Effects of crop rotation and soil tillage on suppressing the syndrome “basses richesses” vector Pentastiridius leporinus in sugar beet

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Research Article

Keywords: Pentastiridius leporinus, life cycle, host plant, vector control, maize, field trials

Posted Date: August 17th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1956648/v1

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Abstract

The planthopper *Pentastiridius leporinus* (Hemiptera: Cixiidae), is the most important vector of syndrome “basses richesses” (SBR) in sugar beet; it leads to severe economic losses. Planthoppers have probably adapted recently from reed grass (*Phragmites australis*) to sugar beet - winter wheat crop rotations. So far, no reliable vector control strategies are available. In this study, field assays were performed to test different soil tillage methods and crop rotations as potential management options. Barley, spring wheat, maize, bare soil and winter wheat (control) were grown using different soil tillage treatments (ploughing and cultivator) after sugar beet harvest. In five of seven field trial sites, reduced tillage did not differ from ploughing and, in two trial sites, it resulted in even higher numbers of emerging planthoppers. In nearly all field sites, consistent and significant reductions (up to 98.9%) of emerging adults were detected in the bare soil and maize treatments when compared to winter wheat. The survival and development of first instar nymphs were then studied on wheat, barley, and maize seedlings in the laboratory to confirm the field observations. The lowest survival was found in nymphs feeding on maize seedlings (4.2%), while 29.2% survived on barley, and 66.7% on wheat, over a period of 300 days. Almost 90% of nymphs in the maize treatment died after eight days. These results suggest that maize is a poor host for *P. leporinus* first instar nymphs. A combination of soil tillage and late sown specific spring crops are discussed as options for *P. leporinus* control.

Key Message

- Variations in soil tillage after sugar beet harvest and before winter wheat cultivation may be an approach to reduce emerging adult *leporinus* – as results are highly variable additional environments need to be analysed in future trials.
- Changing crop rotations or leaving soil bare after sugar beet harvest may represent suitable approaches to control *leporinus* as an SBR vector in sugar beet.
- Maize seedlings are poor host plants for first instar *leporinus* nymphs.

Introduction

Syndrome “basses richesses” (SBR) is a sugar beet (*Beta vulgaris*) disease, which leads to severe reductions of up to 5% (absolute) in sugar content in the tap roots and yield losses up to a maximum of 25% (Gatineau et al. 2002; météy et al. 2007a; Bressan et al. 2008). Symptoms are chlorosis and necrosis of the older leaves, asymmetrically formed younger leaves and necrosis in the vascular bundles of the tap roots (Gatineau et al. 2002). Two prokaryotic bacterial pathogens, the γ-3-proteobacterium, *Candidatus Arsenophonus phytopathogenicus*, and phytoplasma *Candidatus Phytoplasma solani* in the stolbur group (16SrXII), are considered causal agents of SBR (Gatineau et al. 2001, 2002; Firrao et al. 2005; météy et al. 2007b; Bressan et al. 2011). The proteobacterium seems to play the major etiological role (Gatineau et al. 2002; Pfitzer et al. 2020). According to Séméty et al. (2007a), both pathogens cannot be cultivated *in vitro* and are exclusively transmitted by insect vectors. Bacteria from the genus *Arsenophonus* are
endosymbionts of planthoppers in the family Cixiidae (Auchenorrhyncha: Hemiptera, Fulgoromorpha) (Bressan 2014). Bressan et al. (2007) showed that the phloem feeding planthopper Pentastiridius leporinus (Hemiptera: Cixiidae), is the most important SBR vector in France, and Pfitzer et al. (2020) also confirmed this role for Southwest Germany. According to Holzinger et al. (2003), one of the natural hosts of adult P. leporinus is reed (Phragmites australis), while the host plant species of the larvae are still unknown. The host shift towards sugar beet and cereals, winter wheat (Triticum aestivum) and barley (Hordeum vulgare), has probably occurred only recently, since P. leporinus had not been reported as a pest in sugar beet before becoming an economic problem in eastern France (Bressan 2009; Bressan et al. 2009a, 2011). In early summer, adults migrate to sugar beet fields where the female oviposits into the soil. The hatching nymphs first develop on sugar beet roots and, after harvest in early autumn, continue their development on winter wheat, which most frequently follows sugar beet in the crop rotation (Bressan et al. 2011). Overall, five immature stages have been described for P. leporinus (Pfitzer et al. 2022). Bressan (2009) assumed that nymphs probably diapause in late autumn and winter due to the low temperatures. From the end of May until the beginning of July, the insects complete their development into adults in cereal crops and migrate to sugar beet fields (Bressan et al. 2009a, 2011). So far, P. leporinus is known to produce only one generation per year in Central Europe (Holzinger et al. 2003; Biedermann and Niedringhaus 2004).

According to Sémétey et al. (2007a), the first observation of SBR symptoms in sugar beet was recorded in 1991 in the eastern French regions of Burgundy and Jura and, by 2004, 1,800 ha had been infected. In Germany, two major affected areas are in Southwest Germany and in the valley of the river Elbe (located in the federal states of Saxony, Saxony-Anhalt and Brandenburg) (Pfitzer et al. 2020; Behrmann et al. 2021). According to Schröder et al. (2012), the first detection of the γ-proteobacterium in German sugar beet fields occurred in 2009. The estimated area of infestation in 2018 was 16,400 ha (Pfitzer et al. 2020) and the further spread of this disease was reported by Behrmann et al. (2021). In Switzerland, the first detection of SBR disease was in 2017 and its fast spread from 3,000 ha to 5,000 ha was reported between 2019 and 2021 (Peter 2020, 2022). Unlike many new invasive pests, P. leporinus has not been introduced but is a widespread native, palearctic species, except in the northern part of the region (Holzinger et al. 2003). Therefore, SBR is a fast-spreading disease that has the potential to affect all sugar beet growing areas.

Clearly, there is a strong need to identify vector control measures for this disease as it poses a major threat to sugar beet production. So far, chemical control of P. leporinus has not been effective under field conditions, since applications of insecticides at two-week intervals during the peak flight activity of P. leporinus were unable to reduce SBR-induced sugar losses (Pfitzer et al., unpublished data). Therefore, alternative methods that also fulfil demands for sustainability need to be explored. Various authors have described agronomical measures or the cultivation of poor host plants to successfully control different planthopper and leafhopper species (Oka 1979; Lamp et al. 1984; Howard 1990). Indeed, the first preliminary indication that soil tillage and crop rotation may negatively affect P. leporinus populations was provided by Bressan (2009) who showed that growing spring barley instead of winter wheat after sugar beet led to 81% and 80% reductions in nymphs and emerging adults, respectively. Furthermore, a
28% reduction in emerging adults in winter wheat was observed following reduced tillage compared to ploughing after sugar beet harvest (Bressan 2009). This field trial was carried out as a single experiment at one location only.

Hence, to obtain further data and a deeper understanding of the potential of agronomical measures to effectively control *P. leporinus*, we carried out two years of field experiments at nine different locations in total. Following sugar beet harvest, growing barley after cultivator or disc harrow soil tillage was compared with winter wheat at two different locations and ploughing was compared with cultivator treatment combined with different crop rotations, such as spring wheat, maize or bare soil, in comparison to winter wheat at seven different locations. Furthermore, the survival and development of *P. leporinus* nymphs reared on wheat, barley, maize and sugar beet seedlings under controlled laboratory conditions were analysed to support the field trial results and to determine the host suitability of these plant species for *P. leporinus* nymphs. Our hypotheses were: i) changes in soil structure due to soil tillage should worsen the living conditions for the nymphs and, thus, reduce the number of emerging adults; ii) delayed sowing of crops after the sugar beet harvest would lead to starvation of the nymphs and reduced adult emergence; and iii) the performance of *P. leporinus* depends on the plant species. Our results show that, in contrast to previous reports, reduced tillage did not differ from ploughing or resulted in even higher numbers of emerging planthoppers. On the other hand, growing maize after sugar beet or leaving the soil fallow with herbicide treatment to suppress weeds, was found to exert a strong impact on reducing *P. leporinus* populations. Maize seedlings were confirmed to be poor hosts for first instar nymphs.

**Material And Methods**

**Effect of soil tillage and crop rotation on the adult emergence of *P. leporinus***

Field trials were set up in different regions of Southwest Germany (Kirchardt, Neckarsulm, Ilvesheim, Dettenheim, Ladenburg, and Untereisesheim) and in the valley of the river Elbe (Arzberg) in autumn 2018 and 2019, following the sugar beet harvest. Trial sites were selected according to the following criteria: i) high presence of *P. leporinus* nymphs in the soil and strong and uniform SBR symptoms; and ii) uniform field topography. The adult emergence of *P. leporinus* was analysed after different soil tillage treatments (ploughing at depths of at least 20 cm and a cultivator at depths up to 20 cm) and the subsequent crops cultivated after the sugar beet harvest (barley or spring wheat in comparison to winter wheat in 2019 as well as maize (*Zea mays*) and bare soil in comparison to winter wheat in 2020) (Table 1). In Kirchardt 2019, winter durum (*Triticum durum*) was cultivated instead of winter wheat. Winter wheat after soil tillage with a cultivator was considered as the control treatment because it is the most common combination after sugar beet harvests in these regions. In our study, reduced tillage was carried out with a rigid tine cultivator instead of a disc harrow, as in the study by Bressan (2009), because this was more common in the regions analysed. In Ladenburg 2019 and Untereisesheim 2019, winter wheat was compared to spring barley; both after reduced tillage. However, in Ladenburg 2019, using a cultivator for soil tillage this was impossible due to the dry soil conditions, so a disc harrow was used instead. Agronomical measurements (time of soil tillage, tilling depths) and plant protection methods (use of
herbicides and fungicides) were performed according to good agronomical practice in the wheat, barley and maize plots. Emerging weeds in bare soil treatment plots were controlled with the herbicides Adengo (Bayer CropScience Deutschland GmbH, Monheim, Germany), Glyphosate or with a combination of Laudis (Bayer CropScience Deutschland GmbH), Spectrum and Bo 235 (both BASF SE, Ludwigshafen, Germany) when necessary. Detailed information is given in Tables 1 and 2.

**Table 1**

Details of experimental field sites

<table>
<thead>
<tr>
<th>Field trial site</th>
<th>Coordinates</th>
<th>Sugar beet harvest</th>
<th>Depths and dates of soil tillage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mouldboard plough</td>
</tr>
<tr>
<td><strong>2018/19</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untereisesheim</td>
<td>49°12’54.1”N 9°11’27.0”E</td>
<td>18/9/18</td>
<td>20 cm, 15/10/18</td>
</tr>
<tr>
<td>Ladenburg</td>
<td>49°27’11.5”N 8°37’55.1”E</td>
<td>17/10/18</td>
<td>*10 cm, 17/10/18</td>
</tr>
<tr>
<td>Arzberg</td>
<td>51°30’31.3”N 13°09’46.0”E</td>
<td>2/11/18</td>
<td>18 cm, 8/11/18</td>
</tr>
<tr>
<td>Dettenheim</td>
<td>49°10’54.9”N 8°23’22.5”E</td>
<td>13/10/18</td>
<td>15 cm, 19/10/18</td>
</tr>
<tr>
<td>Kirchardt</td>
<td>49°12’33.0”N 8°58’20.1”E</td>
<td>1/10/18</td>
<td>10 cm, 5/10/18</td>
</tr>
<tr>
<td><strong>2019/20</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arzberg</td>
<td>51°31’07.6”N 13°07’24.9”E</td>
<td>27/9/19</td>
<td>20 cm, 4/10/19</td>
</tr>
<tr>
<td>Neckarsulm</td>
<td>49°12’19.3”N 9°10’35.1”E</td>
<td>25/10/19</td>
<td>15–20 cm, 30/10/19</td>
</tr>
<tr>
<td>Ilvesheim</td>
<td>49°27’38.3”N 8°34’33.7”E</td>
<td>24/10/19</td>
<td>15 cm, 25/10/19</td>
</tr>
<tr>
<td>Dettenheim</td>
<td>49°10’51.3”N 8°23’31.1”E</td>
<td>25/10/19</td>
<td>10–12 cm, 26/10/19</td>
</tr>
</tbody>
</table>

*Disc harrow was used instead of the rigid tine cultivator in the Ladenburg testing site
Table 2
Dates and depths of seedbed preparation in the different field trial sites. Seedbed preparation was carried out with a rotary harrow at each testing site.

<table>
<thead>
<tr>
<th>Field trial site</th>
<th>Depths and dates of seedbed preparation</th>
<th>Depths and dates of sowing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Winter wheat</td>
<td>Spring wheat</td>
</tr>
<tr>
<td>2018/19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untereisesheim</td>
<td>4–5 cm, 16/10/18 2–3 cm, 16/10/18</td>
<td>4–5 cm, 28/2/19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2–3 cm, 28/2/19</td>
</tr>
<tr>
<td>Ladenburg</td>
<td>3–5 cm, 2/11/18</td>
<td>3–5 cm, 23/2/19</td>
</tr>
<tr>
<td></td>
<td>5 cm, 2/11/18</td>
<td>5 cm, 23/2/19</td>
</tr>
<tr>
<td>Arzberg</td>
<td>4 cm, 8/11/18</td>
<td>4 cm, 4/3/19</td>
</tr>
<tr>
<td></td>
<td>2 cm, 8/11/18</td>
<td>2 cm, 4/3/19</td>
</tr>
<tr>
<td>Dettenheim</td>
<td>3–5 cm, 4/11/18</td>
<td>8 cm, 26/2/19</td>
</tr>
<tr>
<td></td>
<td>2 cm, 4/11/18</td>
<td>2 cm, 26/2/19</td>
</tr>
<tr>
<td>Kirchardt</td>
<td>5 cm, 8/10/18</td>
<td>5 cm, 13/3/19</td>
</tr>
<tr>
<td></td>
<td>2–3 cm, 8/10/18</td>
<td>2–3 cm, 13/3/19</td>
</tr>
<tr>
<td>2019/20</td>
<td>Winter wheat</td>
<td>Maize</td>
</tr>
<tr>
<td>Arzberg</td>
<td>4 cm, 26/10/19</td>
<td>8 cm, 14/4/20</td>
</tr>
<tr>
<td></td>
<td>2 cm, 26/10/19</td>
<td>8 cm, 15/4/20</td>
</tr>
<tr>
<td>Neckarsulm</td>
<td>4–5 cm, 31/10/19</td>
<td>10 cm, 11/4/20</td>
</tr>
<tr>
<td></td>
<td>3 cm, 31/10/19</td>
<td>8 cm, 14/4/20</td>
</tr>
<tr>
<td>Ilvesheim</td>
<td>8 cm, 26/10/19</td>
<td>8 cm: 1/4/20, 8/4/20</td>
</tr>
<tr>
<td></td>
<td>3 cm, 26/10/19</td>
<td>8 cm, 11/4/20</td>
</tr>
<tr>
<td>Dettenheim</td>
<td>3–5 cm, 27/10/19</td>
<td>8–10 cm, 9/4/20</td>
</tr>
<tr>
<td></td>
<td>2 cm, 27/10/19</td>
<td>8 cm, 11/4/20</td>
</tr>
</tbody>
</table>

The field trial layout used a completely randomized block design. A plan of the layout is provided in Fig. S1. In 2019 the trials were designed with three blocks. This was increased to four blocks in 2020, due to the nonhomogeneous nature of *P. leporinus* adult emergence observed in 2019. Plot width was 6 m except at the sites Untereisesheim 2019 (3 m), Ladenburg 2019 (3 m) and Arzberg 2020 (12 m).

**Quantification of field-emerging** *P. leporinus* **adults**
Three cages (base area approx. 3.6 m² with a 1.5 m maximum height, Fig. S2) were set up in each plot to catch *P. leporinus* adults emerging from the soil. The cages were placed 10 m apart. An additional cage per plot was erected in Arzberg in 2020. All cages were covered with a 1.35 mm mesh gauze (FA.BIO 01 Kulturschutznetz Rettichnetz, Hartmann-Brockhaus, Pfaffenhofen-Wagenhofen, Germany) with gauze ends buried in the ground. Inside each cage, a 10 x 25 cm yellow sticky trap (Gelbe Insekten-Leimtafeln, Aeroxon Insect Control GmbH, Waiblingen, Germany) was attached at the top to catch emerging adults. Each sticky trap was replaced once per week. Counting of adult *P. leporinus* started on 13/5/19 in Arzberg and on 14/5/19 in all other field trial sites in 2019, and on 29/4/20 in Arzberg and on 22/4/20 in all other field trial sites in 2020. On 29/6/20 in Neckarsulm, Ilvesheim and Dettenheim and on 30/6/20 in Arzberg, maize plants were cut to a maximum height of 1 m, to avoid that the plants reached the top of the cages and to ensure that emerging adult *P. leporinus* were still attracted by the sticky traps while the maize plants stayed alive.

**P. leporinus identification**

All trapped planthopper adults were identified to genus level according to their scutellum, vertex, pronotum and hind tarsus, using a stereomicroscope. From each field trial site, a representative sample of 25 male *Pentastiridius* sp. were then identified to species level according to their genital morphology. The key of Biedermann and Niedringhaus (2004) was used. All analysed insects in the genus *Pentastiridius* sp. were identified as *P. leporinus*, so, therefore, we counted all *Pentastiridius* sp. as *P. leporinus*.

**Performance of nymphs on different host plants**

The survival and development of *P. leporinus* nymphs on different plant species was analysed under controlled environmental conditions (20.9 ± 1°C, 45 ± 11.6% relative humidity, 24 h darkness). Plants at the seedling stage (4–10 days old) were then offered to nymphs, as this is the stage under field conditions when they have their first contact. The parental adult generation was caught between 19/6 and 20/6/2020 in a sugar beet field (49°12'17.2"N 9°10'48.8"E). Oviposition was performed on sugar beet plants under controlled environmental conditions and egg batches were kept on Petri dishes until the nymphs hatched. Directly after hatching, the first instar nymphs were individually transferred with a small paint brush to wheat (cv. Dekan, KWS SAAT SE & Co. KGaA, Einbeck, Germany), and barley (cv. Orbit, KWS SAAT SE & Co. KGaA) or maize (cv. Ronaldino, KWS SAAT SE & Co. KGaA) plants. Seeds were not treated with insecticides or fungicides. Plants were grown in conical 600 mL plastic pots (11 cm upper diameter; 9 cm height) that were filled with sand (diameter 0–2 mm) under controlled environmental conditions (as described above, with a 16:8 h light/dark photoperiod and 80 µmol (s m²)⁻¹ light intensity). Light was provided by full-spectrum LED lights (‘Bioledex GoLeaf E2 LED Pflanzenleuchte Vollspektrum 120cm 50W IP44’, DEL-KO GmbH, Germany). After 4–10 days, the seedlings were carefully removed from the sand and cleaned with tap water. A total of 24 nymphs were analysed per plant species (four nymphs each from six different parental females) to ensure genetic variation of specimens.
The experiment was carried out in 11 x 8 x 5 cm plastic containers with 25 g of substrate [(3:1 parts mixture of Fruhstorfer Erde Typ P 25 (HAWITA Gruppe GmbH, Vechta, Germany) and sand (diameter 0–2 mm)]. All boxes were closed with lids, which had tiny holes inserted to allow gas exchange. Seedling roots were placed on top of the substrate to allow the nymphs direct feeding access. Boxes received two seedlings of the wheat and barley treatments and one seedling of the maize treatment. Each box was inoculated with a single nymph. Seedlings were replaced when the first signs of deterioration were observed or after a maximum of eight days. The substrate was kept moist and was replaced after four weeks. The experiment was carried out under controlled environmental conditions. During the first 160 days of the trial, the survival and development of the nymphs were evaluated every second day using a stereomicroscope. Due to slow development and low mortality in the higher nymphal instars, the evaluation was carried out twice per week, starting after 160 days. Nymphal development was evaluated by observation of moulting and measurement of head capsule width according to Pfitzer et al. (2022).

Statistical analysis
Statistical analysis was carried out using SAS 9.4 (SAS Institute Inc., Cary, USA). The total number of field-emerged *P. leporinus* adults per m² was analysed. The mean values of the emerged adults were calculated from three or four cages within each plot. Those means were then used for a two-way analysis of variance (ANOVA) and tested for their homogeneities of variance and normal distribution. Least square means were determined with LSMEANS in the PROC GLIMMIX procedure separately for each testing site to analyse for significant differences at the p < 0.05 significance level. The assumption of the homogeneity of the variances was not fulfilled in the field trials, where the role of soil tillage and cultivation of winter wheat was compared to spring wheat or to maize and bare soil. To fulfil the assumption of homogeneity of variances, the data were square root transformed to analyse for significant differences. The data were then back transformed to calculate the estimated mean values and standard errors in each treatment and for each testing site. For the results of the field trials, where the role of cultivation of winter wheat compared to barley was tested, the values were not transformed as the assumptions of their homogeneities of variance and normal distribution were fulfilled (SAS Institute Inc. 2022).

Cox proportional hazard models and PROC PHREG were used in SAS 9.4 for survival analysis of the no-choice oviposition experiment. Hazard ratios (HR) > 1 or < 1 describe the higher or lower probabilities of mortality compared to the referred treatment. To obtain a letter code in this experiment, survival was also analysed using PROC LIFEREG in SAS 9.4. The Weibull, exponential, and logistic distributions of the data were tested. The Akaike information criterion (AIC) was lowest for the Weibull distribution and assumed for this analysis. Least square means were determined with LSMEANS, to identify significant differences between the treatments at p < 0.05 (SAS Institute Inc. 2022).

Results

**Effect of soil tillage and crop rotation on adult emergence of *P. leporinus***
In field trials, the effects of different soil tillage practices (plough, disc harrow and cultivator) were measured, and five different cropping rotations (barley, spring wheat, maize or bare soil in comparison to winter wheat) with different sowing dates for the number of emerging *P. leporinus* adults, were tested.

**Role of cultivation of winter wheat compared to barley**

In this study, the effects on two different early sown spring crops (barley: sown between the end of February to the end of March, and spring wheat: sown between the end of February to mid-March) were evaluated. Within each site the emergence of adults was quite variable. On the two testing sites, the emergence of adults from barley was compared to winter wheat (Fig. 1). There were no differences in the beginning dates for the emergence of adults (3/6/19) and peaks (24/6/19) were shown between the treatments on both trial sites (Figs. 1a, 1b). The sampling period ended on 8/7/19 before the barley and wheat were harvested. For Untereisesheim 2019, a significantly lower adult emergence rate (-36.3%) was observed in barley, compared to winter wheat (Fig. 1c). In contrast, a 23.9% higher number of *P. leporinus* adults emerged from the barley treatment in Ladenburg 2019, after soil tillage with disc harrows; this was not significantly different from the wheat (Fig. 1d).

**Role of soil tillage and cultivation of winter wheat compared to spring wheat**

On the three field trial sites, cultivation of spring wheat was compared to winter wheat after soil tillage treatments by plough or cultivator (Fig. 2). The first adults were caught on 3/6/2019 in Dettenheim 2019, on 11/6/2019, in Arzberg 2019 and on 17/6/2019 in Kirchardt 2019 (Figs. 2a-c). For Arzberg 2019 and Dettenheim 2019, the highest rates of emerging adults were observed on 17/6/2019 in all treatments, except on 24/6/2019 in Dettenheim 2019 in the plough- spring wheat treatment. In Kirchardt 2019, the peak number of emerging adults was detected on 24/6/2019 in winter wheat and spring wheat after ploughing, and then on 1/7/2019 in winter wheat and spring wheat after soil tillage by cultivator. Adult sampling ended on 8/7/2019 in Dettenheim 2019 and Kirchardt 2019 and on 15/7/2019 in Arzberg 2019, when only very low numbers of emerging adults were detected.

In general, spring wheat cultivation did not lead to significant reductions in *P. leporinus* adult emergence. Only in Arzberg 2019, was a significant reduction (-60.7%) observed in spring wheat, after soil tillage with cultivator, compared to winter wheat (Fig. 2d). In Dettenheim 2019, no significant effects were observed due to soil tillage (Fig. 2e). A low adult emergence was observed in Kirchardt 2019 and this resulted in a low value (Fig. 2f).

**Role of soil tillage and cultivation of winter wheat compared to maize and bare soil**

*P. leporinus* adult emergence in maize was analysed on four field trial sites, representing a late sown (mid-April) spring crop, compared to winter wheat and on three of these sites in addition to bare soil (negative control), to analyse the absence of crops whose roots could be used as a food source for the nymphs.
The treatments were analysed after soil tillage treatment from ploughing or cultivator use (Fig. 3). On all field trials sites, the adult emergence from maize and bare soil were lower compared to winter wheat. The initial *P. leporinus* adult emergence varied between the different field trial sites (Fig. 3a-d): 4/5/2020 in Neckarsulm 2020, 12/5/2020 in Dettenheim 2020 and Ilvesheim 2020, and 31/5/20 in Arzberg 2020. In general, the peaks of adult emergence in the maize and bare soil treatments were observed to be earlier compared to the wheat treatments, then followed by a strong decline in the emergence of adults.

Consistent and significant reductions were observed on all field trial sites in the maize and bare soil treatments, compared to the winter wheat treatments. The strongest effects were observed in Neckarsulm 2020 (-98.3% and −98.9% adult emergence from maize and bare soil, respectively, compared to winter wheat, both after soil tillage by cultivator). Exceptions were observed in Arzberg 2020, where the plough-winter wheat treatment was not significantly different from all other treatments and in Dettenheim 2020, where no significantly different results were observed between the ploughed winter wheat and cultivator-bare soil treatments compared to all other treatments. In Arzberg 2020, Ilvesheim 2020 and Neckarsulm 2020, the reductions in maize compared to wheat were 83.8%, 88.3% and 79%, respectively, after ploughing and 88.5%, 92.4% and 86.2%, respectively, after soil tillage with a cultivator. In Ilvesheim 2020, the mean number of emerged adults was significantly higher in the plough-bare soil treatment, compared to the plough-maize and cultivator-maize treatments. In Neckarsulm 2020, the numbers of emerging adults were significantly higher in the plough-bare soil treatment, compared to all other maize and bare soil treatments. 

**Role of soil tillage**

The results from the figures shown above are described here, with a focus on soil tillage, to analyse the first hypothesis, that changes in soil structure due to soil tillage worsen the living conditions for the nymphs and, thus, reduce the number of emerging adults. From a total of seven trial sites, the effects of soil tillage (plough compared to cultivator) on *P. leporinus* adult emergence from winter wheat were analysed.

Variations in soil tillage were identified as a measure for *P. leporinus* control under practical conditions. In two of the seven field trial sites, significant reductions of emerging adults were observed in winter wheat after soil tillage with a plough compared with the cultivator (Dettenheim 2019: -87.9% and Neckarsulm 2020: -42%; Figs. 2e, 3f). On the other testing sites, no significant differences were shown between the winter wheat treatments, although the absolute reductions and differences in the temporal pattern between the soil tillage treatments were quite high. In Arzberg 2019 (Fig. 2d), Arzberg 2020 (Fig. 3e) and Dettenheim 2020 (Fig. 3h), the reductions observed of adults emerging from winter wheat after ploughing compared to cultivators were −39.3%, −44.4% and −58.4%, respectively. Ilvesheim 2020 was the only testing site where the numbers of emerging adults in winter wheat were higher after ploughing compared to cultivator use (+18.5%).

In Dettenheim 2019, the reduction in spring wheat after ploughing compared to the cultivator was 78.9%, although this effect was not significant (Fig. 2e). A significant reduction in effect after cultivator soil
tillage compared to ploughing was observed from bare soil in Ilvesheim 2020 and Neckarsulm 2020.

Based on the assumption that nymphs were distributed uniformly between the plots, the numbers of adults that emerged from winter wheat after soil tillage with cultivators were extrapolated to one/ha according to Bressan (2009). This was a highly variable between testing sites: The smallest populations were 2,261 adults per ha in Kirchardt 2019 and 33,598 per ha in Dettenheim 2020. The largest populations were found in Untereisesheim 2019 (601,435 per ha) and Neckarsulm 2020 (402,017 per ha).

**Performance of first instar nymphs on different plant species in a no-choice experiment**

The performance of *P. leporinus* was assessed on three different crop plants (wheat, barley, and maize). The strongest effects on survival were observed at the beginning of the trial (Fig. 4). After eight days, the survival rates already showed strong differences and were 91.7% in wheat, 87.5% in barley and 12.5% in maize. After 300 days, the survival rate was 66.7% in wheat, 29.2% in barley and 4.2% in maize. The mortality rates of nymphs kept on barley (HR: 2.6, Cox proportional hazard model, p = 0.0254) and maize (HR: 10, Cox proportional hazard model, p < 0.0001) plants, were each significantly higher compared to nymphs kept on wheat. The mortality rates of nymphs kept on maize were also significantly higher (HR: 3.7, Cox proportional hazard model, p = 0.0001) compared to barley.

The development of the different *P. leporinus* life stages on the plant species was analysed in detail and the results are shown in Fig. 5. After 169, 121 and 158 days the first adults were observed in wheat, barley, and maize, respectively. Despite the survival rate of nymphs being lower in barley compared to wheat, the development in barley was slightly faster. On maize seedlings, only 8.3% of the specimens reached the second or higher nymphal instars. In addition, most deaths in nymphs on barley occurred in the first and second nymphal instars, whereas instars three to five were less vulnerable.

**Discussion**

SBR is a fast-spreading bacterial disease that poses a serious threat to the sugar beet industry. Hence, there is a strong need for the development of vector control measures. So far, the only vector control approaches described have been from agronomical measures, due to the variations in sugar beet cropping rotations or soil tillage, which were reported from a single field site by Bressan (2009). In our experiments, the experimental setups were able to show clear effects due to soil tillage and cropping rotation. This study aimed to analyse if disturbances in the nymphal habitats due to soil tillage or missing or unsuitable food conditions worsened the development of *P. leporinus* nymphs.

This paper is the first report of *P. leporinus* control using agricultural measures (maize or bare fallow) through reproducible results demonstrated in different environments (three and four trials sites). These findings were supported by a no-choice experiment under controlled environmental conditions and suggests that maize might be a suboptimal host. In addition, variations in soil tillage after sugar beet harvests also led to reduced numbers of emerging adults.
The first hypothesis was that changes in the soil structure due to soil tillage worsened the living conditions for the nymphs and this reduced the number of emerging adults. It can only be speculated about possible reasons for the effects caused by soil tillage. The reduction effects of reduced tillage (10–15 cm depth for disc harrows) compared to mouldboard ploughing (25 cm depth) after sugar beet harvests, as reported by Bressan (2009), were not confirmed in this study. However, reduced tillage was carried out using a rigid tine cultivator (20 cm maximum tillage depth) in our experiments. Mouldboard ploughing (20 cm minimum tillage depth) could probably transfer sugar beet harvest residuals into deeper soil layers or induce microbial degradation of harvest residuals and this may have led to the significantly reduced numbers of *P. leporinus* adults that emerged from winter wheat after ploughing compared to cultivator tillage for two of the seven field trial sites. Furthermore, ploughs and cultivators were used at different tillage depths, so it cannot be shown whether the effects observed were caused more from the effect of soil tillage than the tilling depths. We did not identify any soil tillage practices that gave consistent results in all field trial sites and so we conclude that the reduction effect of soil tillage against *P. leporinus* depends more on the individual field and soil conditions or the soil structure (e.g., pores or cavities in the soil), which need to be analysed in detail in further studies.

Generally, the highest adult emergences in the field trials (up to 601,435 adults per ha), and the highest survival and development rates to the adult stage in the wheat treatments, underline previous findings that wheat is a good host plant species for *P. leporinus* nymphs (Bressan 2009, Pfitzer et al. 2022). Spring wheat cultivation did not result in consistently reduced numbers of adults emerging. Therefore, we can conclude that the delayed sowing of spring wheat (end of February to mid-March) did not lead to possible starvation of the nymphs and, thus, did not lower the numbers of adults that emerged (second hypothesis) compared to winter wheat cultivation (sowing: October/November). The reduced adult emergences observed on one of the two trial sites from the barley treatments and the lower performance of *P. leporinus* nymphs on barley seedlings, may indicate that barley seedlings are less favourable hosts for first instar nymphs. However, the strong effects (~80% of adult emergences from barley compared to winter wheat), reported from a single location by Bressan (2009), were not confirmed in this study. This underlines the necessity to re-investigate this hypothesis in additional environments.

The lowest survival rate and adult emergence of *P. leporinus* first instar nymphs on maize seedlings in our laboratory experiment led to the conclusion that maize seedlings are poor hosts. However, we are aware of the caveat that in the field, the nymphs feeding on maize roots would be older. In further studies the performance of older nymphal instars need to be evaluated to support the conclusion that maize is generally a poor host for *P. leporinus* development. Planthopper control of species with aboveground or sub-soil nymphal stages due to cultivation of non-host plant species or keeping land free from vegetation has been described in earlier literature. For instance, *Nilaparvata lugens* (Hemiptera: Delphacidae), a major pest in rice, was reared on 34 different weed and grass species (Oka 1979). It was found that on most plant species the planthopper produced nymphs but nearly all died. Hence, it was concluded that cultivation of non-host plant species or bare fallow land were measures for sustainable vector control. *Myndus crudus* (Hemiptera: Cixiidae) is a vector in the lethal yellowing of palms and the nymphs feed on the roots of grasses. Howard (1990) identified that certain grass species reduced the number of emerging
adults when sown in field trials under coconut palms and in container trials, in comparison to St. Augustine grass (*Stenotaphrum secundatum*) that was used as the control. Significant reductions in adult plant hopper and leaf hopper (Auchenorrhyncha) specimens per m² in alfalfa were also shown by Lamp et al. (1984) when grass herbicides where applied which reduced the biomass of grass weeds as host plants.

Interestingly, maize was the last-sown (mid-April) spring crop in the field trials and no clear differences were observed between bare fallow and maize. These findings lead to the question of whether late sown spring crops in general have the potential to reduce *P. leporinus* populations, due to possible starvation of the nymphs. To avoid bare soil after sugar beet harvest, which could lead to increased erosion and does not lead to an income for the farmer, break crops could be cultivated after sugar beet harvests and before late sown crop species are established. In further studies, the reduction effects and host suitability of break crops, or potential crops, cultivated after sugar beet harvests need to be analysed to identify further non-host plant species, which can be integrated into sugar beet cropping rotations for control of *P. leporinus*. Although possible, maize cultivation in sugar beet crop rotations can lead to several problems. For example, the intensity of late root rot disease caused by *Rhizoctonia solani* can be increased when maize is integrated into sugar beet crop rotations and this can harm sugar yields (Buhre et al. 2007).

A low number of *P. leporinus* adults also emerged from the bare soil treatment. Possible explanations are that the harvest residuals from sugar beet tap roots or weed roots were food sources for the nymphs. When the cages were set up in the plots (mid-April 2020), at some places in the soil, nymphs were found near sugar beet harvest residuals (not shown). In addition, in a laboratory rearing, *P. leporinus* nymphs exclusively developed on sugar beet tap roots (Ptzer et al. 2022). Weeds can be almost excluded as a possible food source for the nymphs since the plots were kept weed-free with herbicides. Further studies should evaluate the role of sugar beet harvest residuals and weeds for the nymphal development of *P. leporinus*, e.g., by adding harvest residuals to the plots or comparison with weed infested bare soil.

In summary, this study provides information about *P. leporinus* management options in sugar beet cropping rotations and the host suitability of different crop species for *P. leporinus* nymphs. We conclude, that *P. leporinus* control should focus on the cultivation of non-host plant species or late sown spring crop species after sugar beet cultivation. Additionally, the observed effects from ploughing need to be further investigated. The general aims should be to worsen the conditions for nymphal development and to identify poor host plant species for *P. leporinus* to avoid providing a food source for the nymphs through agricultural practices.

**Declarations**

**Funding** This research was funded by Kuratorium für Versuchswesen und Beratung im Zuckerrübenanbau (Mannheim, Germany), the Institute of Sugar Beet Research (Göttingen, Germany), and the Division of Agricultural Entomology (Göttingen).

**Conflict of interest** All authors declare that there are no conflicts of interest.
Financial interests The authors have no relevant financial or non-financial interests to disclose.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by René Pfitzer, Patrick Häußer mann, Thilo Häuser and André Rinklef. Michael Rostás, Klaus Schrameyer, Ralf T. Voegele, Johann Maier and Mark Varrelmann supplied materials for the experiments and René Pfitzer, Patrick Häußer mann, Thilo Häuser and André Rinklef analysed the results. The first draft of the manuscript was written by René Pfitzer and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of data and material Research data are not shared.

Ethics approval All applicable international, national and institutional guidelines for the care and use of animals were considered in the present investigation.

Informed consent All authors of this manuscript accepted that the paper is submitted for publication in the Journal of Pest Science and reported that this paper has not been published or accepted for publication in another journal, and it is not under consideration at another journal.

Acknowledgements This research was funded by Kuratorium für Versuchswesen und Beratung im Zuckerrübenanbau (Mannheim, Germany), the Institute of Sugar Beet Research (Göttingen, Germany), and the Division of Agricultural Entomology (Göttingen). We thank Bianca Tappe, Chelsea Schreiber, Gül sen Aydin, Jonas Watterott, Jutta Schaper (Department of Agricultural Entomology, Göttingen) and Erik Fleischer, Gina Preuss, Laura Schott, Matthias Zöller, Philipp Zeier, Veit Nübel and Werner Stohr (Südzucker AG, Mannheim) for technical support. We are grateful to Bernd Kindler (Agrargenossenschaft Arzberg eG, Arzberg, Germany), Achim Roth (Dettenheim, Germany), Steffen Linnenbach, Thomas Maas (both Ladenburg, Germany), Karlheinz Horn (Neckarsulm, Germany) and Eduard Steigerwald (Südzucker AG, Mannheim), for setting up the experiments on their fields.

References


Figures
Temporal patterns of Pentastiridius leporinus adult emergence at the trials sites: (a) Untereisesheim; and (b) Ladenburg in 2019. Treatments were CW = cultivator + winter wheat, CB = cultivator + barley, DW = disc harrow + winter wheat, DB = disc harrow + barley. Figures (c+d) show total mean numbers of emerged Pentastiridius leporinus depending on trial site and treatment. Bars indicate standard errors.
Mean values with the same letter within a site are not significantly different at \( p < 0.05 \), based on two-way ANOVA.

**Figure 2**

Temporal patterns of *Pentastiridius leporinus* adult emergence in the trials sites in 2019: (a) Arzberg; (b) Dettenheim; and (c) Kirchardt. Treatments were PW = ploughing + winter wheat, CW = cultivator + winter wheat, PS = ploughing + spring wheat and CS = cultivator + spring wheat. Figures (d-f) show the estimated total mean numbers of emerged *Pentastiridius leporinus* depending on trial site and treatment. Bars indicate standard errors. Mean values with the same letter within a site are not significantly different at \( p < 0.05 \) based on two-way ANOVA. The y-axis scales in the graphs of Kirchardt in 2019 (c, f) differ from the graphs of the other trial sites due to lower numbers of that adults emerged.
Figure 3

Temporal patterns of *Pentastiridius leporinus* adult emergence in the trial sites: (a) Arzberg; (b) Neckarsulm; (c) Ilvesheim; and (d) Dettenheim in 2020. Treatments were PW = ploughing + winter wheat, CW = cultivator + winter wheat, PM = ploughing + maize, CM = cultivator + maize, P- = ploughing + bare soil and C- = cultivator + bare soil. Figures (e-h) show estimated total mean numbers of emerged *Pentastiridius leporinus* depending on the trial site and treatment. Bars indicate standard errors. Mean
values with the same letter within a field trial site are not significantly different at \( p < 0.05 \) based on two-way ANOVA. The y-axis scales in the graphs of Dettenheim 2020 (d, h) differ from the graphs of the other trial sites due to lower numbers of adults emerged.

**Figure 4**

Survival of Pentastiridius leporinus first instar nymphs on seedlings of wheat, barley, and maize \((n = 24)\). Survival rates with the same letter were not significantly different at \( p < 0.05 \), based on least square means that were determined with LSMEANS in PROC LIFEREG in SAS 9.4.
Figure 5

Development of Pentastiridius leporinus first instar nymphs on wheat, barley, and maize (n = 24)

Supplementary Files

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