7. Herbivore-induced resistance: differential effectiveness against a set of microbial pathogens in *Arabidopsis thaliana*

De Vos M.[1], Van Oosten V.R.[1,2], Van Pelt J.A.[1], Van Loon L.C.[1], Dicke M.[2] and Pieterse C.M.J.[1]
[1]Section Phytopathology, Faculty of Biology, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands. [2]Laboratory of Entomology, Wageningen University, P.O. Box 8031, 6700 EH Wageningen, The Netherlands; e-mail: M.deVos@bio.uu.nl; http://www.bio.uu.nl/~fytopath

Abstract

Plants possess inducible defence mechanisms to protect themselves against different types of microbial pathogens and herbivorous insects. Defences induced against pathogens and insects are often incompatible. A major question in plant defence research is: how are plants capable of integrating signals induced by either microbial pathogens or insects into defences that are specifically active against the attacker? Three plant signalling molecules play a dominant role in the regulation of defences against both microbial pathogens and insects: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). Cross-talk between SA-, JA- and ET-dependent signalling pathways is thought to be involved in fine-tuning the defence reaction, leading to activation of an optimal mix of defences to counteract the intruder. Here we studied the effect of herbivore-induced resistance in *Arabidopsis thaliana* against a range of microbial pathogens.

Introduction

In *Arabidopsis*, the signal molecules JA, ET and SA have been shown to play important roles in defence against both microbial pathogens and herbivorous insects [2]. Therefore, it is postulated that resistance against insects and pathogens functions partly *via* similar defence signalling pathways. In this study, we examined the spectrum of effectiveness of herbivore-induced resistance against a set of microbial pathogens. To this end, *Arabidopsis* plants were infested by larvae of the cabbage white butterfly *Pieris rapae*. Subsequently, we monitored the production of JA, ET and SA in time and tested the effectiveness of *P. rapae*-induced defence against the bacterial leaf pathogens *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) and *Xanthomonas campestris* pv. *armoraciae* (*Xca*), the fungal leaf pathogen *Alternaria brassicicola*, and the viral pathogen Turnip crinkle virus (TCV).

Signal signature of *Pieris rapae*-induced *Arabidopsis*

To activate herbivore-induced resistance, five first-instar (L1) larvae of *P. rapae* were allowed to feed for 48 hours on 5-week-old *Arabidopsis* Col-0 plants as described previously [6] (Fig. 1A). Subsequently, the production of JA, ET and SA was monitored as described previously [3]. Feeding by *P. rapae* resulted in a slight increase in ET production and a significant increase in the level of JA, while SA levels did not differ from control plants (Table 1). Furthermore, analysis of the mRNA levels of the SA-, JA- and/or ET-responsive genes *PR-1*, *VSP2*, *PDF1.2* and *HEL* revealed that *P. rapae* feeding predominantly activates a JA-dependent signalling pathway (Fig. 1B).

Table 1. JA, ET and SA accumulation in response to infestation by 5 first-instar larvae of *P. rapae*

Signal molecule	Treatment	Hours after infestation			
		0	12	24	48
JA, ng/g FW	Control	7.9	8.7	9.9	5.5
	P. rapae	7.9	49.5	25.3	104.9
ET*), nl/g FW	Control	0	29.8	31.9	44.9
	P. rapae	0	39.0	43.9	63.9
SA, ng/g FW	Control	155.9	47.2	93.8	60.4
	P. rapae	155.9	43.3	92.4	63.1

*) Cumulative ET production in time.

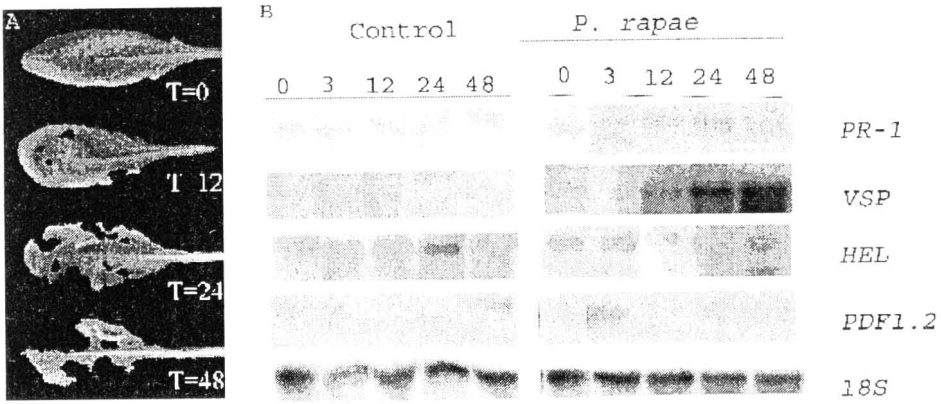

Fig. 1. A: Damage caused by *P. rapae* during 48 hrs of feeding. B: Transcript levels of SA-responsive *PR-1*, JA-responsive *VSP2*, ET-responsive *HEL and* JA/ET-responsive *PDF1.2* in control and *P. rapae* infested plants.

Local herbivore-induced resistance against bacterial pathogens

To assess the effectiveness of *P. rapae*-induced defence against the bacterial pathogens *Pst* and *Xca*, *P. rapae* L1 caterpillars were allowed to feed for 24 hours on the leaves of 5-week-old Col-0 plants. Subsequently, the larvae were removed and the leaves were challenge inoculated with either *Pst* or *Xca* as described previously [5]. Three days later, the severity of the disease symptoms were assessed for both local (= primary damaged) and systemic (= induced, undamaged) leaves. Fig. 2 shows that *P. rapae*-induced defence resulted in a significant reduction of

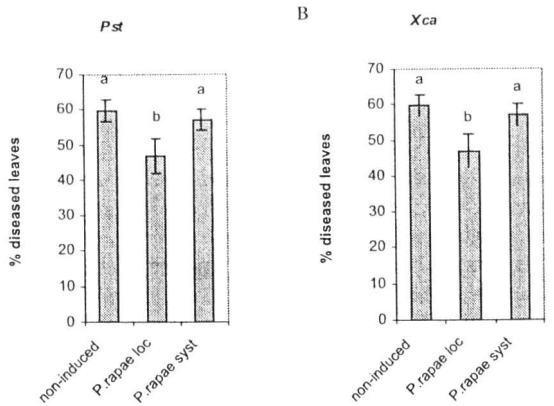

Fig. 2. Disease severity in control and *P.rapae*-induced plants at 3 days after inoculation with *Pst* (A) or *Xca* (B). Induced resistance against *Pst* and *Xca* is only apparent locally (*P.rapae* loc), while undamaged leaves of *P.rapae*-induced plants (*P.rapae* syst) were not protected. Different letters indicate significant differences between treatments (Fisher's LSD test, $\alpha = 0.05$).

disease symptoms caused by both *Pst* and *Xca*. However, the effect was only apparent in local, primary damaged leaves and not in the systemic leaf tissues, suggesting that the resistance attained is only expressed locally.

Systemic herbivore-induced resistance against TCV

To assess the effectiveness of *P. rapae*-induced defence against TCV, *P. rapae* L1 caterpillars were allowed to feed for 24 h on leaves of 5-week-old Di-0 plants. Subsequently, the larvae were removed and the leaves were challenge inoculated with TCV as described previously [5]. Three days later, lesion diameter was measured and RNA was extracted to quantify the level of TCV multiplication. *P. rapae* feeding triggered a significant reduction of the TCV-induced lesion diameter (Fig. 3A) and clearly inhibited TCV multiplication (Fig. 3B). This effect was apparent both locally and systemically.

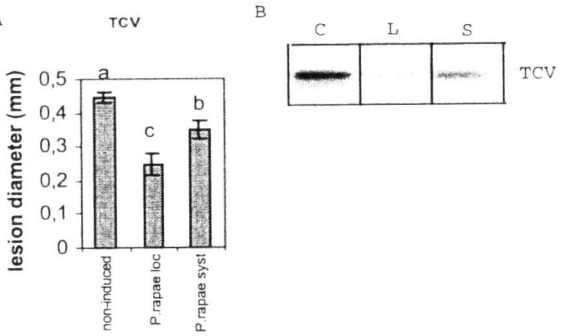

Fig. 3. A: Disease severity measured as lesion diameter caused by the TCV-induced hypersensitive response of *Arabidopsis* accession Di-0. Different letters indicate statistically significant differences between treatments (Fisher's LSD test, $\alpha = 0.05$).
B: Northern blot analysis of TCV RNA. C = non-induced leaves; L = *P. rapae* induced local (damaged) leaves; S = *P. rapae* induced systemic (undamaged) leaves.

Herbivore-induced defence is not effective against *Alternaria brassicicola*

To determine the effectiveness of herbivore-induced defence against the fungus *A. brassicicola*, *pad3-1* plants (in Col-0 background) were infested by *P. rapae* as described above. Subsequently, induced and non-induced plants were challenged with the fungal pathogen *A. brassicicola* and assayed for disease severity as described previously [4, 5]. We found that the disease rating of *A. brassicicola* infection did not differ between induced and non-induced plants, indicating that *P. rapae*-induced defences are not effective against this pathogen (data not shown).

Discussion

Previously, we studied the effectiveness of JA/ET-dependent, *Pseudomonas fluorescens*-induced systemic resistance (ISR), and SA-dependent, pathogen-induced systemic acquired resistance (SAR) against *Pst*, *Xca*, *A. brassicicola* and TCV [5]. Here we show the effectiveness of herbivore-induced resistance against these pathogens. We provide evidence that *P. rapae* triggers a defence response that is effective locally against *Pst* and *Xca* and both locally and systemically against TCV. In contrast, *P. rapae*-induced defence was not effective against *A. brassicicola*. These results seem to contradict previous findings. First of all, *P. rapae*-induced defence is associated with enhanced JA production, which has been shown to be effective against *A. brassicicola* [4, 5]. However, in our experiments, this effect was not apparent. A possible explanation is that the amounts of JA produced are not sufficient to inhibit *A. brassicicola* infection. Secondly, resistance against TCV has been shown to be regulated through SA-dependent defences [1, 5]. However, *P. rapae* infestation neither resulted in the enhanced accumulation of SA, nor in the increased expression of SA-responsive *PR-1* gene expression. Apparently, other unknown defence mechanisms can contribute to resistance against TCV as well.

References

1. Kachroo P. et al. (2000) Plant Cell 12: 677-690.
2. Pieterse C.M.J. and Van Loon L.C. (1999) Trends in Plant Sci. 4: 52-58.
3. Pieterse C.M.J. et al. (2000) Physiol. Mol. Plant. Pathol. 57: 123-134.
4. Thomma B.P. et al. (1998) Proc. Natl. Acad. Sci. USA 95: 15107-15111.
5. Ton J. et al. (2002) Mol. Plant-Microbe Interact. 15: 27-34.
6. Van Poecke R.M.P. and Dicke M. (2002) J. Exp. Bot. 53: 1793-1799.