

## SHORT COMMUNICATION

**Agronomic practices as potential sustainable options for the management of *Pentastiridius leporinus* (Hemiptera: Cixiidae) in sugar beet crops**

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**Abstract**

Cixiid planthoppers (Hemiptera: Fulgoromorpha: Cixiidae) have been shown to vector phloem-limited prokaryotes associated to prominent plant diseases world-wide. However, little information is available on the management of such insects that spend a significant part of their life cycle underground as nymphal stages. Preliminary assays were carried out to analyse the potential of some agronomic practices to reduce the underground populations of *Pentastiridius leporinus*, a cixiid vector of plant pathogenic bacteria to sugar beets that completes its life cycle in the cropping rotation sugar beet-winter wheat. A first field assay was carried out to test the effect of spring barley as an alternative crop to winter wheat, a second one analysed the effect of reduced tillage as alternative to ploughing. Planthopper abundance, as evaluated by using horizontal sticky sheets to intercept flying adults that emerged from the soil beneath traps, showed a reduction of 80% and 28% of planthoppers when barley was grown instead of wheat and when reduced tillage was applied instead of ploughing, respectively. Barley roots had 81% fewer nymphs than did wheat roots. Indirectly this study demonstrates how conventional cultural practices for sugar beet production can promote the establishment of *P. leporinus* populations in sugar beet crops in eastern of France.

**Introduction**

Cixiid planthoppers (Hemiptera: Fulgoromorpha: Cixiidae) are considered to be important pests because of their ability to transmit phloem-restricted prokaryotes causing prominent plant diseases from temperate to tropical areas world-wide (e.g. Howard et al. 1983; Liefing et al. 1997; Danet et al. 2003; Jovic et al. 2007).

The syndrome 'basses richesses' is an emerging disease of sugar beet (*Beta vulgaris* L.) spread in the regions of Burgundy and Franche-Comté in the east of France. The disease causes a reduced content of sugar on infected sugar beet tap roots (Richard-Molard et al. 1995); disease incidences vary greatly

across fields and growing seasons, but almost all plants can be affected in some heavily diseased fields (Richard-Molard et al. 1995).

The disease has been shown to be associated primarily with a  $\gamma$ -3 proteobacterium, here called SBR bacterium, and secondarily with a stolbur phytoplasma (Gatineau et al. 2001, 2002; Sémétey et al. 2007a,b; Bressan et al. 2008). Both pathogens are transmitted to sugar beet by a cixiid planthopper, *Pentastiridius leporinus* (L.) which was first presumed to be *Pentastiridius beieri* (Wagner) (Gatineau 2002; Bressan et al. 2009a).

Although very little is known about *P. leporinus* host range, the planthopper species has been described for having a Palearctic distribution (Ossiannilsson 1978;

Nickel et al. 2002; Holzinger et al. 2003). Plant pathogenic bacteria sharing near 100% sequence homology in the 16Sr RNA gene with SBR bacterium have been characterized from strawberries (*Fragaria* spp.) in Italy (Terlizzi et al. 2007), and on sugar beet in Hungary (O. Sémétey, INRA, personal communication) suggesting that the pathogen has also a broad distribution and that its host range is not restricted to sugar beet. Thus, because of the wide distribution of both plant pathogen and insect vectors the disease has the potential to spread across the major sugar beet growing areas of Europe.

Sugar beet is generally sown at beginning to middle of March and usually harvested from the following October through November. Generally, the crop is rotated in a 3-year interval and therefore alternated with other crops in the same field. Winter wheat is the most common crop that is rotated with sugar beet harvests; an alternative crop to winter wheat is spring barley, which is generally cultivated when unfavourable climatic conditions do not permit to sow wheat in the autumn.

Moldboard ploughing soil to a depth of 20–25 cm usually follows sugar beet harvests. Reduced tillage is an alternative cultural practice to deep ploughing, and can be performed by using disc ploughs that slice into and disperse soil without completely inverting the soil. Reduced tillage is generally accomplished at a lower depth than ploughing, and significantly diminishes tilling costs because of its lower energy requirement compared to deep ploughing.

Studies have shown that *P. leporinus* completes its life cycle between the cropping rotation of sugar beet and winter wheat (Gatineau 2002; Sémétey 2006; Bressan et al. 2009a,b). Consistent with the life cycle of cixiid planthoppers from temperate areas (e.g. Holzinger et al. 2002) the species is univoltine, living for most of the year underground throughout their nymphal stages. Between the middle of June through the end of July emerging adults migrate from winter wheat (cultivated in the previous year to sugar beet) to newly sowed sugar beet fields (Bressan et al. 2009b). Females lay eggs nearby sugar beet tap roots and hatching nymphs develop thereafter by sucking sugar beet root sap. Because of the declining temperatures in autumn (October–November), nymphs are probably induced to diapause, at the same time sugar beet tap roots are harvested and winter wheat is sown right after that soil has been tilled. Post-diapausing nymphs complete their development by spring–summer time presumably by feeding on wheat roots. To complete the life cycle, adults that emerge from the

wheat migrate to the newly sown sugar beet crops (Bressan et al. 2009b). Migrant adults are infective (A. Bressan, unpublished data) and can inoculate the pathogen to sugar beets, thus increasing the spread of the disease.

Thus far, the sole management option available to sugar beet growers to diminish spread of SBR disease is the insecticidal suppression of migrating adults on sugar beet fields. Pyrethroids (Soderlund and Bloomquist 1989) are the only insecticides allowed to be deployed against the planthopper. These insecticides are effective in killing planthoppers, but they have a limited persistence in field conditions (M. Richard-Molard, Institute Technique de la Betterave Industrielle, personal communication).

There is therefore a need to identify new control strategies to manage the planthopper and pathogenic bacteria it spread. This research reports a preliminary study on the effect of agronomic practices to diminish the nymphal populations of *P. leporinus*. Two separate field assays were developed to analyse the effect of spring barley as an alternative crop to winter wheat, and reduced tillage as alternative practice to ploughing on the abundance of subterranean nymphs and emerging adults.

## Materials and Methods

To assess the influence of the crop following sugar beet harvests on the abundance of emerging planthoppers, a rectangular north–south oriented, 130 m × 250 m sugar beet field located at 47°00′53″N, 5°42′40.24″E in the Jura Department (Franche-Comté Region, France) was selected as the research site in 2005. The northern side of the sugar beet field was not treated with insecticides, and was monitored for planthoppers from June 9 to the end of July by using 7 vertical sticky traps. These were made of transparent PVC sheets (300 mm long, 210 mm wide and 0.2 mm thick) coated on each side with Soveurode® aerosol (Scotts®, Biosystèmes, France). Binder clips fastened the top and bottom of each trap to a 5-mm diameter, 600-mm long fibreglass stake. Traps were posted every 10 m along an east–west line parallel and 40 m south of the northern edge of the field and were maintained above the vegetation layer by raising them on the stakes as the plants grew. Planthoppers were counted and removed from traps twice a week and traps were replaced every 7–10 days.

Sugar beets tap roots were harvested on 23 September, 2005; thereafter the entire experimental field was ploughed to a 25-cm depth. Four contiguous, east–west oriented, 18-m wide by 120-m long blocks, were arranged on the experimental field, each block

comprised two 18-m wide by 60-m long plots. Within each block, treatments (winter wheat or spring barley) were randomly assigned to one or the other plot.

Within assigned plots, wheat was sown right after tilling and spring barley was sown about 4 months later in accordance with typical cultural practices adopted in the region for these two crops (table 1). In order to examine the abundance of nymphs underground, wheat or barley plots were sampled to estimate nymphal densities between 17 May through 3 June, 2006, which preceded adult emergence. Samples consisted of four randomly selected soil removals of about 1250 cm<sup>3</sup> each (50 cm<sup>2</sup> × 25 cm deep). Soil was dug out with a spade and put into a basket, where it was carefully disaggregated and number of identified nymphs was recorded.

Because of the difficulties of quantifying nymphal populations in the soil, sticky traps were used to sample for emerging adults on cereal plots. Traps were made with same transparent PVC sheets described previously; however, they were oriented horizontally directly above the vegetation layer with the downward side of the sheets coated with glue to intercept flying adults that emerged from the soil beneath the trap. In order to keep the sheets horizontal, they were mounted on the top of 7-mm diameter and 1000-mm long fibreglass stakes through a support made by two crossed 5-mm diameter fibreglass stakes that permitted to maintain sheets flat. Starting on 3 June, 2006 and right before adult emergence, seven to eight horizontal sticky traps were positioned every 6–7 m and for the entire length of each plot. A total of 56 horizontal traps were inspected for trapped insects twice a week. Insects were removed from each trap with forceps and captures were recorded. Glued sheets were changed every 7–14 days.

To assess the influence of tilling practices following sugar beet harvesting on the abundance of emerging planthoppers, a rectangular (longest side oriented north–south), 162 m × 288 m sugar beet field located at 47°00′53.21″N, 5°42′51.28″E in the

Jura Department (Franche-Comté Region, France) was selected as the research site in 2006. The southern half of the sugar beet field that was selected to conduct the assay was not treated with insecticides and was monitored for presence of planthoppers by using eight vertical sticky traps previously described. Traps were posted every 10 m along a north–south oriented line parallel and 80 m at the east of the western side of the field. Starting by 9 June 2006 through the end of July planthoppers were counted and removed from traps once a week and sheets were replaced every 7–10 days.

After sugar beet harvesting the experimental field was organized with a randomized block design with four contiguous, north–south oriented, 18-m wide by 120-m long blocks. Each block comprised two 18-m wide by 60-m long plots. Within each block, treatments were randomly assigned to one or the other plot. Treatments were 25-cm depth soil moldboard ploughing and 10- to 15-cm deep disc ploughing. All other cultural practices were the same for both treatments (table 2). Wheat was sown in the entire experimental field.

Starting on 3 June, 2006, which was just before adult emergence, four horizontal sticky traps were posted every 10 m within each plot. Sampling was carried out as previously described, except that traps were inspected weekly.

To analyse the abundance of planthoppers colonizing sugar beet fields, cumulative counts of insects on each trap throughout the sampling period and means with respective standard deviations were calculated. To analyse patterns of adults emerging from experimental fields over time, mean number of emerging adults per trap were calculated per each treatment (winter wheat vs. spring barley and ploughing vs. minimum tilling) and plotted over the sampling period. ANOVA was used to examine differences among mean captures of emerging planthoppers among treatments (winter wheat vs. spring barley or ploughing vs. minimum

**Table 1** Cultural practices in experimental field selected to analyse the effect of spring barley as alternative crop to winter wheat on the abundance of emerging *Pentastiridius leporinus*

Winter wheat		Spring barley	
Date	Cultural practice	Date	Cultural practice
23/9/2005	Sugar beet harvesting	23/9/2005	Sugar beet harvesting
27/9/2005	Moldboard ploughing	27/9/2005	Moldboard ploughing
12/10/2005	Roto-tilling and sowing	18/2/2006	Sowing

**Table 2** Cultural practices in experimental field selected to analyse the effect of reduced tillage as alternative practice to ploughing on abundance of emerging *Pentastiridius leporinus*

Ploughing		Reduced tillage	
Date	Cultural practice	Date	Cultural practice
08/10/2006	Sugar beet harvesting	08/10/2006	Sugar beet harvesting
16/10/2006	Moldboard ploughing	15/10/2006	Disc ploughing
18/10/2006	Roto-tilling and sowing	18/10/2006	Roto-tilling and sowing
18/10/2006	Soil compacting	18/10/2006	Soil compacting

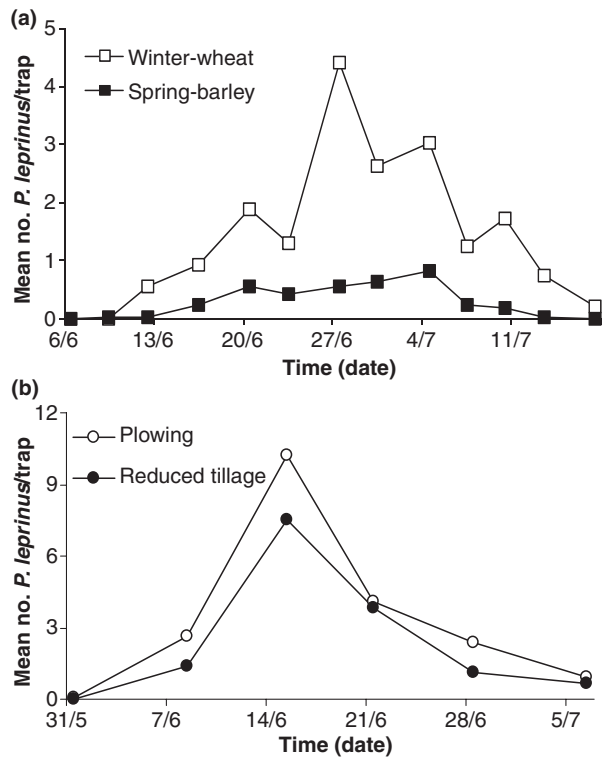
tilling). Numbers of planthoppers caught on each trap were summed over the sampling dates for both assays. Data from assay comparing insect abundance on wheat and barley plots were transformed as  $\log(x + 1)$  to normalize data distribution. To test for statistical differences between abundance of underground nymphs counted on winter wheat or spring barley plots, a Mann–Whitney *U*-test was adopted as data were not distributed normally. Statistical analyses used SIGMASTAT version 3.5 (Systat Software, Inc. Chicago, IL).

## Results

Both sugar beet fields selected to conduct agronomic assays were colonized by *P. leporinus*; however, sticky traps captured more than three times the total number of adults in the experimental field selected to analyse the effect of the crop rotation than the one selected to study the effect of tilling practices. Mean numbers of adults per trap cumulated over the season were  $462.3 \pm 83.6$  (SD) and  $144 \pm 58.7$  (SD) for the first and second fields, respectively.

For the crop rotation assay, the mean number of nymphs identified in soil samples was  $0.62 \pm 0.72$  (SD) and  $3.37 \pm 2.3$  (SD) for barley and wheat respectively. Highly significant differences were detected between these two treatments by using Mann–Whitney *U*-test ( $U = 362$ ,  $n_{1/2} = 16$ ,  $P < 0.001$ ). Based on the sample means and assuming uniform distribution of nymphs across the plots, the number of nymphs per hectare can be projected to be about 24 800 for barley and 134 800 for wheat. The patterns of emerging *P. leporinus* intercepted on horizontal traps on wheat and on barley plots over time were similar for the two treatments (fig. 1a); however, emerging adults from barley were about 80% less than those from wheat. Mean captures per trap cumulated over the season were  $3.63 \pm 2.5$  (SD) and  $18 \pm 8.9$  (SD) for barley and wheat plots respectively. Means were statistically different when compared with ANOVA ( $P < 0.001$ , table 3). Differences in the relative abundance of nymphs and adults were similar with a ratio barley : wheat of 1 : 5.4 and 1 : 4.9 respectively.

For the tillage assay, the patterns of adult emergence were similar for deep ploughing vs. disc ploughing (reduced tillage) (fig. 1b), but the number of emerging planthoppers trapped from reduced-tillage plots were about 23% less than those from deep ploughing. Mean number of captures per trap for the season were  $20.25 \pm 8.3$  (SD) and  $14.5 \pm 6$  (SD) for plough and reduced-tillage plots respectively.



**Fig. 1** (a) Temporal pattern of *Pentastiridius leporinus* adults emerging from spring barley and winter wheat plots. (b) Temporal pattern of *P. leporinus* adults emerging from wheat plots whose soil was mold-board- or disc plough (reduced tillage).

**Table 3** Results of anova on mean number of *Pentastiridius leporinus* intercepted on horizontal sticky traps

Source of variation	Degree of freedom	Sums of squares	Mean squares	F	P-value
Treatment	1	6.045	6.045	153.124	<0.001
Blocks	3	0.245	0.0817	2.069	0.116
Experimental error	3	0.74	0.247	6.247	0.001
Sampling error	52	2.053	0.0395		

Treatments were spring barley and winter wheat crops cultivated after sugar beet harvesting. Experimental design was a randomized blocks.

Mean values were statistically different when compared with ANOVA ( $P = 0.037$ , table 4). Probably due to a low density of nymphs underground, survey of nymphs from soil samples were inconsistent.

## Discussion

Agronomic practices have been proposed to control cixiid planthoppers to mitigate the spread of pathogens that they transmit. For instance, *Myndus crudus* Van Duzee, which has been considered as the main



**Table 4** Results of anova on mean number of *Pentastiridius leporinus* intercepted on horizontal sticky traps posted on wheat plots

Source of variation	Degree of freedom	Sum of squares	Mean squares	F	P-value
Treatments	1	264.5	264.5	4.853	0.037
Blocks	3	196	65.333	1.199	0.331
Experimental error	3	67	22.333	0.41	0.747
Sampling error	24	1308	54.5		

Treatments were ploughing and minimum tilling that were applied to experimental field after sugar beet harvesting. Experimental design was a randomized blocks.

vector of palm lethal yellowing phytoplasma to palms in Florida (Howard et al. 1983) develops as nymphs on roots of grasses, whereas adults feed on palm foliage.

Cultural control practices that have been suggested include the replacement of St Augustine grass [*Stenotaphrum secundatum* (Walter)] favourable for planthopper development with grasses that are poor breeding hosts for the planthopper, as ground cover in coconut plantations (Howard 1990).

Different cultural practices have been tested to control *Hylasthes obsoletus* Signoret, a European cixiid planthopper that transmits stolbur phytoplasma to a large number of cultivated plants including grapevines (*Vitis vinifera* L.) (Maixner 1994). This planthopper breeds on two main host plants: bindweed (*Convolvulus arvensis* L.) and nettle (*Urtica dioica* L.), across several European countries (e.g. Johannesen et al. 2008). Depending on the application time, control of weeds host plants through herbicide applications has been proved to be effective in reducing vector population (Mori et al. 2005). The prevalence of bulbous buttercup (*Ranunculus bulbosus* L.) between vine rows in vineyards serves as a secondary breeding host for the planthopper but is not host of stolbur phytoplasma, significantly reducing the infectivity of the planthopper population within the vineyard compared to those fields where bindweed was prevalent (Weber and Maixner 1998).

This research provides further evidence about the potential of agronomic practices to control cixiid planthoppers. In fact, the use of barley as a rotation crop in lieu of wheat resulted in a reduction of approximately 80% of both *P. leporinus* nymphs and emerging adults. While insecticide treatments applied to sugar beet crops are intended to target migrant adults, non-conventional agronomical practices tested in this research were developed to suppress nymphal populations. Because of their complementarity, both chemical and agronomic

tactics have the potential to be adopted in an integrated pest management program.

This research indirectly suggests that underground nymphal populations of *P. leporinus* have specific requirements in term of host plants and physical soil condition. Ploughing and wheat cultivation after sugar beet harvesting may create subsurface conditions that limit nymphal mortality and as a consequence may promote planthopper populations. As mentioned in the introduction, wheat is the most common rotation crop after sugar beet harvest and ploughing is the most frequent tilling practice. Therefore, the extended adoption of such practices may have contributed to the increase of insect populations and the consequent outbreak of 'basses richesses' disease.

Although this research has not examined soil structures, it is likely that both non-conventional practices tested created a less favourable habitat for nymphs survival. In fact, the soil in the barley cultivated plots appeared to be less structured and had fewer cavities compared with the soil cultivated for wheat. Furthermore, barley roots grew much more swallow compared with wheat roots. An alternative hypothesis would be that barley is a less suitable host plant for planthopper development and survival compared with wheat. It is also possible that both of these factors contributed to the reduced nymphal survival. Possible differences in the physical composition of the soil may also explain differences in planthopper abundance in the tilling assay. Because ploughing generated a highly porous soil, this might have resulted with an optimum habitat for nymphs and have limited nymph mortality to a greater extent compared with disc plough wheat plots, which soil appeared to be much more compacted. Further assays need to be conducted in order to assess how physical soils status and host plants affects *P. leporinus* development and nymphal survival.

Because of the standardization of crop rotation and cultural practices, the syndrome 'basses richesses' disease is a potential threat for sugar beet industry in Europe. As many other diseases transmitted by insects, it is likely that integration of multiple control strategies need to be applied to gather a sustainable management and to impair the expansion of the disease to new regions.

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