access period (IAP) to sugar beet were performed in 2004 in a growth chamber (23 ± 1 °C, l16:d8). Plants were 1 month-old seedlings. In a first series, one field-collected *Pentastiridius* sp. specimen was confined per plant for an IAP of 1, 1.25, 1.5, 2, 3, 4, 6, 11 or 16 days. All experiments were started simultaneously. At the end of each IAP, insects were collected. Inoculated sugar beets were sprayed with 0.5 g l^{-1} of Dichlorvos (Bayer) and transported to an insect-proof greenhouse (26 ± 5 °C, natural light) until expression of SBR symptoms. Ten plants were used per treatment and 10 were left free of inoculation for the control. In all 100 sugar beet seedlings were used and were annotated for symptoms after an incubation period of 3–4 months, sugar content was measured and DNA was extracted from each plant as described. A second series of assays consisted of confining 1, 5, 10 or 20 field-collected *Pentastiridius* sp. specimens per sugar beet seedling for an IAP of 24 h. All experiments were started simultaneously. Six plants were used per treatment and nine plants were left uninoculated for the control. In all 33 sugar beet seedlings were used in 2004. Symptoms were annotated after an incubation period of 3–4 months, sugar content was measured and DNA was extracted from each plant as described.

DNA extraction

Total DNA was extracted from 1.5 g of fresh vascular tissue from leaves or tap root of sugar beet or from veins of periwinkle or strawberry, according to the procedure described by Gatineau et al. (2001). Occasionally, tissues from older yellowing leaves, young deformed leaves and tap root of the same plant were extracted separately for assessment of detection sensitivity in the different parts of the plant. All DNA samples were stored in a deep-freezer until use.

PCR detection of phytoplasma in plants and RFLP analysis

Nested-PCR amplification of ribosomal DNA of phytoplasma was used. P1 (Deng and Hiruki, 1991) and P7 (Smart et al., 1996) was the first primer pair; fU5/rU3 (Lorenz, Schneider, Ahrens, & Seemüller, 1995) was the second primer pair. The expected size of the final fU5/rU3 PCR product was 860 bp. PCR conditions used were according to Gatineau et al. (2001). Seven microlitre of the second amplification product were analysed by electrophoresis in 1.2% agarose gel stained with ethidium bromide and visualised under UV light. For RFLP analysis, 10 µl of fU5/rU3 amplification product was 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 UV light.

PCR detection of SBR proteobacteria in sugar beet plants

The four primers fD1, rP1 (Weisburg, Barns, Pelletier, & Lane, 1991), Fra4 and Fra5 (Zreik et al., 1998) were used in combination in direct PCR for amplification of 16S rDNA of SBR proteobacteria (Sémétey et al., 2007). The four different combinations were compared for specificity and sensitivity on field sugar beets. The size of expected PCR products was: fD1-rP1, 1590 bp; Fra5-rP1, 1070 bp; Fra5-Fra4, 551 bp; fD1-Fra4, 950 bp. PCR amplification was performed in 20 µl reaction mixture in a Biometra thermocycler. All primer pairs were used at 1.0 µM in the reaction mixture containing 200 µM of each dNTP, 78 mM Tris HCl, pH 8.4, 5 mM $MgCl_2$, 0.1% Triton X100, 200 µg ml^{-1} Bovine serum albumin (BSA), 2 units of *Taq* DNA polymerase (Q. BIOgene, Illkirch, France) and 1 µl of target DNA. PCR conditions for amplification of Fra5-rP1 and fD1-rP1 were as follows: eight cycles of 45 s at 92 °C, 30 s at 58 °C–0.5 °C per cycle and 60 s at 72 °C followed by 27 cycles of 45 s at 92 °C, 30 s at 54 °C and 60 s at 72 °C, and a final extension for 10 min at 72 °C. Conditions for fD1-Fra4 amplification were: 35 cycles of 45 s at 92 °C, 45 s at 54 °C and 60 s at 72 °C: and a final extension for 10 min at 72 °C. Fra5-Fra4 amplification was performed according to Zreik et al. (1998). PCR products (7 µl) were analysed by electrophoresis in 1.2% agarose gels, stained with ethidium bromide and visualized under UV light.

Sequencing of PCR products

Fra5-rP1 PCR products from samples of 22 sugar beets taken in France (Table 1) were directly sequenced. Sequencing reactions were performed by MWG AG Biotech (Ebersberg, Germany). Nucleotide sequences were analyzed with CLUSTAL W Multiple Sequence Alignment Programme (version 1.8, European Bioinformatics Institute website) (Higgins and Sharp, 1988). Sequence homology analysis with sequences in GenBank Database was done using the BLAST2 programme (Altschul et al., 1997).

Results

PCR detection of SBR proteobacteria in diseased sugar beets

PCR products obtained using fD1/rP1, Fra5/rP1, fD1/Fra4 and Fra5/Fra4 primer pairs are shown in Fig. 2A–C, respectively. Two DNA fragments of about 1,590 and 1,900 bp were amplified with primer pair fD1/rP1 from all DNA samples including healthy sugar beet controls but not from a water control (results not shown). No amplification was obtained with DNA from healthy sugar beet (lane 1 and 2) or from stolbur—infected periwinkle (lane 13) with three

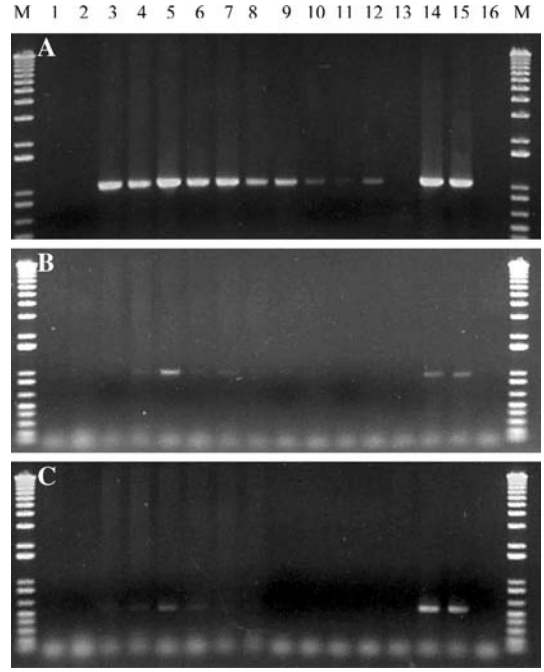

Fig. 2 Agarose gel electrophoresis of PCR products obtained with **A**: Fra5-rP1, **B**: fD1-Fra4 and **C**: Fra5-Fra4 primer pairs. M, molecular weight ladder (1kb Plus DNA Ladder, Invitrogene). Lanes 1–2, healthy sugar beet control. Lane 3–12, SBR-affected sugar beets from different origins: lane 3, Eperney sous Gevrey, 2004; lane 4, Epoisses, 2003; lane 5, *Pentastiridus* sp.-exposed plant, 2004; lane 6, Longwy sur le Doubs, 1997; lane 7–8, Longwy sur le Doubs, 2000; lane 9–12, Epoisses, 2004, roots, young leaves, intermediate leaves and old leaves of the same plant, respectively. Lane 13, STOL C-infected periwinkle. Lane 14, SBR proteobacteria infected periwinkle. Lane 15, strawberry plant infected with ''*Candidatus* P. fragariae''. Lane 16, water

primer pairs Fra5/rP1 (Fig. 2A), fD1/Fra4 (Fig. 2B) and Fra5/Fra4 (Fig. 2C). A product of the expected size for each primer pair (Fra5-rP1, 1070 bp; Fra5-Fra4, 551 bp; fD1-Fra4, 950 bp) was obtained with control DNA from SBR proteobacteria infected periwinkle (lane 14) and *Ca*. Phlomobacter fragariae-infected strawberry (lane 15). No signal was obtained from the water control (lane 16). Only a few positive detections were obtained with fD1/Fra4 and Fra5/Fra4 primer pairs from SBR symptomatic plants (Fig. 2B, C, respectively, lane 3–12). By contrast, the primer pair Fra5/rP1 (Fig. 2A) permitted detection of SBR proteobacteria in all samples from symptomatic sugar beets from the fields (lane 3, 4, 6 to 12) or submitted to inoculation with *Pentastiridius* sp. (lane 5). SBR was detected in tap roots of symptomatic sugar beets from different sites and years (lane 3, 4, 6 to 9) and in leaves showing symptoms (lane 10–12). However, the reliability of detection was higher in tap roots than in leaves of the same plant (lane 9–12).

The Fra5-rP1 fragment was amplified and sequenced from the 22 SBR-affected sugar beets from different origins shown in Table 1. A 950 bp sequence was obtained by direct sequencing of each amplicon from the 22 samples. Total (100%) identity was found between each sequence and with 16S rDNA of SBR proteobacteria (AY057392) (results not shown). Hence Fra5-rP1 amplification was considered in the rest of the work as a reliable method of detection of SBR proteobacteria in sugar beet.

Monitoring of SBR pathogens

Table 2 shows results of PCR amplification with primers Fra/rP1 and stolbur phytoplasma detection in a collection of DNA samples from naturally infected field-collected sugar beets or sugar beets experimentally inoculated with *Pentastiridius* sp., since 1997. A stolbur phytoplasma was detected in a few samples of field-grown sugar beets (Table 2), in 1997, 2000 and 2004 while SBR proteobacteria was detected in 40–70% of sugar beet collections from all years and all sites. Double infection was observed in a few plants collected in 1997 and 2000. When healthy sugar beets were submitted to free-access feeding with numerous *Pentastiridius* sp. delivered under insect-proof tunnels (Table 2), all symptomatic plants showed severe symptoms (score 4, data not shown) and all tested plants were positive for SBR proteobacteria. The rate of positive detection of SBR proteobacteria was higher for the latter inoculated plants (29 PCR positive out of 29 tested, 100%) than for field-collected sugar-beets selected on the basis of visual assessment of leaf symptoms (287 PCR positive out of 525 tested, 55%).

Table 2 Results of PCR amplification with Fra5-rP1 and PCR-RFLP detection of stolbur phytoplasma in a collection of DNA samples from sugar beets showing SBR symptoms from different fields and years or submitted to *Pentastiridius* sp. inoculation under insect-proof tunnels

Year	Sugar beet plants from SBR-affected fields			Sugar beet plants exposed to *Pentastiridius* sp.	
	Fra5/rP1 positive/tested (%)	Sp[a] positive/tested (%)[b]	Double infection[c] (%)	Fra5/rP1 positive/tested (%)	Sp[a] positive/tested (%)
1997	20/50 (40)	5/50 (10)	2/50 (4)	–	–
2000	34/50 (68)	4/50 (8)	1/50 (2)		
2002	77/110 (70)	0/110 (0)	–	10/10[d] (100)	0/10 (0)
2003	38/93 (41)	0/93 (0)	–	–	–
2004	118/222 (53)	1/222 (0.5)	0/222 (0)	19/19[d] (100)	0/19 (0)
Total	287/525 (55)	10/525 (2)	5/525 (0.6)	29/29 (89)	0/29 (0)

[a] Sp = Stolbur phytoplasma

[b] Nested P1/P7–U5/U3 PCR detection and RFLP analysis

[c] Plants positive with both PCR assays

[d] All the sugar beets expressed strong SBR symptoms (score 4)

Association of SBR proteobacteria with SBR symptoms

Figure 3A shows percentage of detection of SBR proteobacteria (on the basis of Fra5-rP1 amplification) and sugar content value, each expressed as a function of severity of SBR-symptoms (scored 0–4) in 192 sugar beets harvested at random from three SBR affected plots in 2004. The higher the score for symptom severity, the higher was the percentage of SBR proteobacteria-positive plants and the lower the sugar content value. Phytoplasmas were not detected in any of these 192 sugar beets.

Figure 3B shows sugar content and severity of SBR symptoms in experimentally inoculated plants as a function of the length of IAP with confinement of a single specimen of *Pentastiridius*

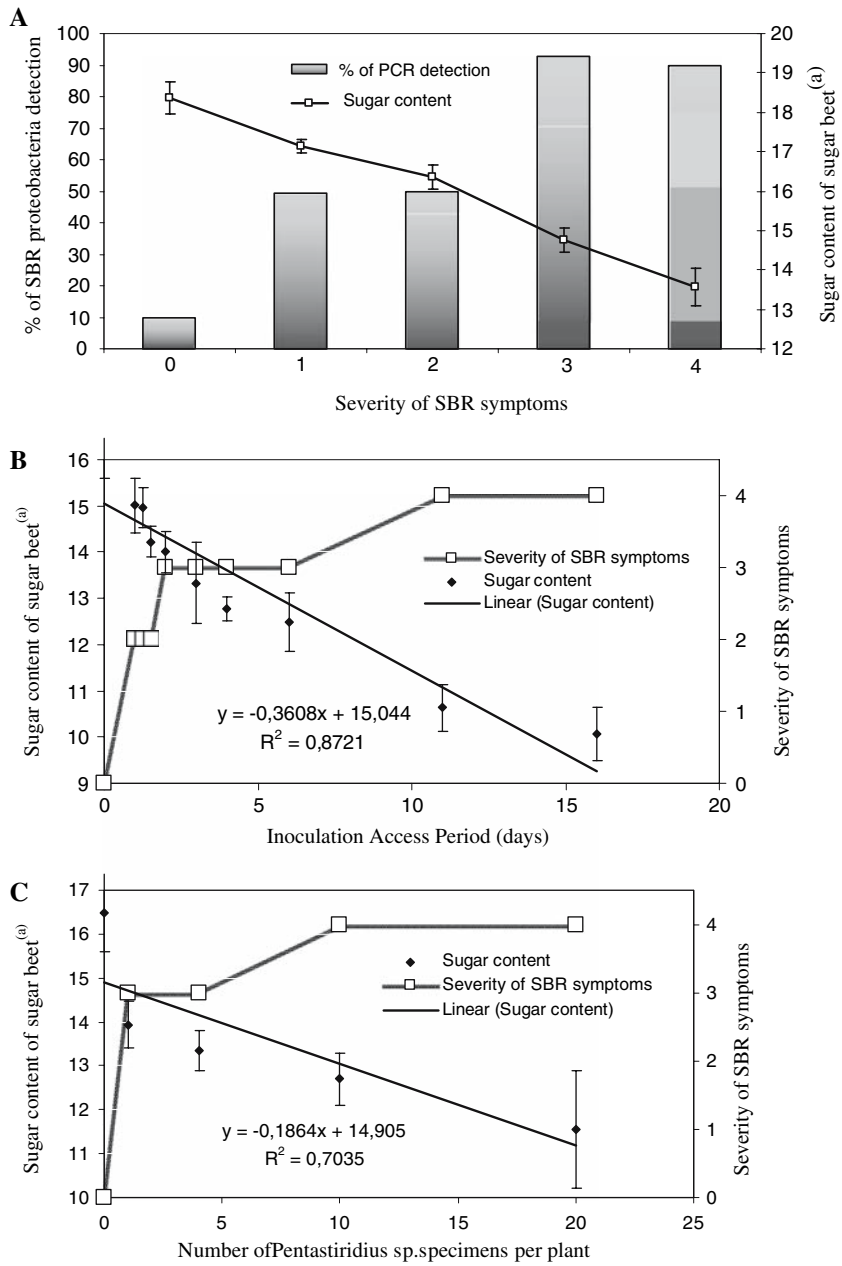

Fig. 3 (**A**) Relationship between severity of SBR symptoms, the rate of PCR detection of SBR proteobacteria with Fra5/rP1 primers and sugar content in field sugar beets. A total of 192 plants were analysed. (**B**) Effect of the length of inoculation access period (IAP) by one *Pentastiridius* sp. specimen per sugar beet seedling on severity of SBR symptoms and on sugar content. A total of 120 plants were analysed. (**C**) Effect of the number of *Pentastiridius* sp. specimens confined per sugar beet seedling for 24 h—IAP on severity of SBR symptoms and on sugar content. A total of 33 plants were analysed. *Note*: [(a)] represents % sugar in dry weight, vertical bars show standard deviation

sp. per healthy seedling. One-day IAP was enough for transmission of SBR proteobacteria. For each plant the score for symptom severity and the sugar content value were correlated with the length of IAP for a single insect specimen. No phytoplasma were found in inoculated sugar beets (results not shown).

Figure 3C shows that both the score for symptom severity and the sugar content value were a function of the number of specimens of *Pentastiridius* sp. confined for a 24 h-IAP on sugar beet seedlings. The higher the number of insect specimens per plant, the higher was the score for symptom severity and the lower the sugar content. The severity of SBR symptoms was again related to frequency of detection of SBR proteobacteria. No phytoplasma were detected (data not shown).

Discussion

The syndrome 'Basses richesses' of sugar beet (SBR) has been associated with two uncultivated phloem-restricted plant pathogenic prokaryotes: a γ-3 proteobacterium and a stolbur phytoplasma, both transmitted by the same planthopper in the genus *Pentastiridius* (Hemiptera, Cixiidae) during feeding on plants (Gatineau et al., 2002; Sémétey et al., 2007). Stolbur phytoplasmas are ubiquitous organisms transmitted by insect vector species that are associated with multiple host plants (Fos, Danet, Zreik, Garnier, & Bové, 1992; Maixner, 1994; Sforza, Daire, Clair, Larrue, & Boudon-Padieu, 1998). Detection of stolbur phytoplasma in SBR-affected sugar beets and in specimens of *Pentastiridius* sp. could be readily obtained with the use of established sensitive PCR methods (Gatineau et al., 2001). However, sensitive and efficient routine diagnostic tools for SBR proteobacteria are still needed to study the inoculum pressure of SBR pathogens and the distribution of the disease in sugar beet crops.

Among the four pairwise combinations of four primers designed on 16S rDNA, the use of PCR with primer pair Fra5/rP1 appeared to be specific and the more sensitive in diseased sugar beets. Positive amplification has been obtained with a collection of DNA samples from diseased sugar beets since 1997 from different areas, and sequencing of PCR products confirmed that the SBR proteobacterium is a unique organism. These results underline the major role of SBR proteobacterium in SBR since 1997. Based on the latter results, we used Fra5/rP1 PCR amplification as a tool for detection of SBR proteobacteria in sugar beet. We showed that positive amplification was related to severity of SBR visual symptoms and reduction of sugar content of the tap root. Positive amplification with Fra5/rP1 was obtained in 90–95% of severely diseased sugar beets from the fields. These observations suggest that absence or lower rate of detection of SBR proteobacteria in sugar beet showing mild SBR symptoms might be due to a low concentration of SBR proteobacteria rather than to the presence of other unidentified plant pathogenic organisms.

Since its first observation, SBR has been described as a disease that fluctuates in occurrence and severity, in space and time (Richard-Molard et al., 1995; Muchembled et al., 1999; Gatineau, 2002). Our data underline the role of vectors in the prevalence of the disease. In transmission trials *Pentastiridius* sp. could transmit the SBR proteobacteria to sugar beet seedlings that developed typical symptoms with IAP as short as 24 h. The longer the IAP or the more numerous the insects confined on seedlings, the higher was the severity of SBR symptoms that developed on inoculated plants. This suggests a direct relationship between the importance and precocity of populations of inoculative *Pentastiridius* in fields and the loss of sugar yield affecting sugar beet crops. Such a relationship could explain differences in the number of SBR-affected plants and the severity of symptoms observed among different sugar beet plots, during the same or different years. Studies of agronomic factors that could interfere with populations and flight activity of *Pentastiridius* sp. are presently underway.

In addition, we demonstrated that it is possible to manipulate experimental vector transmission. This will permit the study of plant colonization and its physiological effects, especially in regard to sugar yield. We might expect that very high rates of naturally affected sugar beet plants with the SBR proteobacteria are related to a very high

rate of infective specimens in populations of *Pentastiridius* sp. Gatineau (2002) provided indirect evidence of vertical transmission of proteobacteria to the progeny from infected females. Occurrence of such vertical transmission and other factors such as acquisition of proteobacteria by larval instars from roots of infected source plants might account for the high prevalence of SBR proteobacteria in affected sugar beet crops.

A high sequence homology of 16S rDNA has been shown between SBR proteobacteria and secondary endosymbionts of hemipters belonging to the prokaryote genus *Arsenophonus* (Sémétey et al., 2007). Secondary endosymbiotic bacteria are facultative in insects and can be involved in responses to physiological stress such as resistance of insects to parasitoid infection or recovery from heat stress (Montllor, Maxmen & Purcell, 2002; Oliver, Russell, Moran, & Hunter, 2003). The use of PCR amplification of bacterial 16S rDNA to investigate the presence of SBR proteobacteria in insect specimens could be hampered by the presence of such endosymbionts (Sémétey et al., 2007). More specific detection tools for use in the study of transmission biology of SBR pathogens are under development.

SBR symptoms associated with phytoplasma and with SBR proteobacteria are very similar (Gatineau et al., 2002). A study of plant colonization, pathogenicity and sugar yield in stolbur phytoplasma-infected sugar beets should be conducted, even though a low frequency of such infection was found during the course of the present study. As many as 13% of stolbur-infected *Pentastiridius* sp. specimens were recorded in captures done in 1997 (Gatineau et al., 2001). Vertical transmission of phytoplasmas in insects is not the rule (Tedeschi, Ferrato, Rossi, & Alma, 2006). It was assumed (Gatineau, 2002) that the rate of phytoplasma-infected *Pentastiridius* sp. specimens depended on the presence and abundance of stolbur-infected weeds on the route of young adults during migration flights in spring. If so, prevalence of stolbur phytoplasma in SBR affected sugar beets should depend on the abundance of host plants in the environment of sugar beet plots.

In conclusion, this paper presents important information on the prevalence of SBR-associated pathogens and the direct role of SBR proteobacteria in the physiological disorder and economic consequences of SBR disease. It also provides experimental tools to approach the study of a new class of plant pathogenic bacteria.

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