Interactions between *Polymyxa betae* and plant systemic defense ways

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Abstract

Polymyxa betae is the vector of *Beet necrotic yellow vein virus* (BNYVV), the causal agent of sugar beet rhizomania disease. Because of the widespread use of cultivars partially resistant to BNYVV, resistance breaking BNYVV isolates have been reported. In order to develop alternative control strategies, we investigated interactions between *P. betae* and plant defenses. A first set of bioassays was conducted in order to assess *P. betae* infection after the elicitation of inducible defenses in sugar beet. The systemic acquired resistance (SAR) or the induced systemic resistance (ISR) was induced by a treatment of the plants with an analogue of the salicylic acid (SA) or with lipopeptides (LPs) from *Bacillus amyloliquefaciens*, respectively. While *P. betae* infection, quantified by real time PCR, was not reduced in plants treated with SA, the intensity of infection was significantly lower (up to 95 % of reduction) in plants after a treatment with LPs. LPs were shown to effectively induce systemic resistance in both roots and leaves of sugar beet, resulting in a reduction in *P. betae* infection. The absence of any effect of SAR against *P. betae* could be explained by the coevolution of *P. betae* and sugar beet, allowing the biotrophic endoparasite to bypass self-induced SAR. This hypothesis was validated in another bioassay. SAR was efficiently induced in sugar beet after the infection by *P. betae*, allowing the plants to resist (75% reduction) to a further infection by *Cercospora beticola*. These results suggest that *P. betae* has to be considered not only as a target but also as an actor of plant defenses.

Polymyxa betae, sugar beet and rhizomania

Polymyxa betae (Keskin, 1964) is a protist which belongs to the family of plasmodiophorids. Some members of this family are important pathogens on major crops, as *Plasmodiophora brassicae,* the causal agent of the brassicaceae clubroot and *Spongospora subterranea,* cause of powdery scab of potato. *Polymyxa* is known for its ability to transmit viruses. *Polymyxa betae* is the only known vector of *Beet necrotic yellow vein virus* (BNYVV), the causal agent of rhizomania in sugar beet.

BNYVV has caused major reductions in root yields (up to 80 %) and quality. Up to now, only resistant/tolerant varieties are used to control the disease. However, only few resistance or tolerance genes are known, and resistance breaking isolates of the virus appeared these last years.
In this work, we investigated the relationships between *P. betae* and plant defenses, in order to assess the potential of plant defense based control methods against rhizomania of the sugar beet.

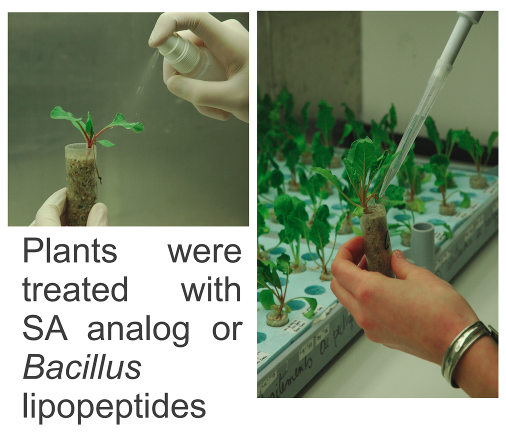
3D view of a *P. betae* zoosporangium

Typical symptoms of rhizomania: main root restriction and lateral rootlets proliferation.

Effect of plant defenses elicitation on *P. betae* infection

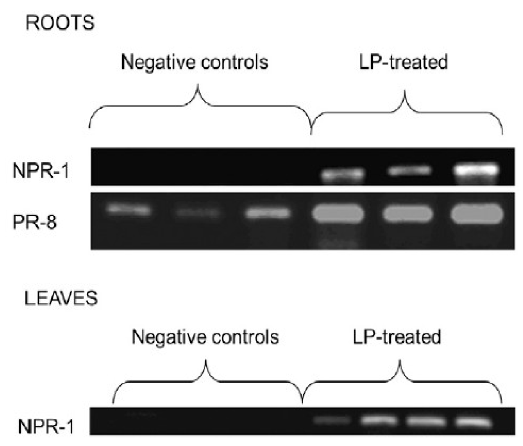
Sugar beets grown in glass tubes

Elicitation →



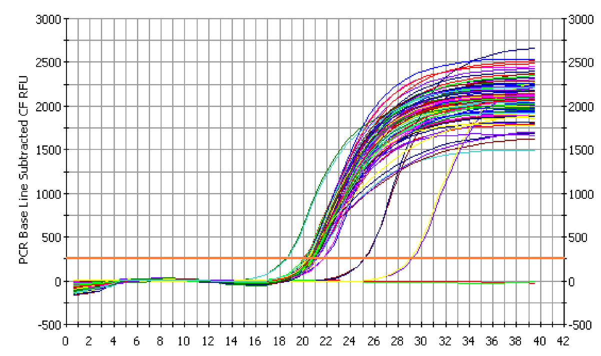
Plants were treated with SA analog or *Bacillus* lipopeptides

Inoculation of *P. betae*, five weeks after sowing. At this time, plant defense elicitation was assessed.



Expression of nonexpressor of pathogenesis-related (PR) genes 1 (NPR-1) and PR-8 in roots and leaves of sugar beet plants, treated or not with lipopeptides previously.

Quantification of *P. betae* by quantitative PCR, one week after inoculation



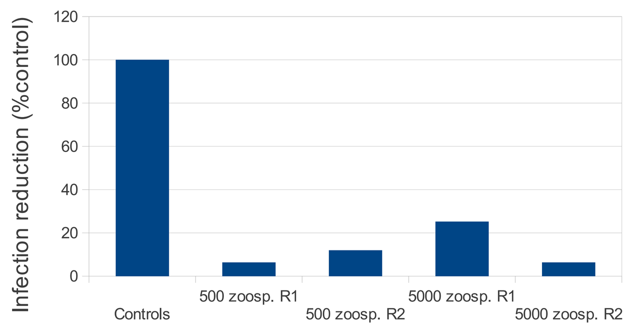
The qPCR targets the ITS region of *P. betae*. These data were normalized with Cq values for the sugar beet glutamine synthetase

RESULTS

No effect was observed on *P. betae* infection after SAR elicitation. On the contrary, plants elicited by Bacillus lipopeptides exhibited strong repression of *P. betae* infection: up to 93 %. Systemic resistance induced by Bacillus lipopeptides thus drastically reduced the infection of sugar beet (Desoignies et al., 2013).

Experiment	Zoospores inoculated	Difference in *P. betae*-normalized C_q	Mean sugar beet C_q	ΔCycles	Reduction factor	Reduction rate
1	500	0.195	20.436	3.98	15.78	93.66
1	5000	0.151	20.32	3.07	8.39	88.08
2	500	0.092	21.6	1.99	3.97	74.82
2	5000	0.169	21.61	3.65	15.67	93.61

The reduction rate is reported as the difference between the *P. betae*-normalized C_q of treated plants and the *P. betae*-normalized C_q of the controls multiplied by the mean of the *Beta vulgaris* C_q. It represents the standardized difference in C_q (ΔCycles). At each amplification cycle, the DNA quantity is multiplied by two. The reduction factor can be obtained as $2^{\Delta Cycles}$. Finally, the reduction rate is obtained b: reduction rate = 1 − 1/reduction factor.



Regarding to the untreated controls, the reduction of infection of sugar beet by *P. betae* was ranged between 74 and 93 % of reduction (500 zoospores) and 88 and 93 % of reduction (5000 zoospores).

As some studies evidenced a direct action of lipopeptides against protists (Jousset et al., 2006), putative direct activity of lipopeptides on zoospores was assessed. No statistical difference was found.

Concentration of cLPs	*n*	Luciferase activity (relative quantification of luminescence)	
		Mean	Standard deviation
0 mg/L	5	31 272.31	8735.19
30 mg/L	5	40 317.13	8243.71
60 mg/L	5	31 469.70	7926.97
120 mg/L	5	32 416.49	6708.03

The viability of zoospores was measured throughout the ATP quantification process in a solution containing the same number of zoospores, but increasing concentrations of lipopeptides.

Eliciting abilities of *P. betae*

Previous research showed that plants infected by *P. betae* exhibited a higher expression of PR1-a, a classical marker of the SAR induction. As *P. betae* is asymptomatic and apparently costless for the plant, we wondered if the protist could act as a sugar beet defenses elicitor and help the plant to prevent infection by harmful pathogens.

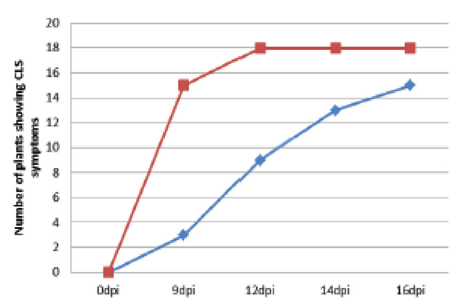
EXPERIMENTAL DESIGN

t_0 — Seeds were sown in glass tubes in sterile quartz (n =18).

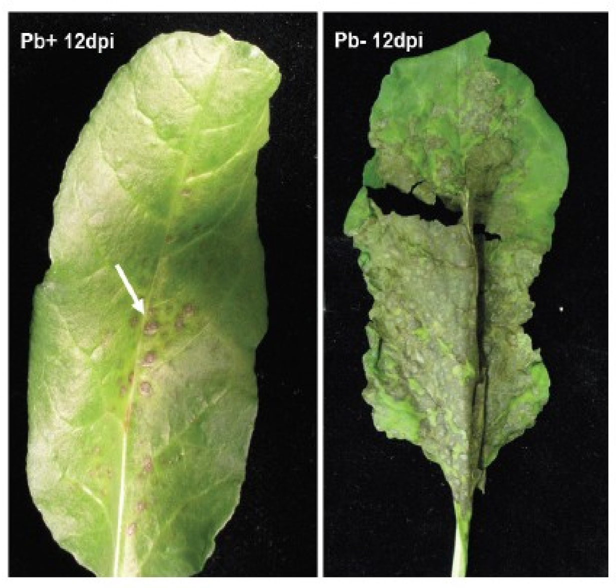
t_1 = 5 weeks — Each test plant was inoculated with 5,000 zoospores. Control plants were treated with the same quantity of Hoagland solution.

t_2 = 7 weeks — Sugar beet plants were challenged with *Cercospora beticola*, a foliar sugar beet pathogen.

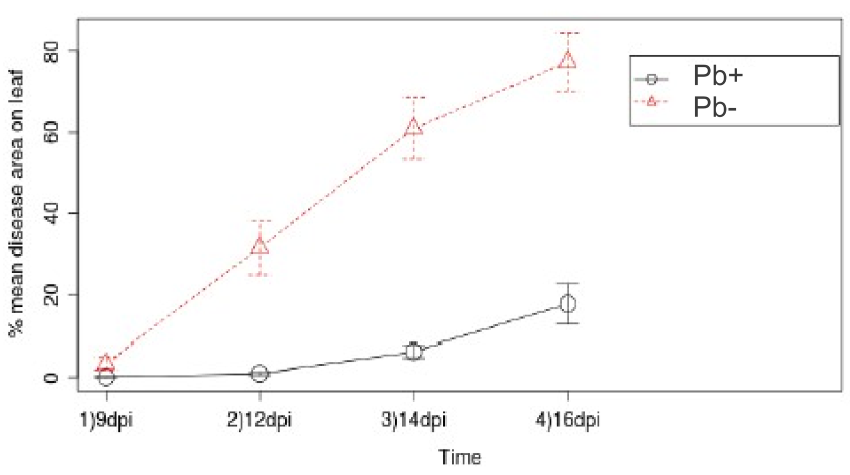
t_3, t_4, t_5 and t_6 — The progress of the Cercospora Leaf Spot was assessed on each plant 9, 12, 14 and 16 days post-*Cercospora* infection.



Progress of CLS incidence on plants previously inoculated (blue) with *P. betae* (Pb+) and on plant non-inoculated (red) with *P. betae* (Pb-).
Nine days after the inoculation of *C. beticola*, the incidence of CLS in *P. betae* inoculated plants was clearly higher than in *P. betae* non-inoculated plants. Twelve days after *C. beticola* inoculation, all Pb- plants showed CLS symptoms. At the end of the experiment (16 dpi), three Pb+ plants remained asymptomatic.



CLS symptoms observed on sugar beet leaves, 12 days after *Cercospora beticola* inoculation, on plants previously inoculated (Pb+) or not (Pb-) with *P. betae.*



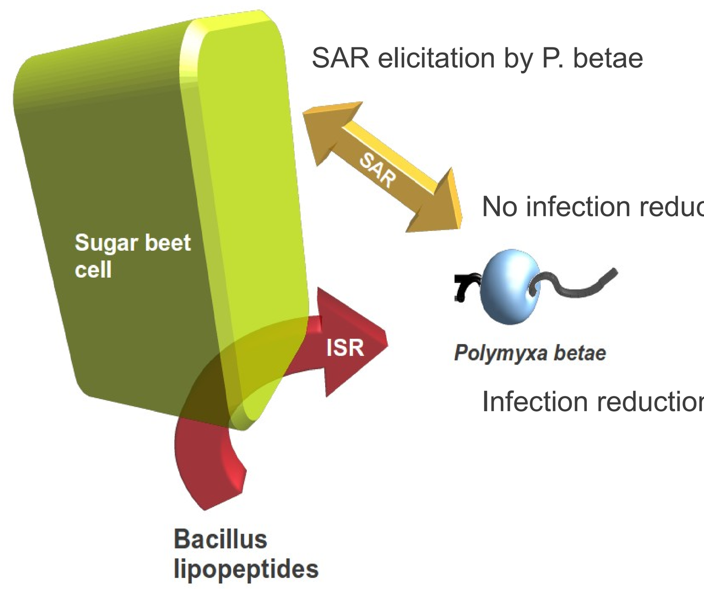
Mean of disease area of Cercospora leaf spot on the most damaged leaves which were previously infected by *P. betae* (circles) or not (triangles). Disease reduction (%) is shown in the table below. Reduction is calculated as 1-(mean disease area Pb+/mean disease area Pb-)

0 dpi	9 dpi	12 dpi	14 dpi	16 dpi
0	98.5	97.2	89.64	77.11

The image analysis with the ASSESS software (Lamari, 2002) confirmed the macroscopic observation. Statistical analysis evidenced differences in the mean percentage of diseased area at each time of observation. The difference was important after nine days (98 % disease reduction in Pb+ plants) and decreased over time to 77 % of disease reduction 16 dpi.

Regarding to the dynamics of disease progress at each time, especially numbers of symptomatic plants and CLS progress leaf by leaf (data not shown), the *P. betae* inoculation prior to *C. beticola* seemed to had double effect: *C. beticola* infection was delayed and disease progression rate reduced. These results could be explained by a SAR response, with thickening of cell walls (delay of infection) and antimicrobial compounds production (reduced progression rate). This hypothesis was strengthened by overexpression of PR proteins after *P. betae* infection.

Discussion



The plant can drastically reduce *P. betae* infection through ISR. Nevertheless, SAR has no impact on the protist. It is quite logical : as SAR is induced by *P. betae*, this one acquired during coevolution resistance mechanisms against SAR products. This is not the case for ISR.
The eliciting abilities bear thus that the protist is an ancient pathogen. Nowadays both plant and protist live together in an apparent peaceful interaction, but evidences of the ancient pathogenicity remain.
Nevertheless this « peaceful » interaction of commensalism is endangered in the nature by the frequent presence of BNYVV. In this view, the impact of *Bacillus* lipopeptides treatment offers new possible way of rhizomania control. The maintaining of the vector population at low level combined with the use of tolerant varieties could reduce the selection pressure on BNYVV and increase the durability of tolerance genes.
This putative impact has to be evidenced by using the complete pathosystem *P. betae*-BNYVV-sugar beet under controlled conditions and in field assays.

References

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