The biology of *Cixius wagneri*, the planthopper vector of 'Candidatus *Phlomobacter fragariae*' in strawberry production tunnels and its consequence for the epidemiology of strawberry marginal chlorosis

Salar; P.[1]; Danet, J.-L.[1]; Pommier, J.-J.[2]; Foissac, X.[1]
[1]UMR1090, INRA and Université Bordeaux2, BP81, F33883, Villenave d'Ornon, France
[2] Hortis Aquitaine, Maison Jeannette, F-24140, Douville, France

Abstract

«Candidatus *Phlomobacter fragariae*» is the prevalent agent of strawberry marginal chlorosis (SMC) and is transmitted by the planthopper *Cixius wagneri*. Because the insect vector biology was unknown, a field experiment was set up to determine if it was able to reproduce on strawberry plants, to determine the number of insect generations per year and the ability of nymphs to transmit SMC. During spring 2004, 80 *C. wagneri* adults were delivered into 4 small insect-proof tunnels containing 30 healthy plants. Fifteen percent of the delivered insect population were carrying the pathogen. In October 2004, only 3 young L1 instar nymphs were found in the first tunnel, demonstrating there were no new insect generations during the summer. In April 2005, 330 *C. wagneri* of early L1 to late L5 nymph instars were collected at the roots of the plants,clearly indicating that a single insect generation had overwintered as larvae andemerged at the following spring. All instars were shown to carry 'Ca. *P. fragariae*' (70 to 75 % of the larvae) and were able to transmit SMC as assessed by transmission assays. An insecticide treatment was applied in March 2005 in a third tunnel and a fourth tunnel was kept as a control. More than 120 *C. wagneri* adults were collected in the control tunnel 4 in June 2005 confirming that an insect generation arose in the tunnel, whereas no insects could be found in the treated tunnel 3. All plants were kept for two years, surveyed for symptom expression and tested for 'Ca. *P. fragariae*' infection by 16S-PCR. Results indicated a reduced mortality and SMC incidence in tunnel 3, and a higher mortality and SMC incidence in tunnel 2 than in tunnel 1, attesting that *C. wagneri* larvae had spread SMC and that an early insecticide treatment could control the disease.

Keywords: Phloem-restricted bacteria, planthopper, insect vector, *Fragaria x ananassa*

Introduction

Marginal chlorosis has affected strawberry production in France since the early eighties (Nourrisseau et al., 1993). A phloem-restricted uncultured bacterium, "Candidatus *Phlomobacter fragariae*" is associated with the disease (Zreik et al., 1998). A large survey conducted from 1996 to 2001 for marginal chlorosis in French strawberry production fields and nurseries revealed that symptoms of SMC were most frequently induced by "Ca *P. fragariae*" in strawberry production tunnels but that the stolbur phytoplasma could also cause SMC in nurseries (Danet et al., 2003). Whereas the transmission of stolbur phytoplasma to strawberry plants is certainly achieved by its main planthopper vector *Hyalesthes obsoletus* (Fos et al., 1992), «Ca. *P. fragariae*» is transmitted in production tunnels by the planthopper *Cixius wagneri* (Danet et al., 2003). To investigate the ability of *C. wagneri* to grow in strawberry production tunnels and of *C. wagneri* larvae to transmit «Ca. *P. fragariae* », we intended to establish infectious *C. wagneri* populations in insect-proof mini-tunnels and recover infectious *C. wagneri* nymphs. The efficiency of an early insecticide treatment to reduce insect vector population and disease spread was also evaluated.

Material and methods

Design of tunnel experiments:Four small insect-proof tunnels were installed in April 2004 in a 6 meter wide plastic tunnel under normal production conditions. Each tunnel contained 30 healthy strawberry plants of the cultivar 'Cijosée'. In May and June 2004, *C. wagneri* were captured in organic tunnels and groups of insects were introduced, with an equal sex ratio, into the 4 insect-proof mini-tunnels. A total of 80 insects were introduced in each tunnel. Twenty insects, representative of the collected population were kept for "Ca P. fragariae" detection. After a period of 4 months (Tunnel 1, October 2004), 10 months (Tunnel 2, April 2005) and 12 months (Tunnel 3 and 4, June 2005), insects were collected using D-Vac aspiration, and plants were pulled out and their root system and surrounding soil examined for the presence of *C. wagneri* larvae. Finally, plants were planted in individual pots, sprayed with insecticide and kept in a greenhouse for 18 months. Mortality and symptoms were recorded after 12 and 18 months. Plants were submitted to "Ca. *P. fragariae*" detection after 12 months of incubation. Tunnel 3, received a single insecticide treatment with endosulfan (organochlorine) in March 2005.

"Ca. *P. fragariae*" transmission assays: *C. wagneri* larvae collected in tunnel 2 in April 2005 were caged on a healthy strawberry plant in groups of 10 (L1-L2 larvae) or 20 (L3, L4-L5 larvae) until all insects died. Plants were then kept in the greenhouse, examined for SMC symptoms and PCR tested for "Ca. *P. fragariae*" infection.

"Ca. *p. fragariae*" detection in plants and insects: Nucleic acids were extracted from strawberry plants and from individual insects as described previously (Maixner et al., 1995). Primers

Fra4 (5' CTCCTCTGTCTCTAAAGG-3') and Fra5 (5'-AGCAATTGACATTAGCGA-3')

from the 16S rDNA sequence of "Ca. *P. fragariae*" were used for the amplification of DNA extracted from strawberry plants under the following conditions: 35 cycles of 1 min at 92 °C, 1 min at 52 °C, and 1 min at 72 °C (Zreik et al., 1998). The amplified DNA was visualized under UV light after electrophoresis on 1 % agarose gels stained with ethidium bromide. Spot-PCR was carried out for "Ca. *P. fragariae*" detection in *C. wagneri* as previously described (Foissac et al., 2000).

Results

Fifteen percent of the *C. wagneri* population introduced into the small tunnels was infected with "Ca *P. fragariae*" as revealed by Spot-PCR detection performed on 20 individuals randomly selected from the collected populations. In October 2004, only 3 larvae of early development stage L1 and an old adult were found at the upper part of the root system. Therefore no C. wagneri summer generation had emerged at fall. In April 2005, 80 larvae of early L1-L2 stages, 110 larvae of L3 stages and 140 larvae of late L4-L5 stages were collected on the soil just under the plastic cover. This result indicated that a single C. wagneri generation is overwintering as eggs or young larvae and is emerging at spring under the production tunnel. Infectivity of insects was assessed by transmission assays to strawberry plants. Six of the 8 plants caged with 10 larvae of stages L1-L2 developed SMC after 6 months and 7 out of 8 tested positive for "Ca *P. fragariae*" infection. Similarly, 5 plants out of 6 that had been caged with 20 L3 larvae finally developed SMC and were infected by "Ca *P. fragariae*". All the 6 plants caged with 20 L4-L5 larvae developed SMC and were infected by "Ca *P. fragariae*". Insects dead during the transmission period were collected and submitted to "Ca *P. fragariae*" PCR detection. It revealed that 70 % of the L3-L4 larvae (developed from L1 & L2), 75 % of L4-L5 larvae (developed from L3) and 19 % of adults (developed from L4 & L5) were infected with "Ca. *P. fragariae*".

In June 2005, no *C. wagneri* could be found in the insecticide-treated tunnel 3, whereas 120 *C. wagneri* adults were captured in the untreated Tunnel 4. This confirmed that *C. wagneri* can reproduce and develop on strawberry plants under production conditions and that an early insecticide treatment can control *C. wagneri* population. In June 2006, 9 plants of the 30 plants of tunnel 3 tested positive for "Ca *P. fragariae*" infection and only 3 % of the plants died after 18 months, while 17 plants of the 30 plants of the control tunnel 4 tested positive for "Ca *P. fragariae*" infection and 50 % died after 18 months.

Discussion

In conclusion, the planthopper *C. wagneri* reproduces on strawberry plants under tunnels in the French strawberry production system. As *C. wagneri* is the vector of "Ca. *P. fragariae*", one of the bacterial agents of SMC, its ability to multiply on strawberry plants explain the epidemic spread of SMC on the crop. One insect generation occured per year and insects overwintered as larvae on plant roots under their plastic covers. Larvae of early stages were infectious and could efficiently transmit the disease. The high proportion of larvae infected by "Ca. *P. fragariae*" was certainly due to the acquisition of the proteobacterium during winter when larvae were feeding on the roots of the infected strawberry plants. However, we cannot exclude the possibility of transovarial transmission of "Ca. *P. fragariae*" by infected females to their progeny. It was also shown that a single insecticide treatment targeting the nymphal stage efficiently reduces the insect population and disease spread. Other means such as biological control of the insect vector should also be investigated in order to provide alternative measures compatible with integrated pest management.

Acknowledgements

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