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Author(s)	R.W.H.M. van Tol, A.T.C. van der Sommen, M.I.C. Boff, J. van Bezooijen, M.W. Sabelis, P.H. Smits
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Plants protect their roots by alerting the enemies of grubs

Rob W.H.M. van Tol,¹ Anton T.C. van der Sommen,² Mari I.C. Boff,^{3,4} Jan van Bezooijen,² Maurice W. Sabelis⁵ and Peter H. Smits³

¹Applied Plant Research, Nursery Stock Research Unit, P.O. Box 118, 2770 AC Boskoop, The Netherlands

E-mail:

r.w.h.m.van.tol@ppo.dlo.nl

²Wageningen-UR, Department of Nematology, P.O. Box 8123, 6700 ES Wageningen, The Netherlands

³Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands

⁴Current address: Universidade do Estado de Santa Catarina – Centro de Ciências

Agroveterinárias, Av. Luis de Camões, 2090, 88520-000, Lages, Santa Catarina, Brazil

⁵Institute for Biodiversity and Ecosystem Dynamics, P.O. Box 94084, 1090 GB Amsterdam, The Netherlands

Abstract

Plant roots in the soil are under attack from many soil organisms. Although many ecologists are aware of the presence and importance of natural enemies in the soil that protect the plants from herbivores, the existence and nature of tritrophic interactions are poorly understood. So far, attention has focused on how plants protect their above-ground parts against herbivorous arthropods, either directly or indirectly (i.e. by getting help from the herbivore's enemies). This article is the first in showing that indirect plant defences also operate underground. We show that the roots of a coniferous plant (*Thuja occidentalis*) release chemicals upon attack by weevil larvae (*Otiorynchus sulcatus*) and that these chemicals thereby attract parasitic nematodes (*Heterorhabditis megidis*).

Keywords

Synomones, semiochemicals, roots, herbivory, entomopathogenic nematodes, vine weevil, *Otiorynchus sulcatus*, *Heterorhabditis megidis*, *Thuja occidentalis*.

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Plants protect themselves against insect herbivores, either directly, e.g. using toxins, or indirectly by promoting the effectiveness of the herbivore's enemies. Indirect plant defences may help explain why insect herbivores are generally predator-controlled and therefore why “the World is green” (Sabelis *et al.* 1999). The evidence for this mode of defence comes from studies on above-ground plant parts (stems and leaves) providing shelter, food or SOS-signals to the herbivore's enemies (Dicke *et al.* 1990; Turlings *et al.* 1995; Takabayashi & Dicke 1996; Sabelis *et al.* 1999). However, roots are a vital, yet vulnerable part of the plant and the role of soil-dwelling natural enemies in suppressing populations of root-feeding insects in natural ecosystems has been shown (Strong *et al.* 1996, 1999). Thus, one may wonder whether plants actively protect their roots by attracting these natural enemies. We tested this

hypothesis by studying the interaction between conifer roots (*Thuja occidentalis*), root-feeding vine weevil larvae (*Otiorynchus sulcatus*) and entomopathogenic nematodes (*Heterorhabditis megidis*). Olfactometry revealed that roots damaged by weevil larvae release exudates that attract parasitic nematodes. These chemicals therefore function as an SOS, signalling the presence of herbivores to their natural enemies.

Little is known of the searching behaviour of entomopathogenic nematodes in the soil, let alone the role of chemical communication. Entomopathogenic nematodes are known to be attracted to undamaged plant roots (Bird & Bird 1986; Choo *et al.* 1989; Lei *et al.* 1992; Wang & Gaugler 1998), to their insect hosts (Schmidt & All 1978; Gaugler *et al.* 1980; Lei *et al.* 1992; Lewis *et al.* 1993) and the associated cues (e.g. CO₂ and faeces) (Schmidt & All 1978;

Gaugler *et al.* 1980; Grewal *et al.* 1993; Lewis *et al.* 1993), but only one study (Wang & Gaugler 1998) suggests that cues from intact and wounded grass roots influence host finding ability of the nematodes. The possibility that plant roots release SOS signals upon being eaten by insects has been largely ignored, even though there is a wealth of evidence for such herbivore-induced signals from leaves (Dicke *et al.* 1990; Turlings *et al.* 1995; Takabayashi & Dicke 1996; Sabelis *et al.* 1999). We hypothesized that this is also possible in the soil environment where plant roots are under insect attack and would be able to maintain their function by the aid of the herbivore's natural enemies.

To test the olfactory behaviour of the nematodes we used a Y-shaped tube, comprising five short tubes (two per arm – 7 cm long, 3.5 cm diam. – and one at the base – 5.5 cm long, 4.5 cm diam.), each filled with silver-sand (moisture content 10% w/w) and closed using nylon gauze to isolate insects and roots, yet allow nematodes to pass through (Boff *et al.* 2001). The short tube on top of each Y-arm was disconnected and incubated for four days with one of the following odour-emitting objects in silver-sand: six weevil larvae, undamaged, mechanically damaged (cutting 10% of root tips) or weevil-damaged (with or without six larvae) roots of one intact thuja plant with its above-ground parts sealed off from the tube. After incubation, the tube parts were connected again on top of the Y-arms and the Y-tube was positioned vertically, arms up, by clamping it to a stand in a climate room (15 °C; L : D = 16 : 8 h). One day thereafter, nematodes (900 in 0.5 mL tap water) were released in a pipette inserted up the middle of the base tube, i.e. 16 cm from the top of the Y tube. One day later, their numbers in either arm were counted after sand-extraction with an independently estimated 90–95% efficiency (Boff *et al.* 2001). Each experiment was replicated at least four times with fresh odour sources, fresh sand and new batches of nematodes. For the choice tests between weevil-damaged

and mechanically damaged or undamaged thuja roots, we first rinsed the roots with tap water, placed them in water for one day and finally replanted the thujas in fresh silver-sand in the Y-tubes. Thus, odours from insects and their faeces are absent during these tests and the observed response of the nematodes must be attributed to odours released from the plant.

We found that nematodes were more attracted to weevil larvae alone and to undamaged *Thuja* roots alone when clean air was the alternative, and that they were more attracted to weevil-infested roots than to larvae alone or roots alone (Table 1). The nematodes also moved to odours from weevil-damaged roots, freed of weevil larvae prior to the experiment, instead of to odours from undamaged or mechanically damaged roots (Table 1).

The attractive plant odour probably does not travel by air to the nematode's sensory organs. GC-MS analysis of volatile chemicals (using Tenax-TA adsorbents-tubes and a thermodesorption cold trap unit) did not reveal differences between treatments. It is probably that the chemicals released from the plant enter the water in the silver sand and diffuse into the Y-tube. There, the response of the parasitic nematodes becomes manifest in a matter of hours after release, as shown by independent olfactometer experiments (three replicates per time treatment). A proportion of the nematodes had entered the *Thuja* Y-arm within 2 h but, even after 6 h, none of them had reached the top tube with the control source, whereas many were already in the top tube with the odour source under investigation. (e.g. in the case of odour from undamaged thuja roots against clean air: 5% after 2 h, 6% after 4 h, 8% after 6 h). It can therefore be safely concluded that the nematodes are attracted to the odour source, rather than arrested after random movement.

These results demonstrate herbivory-induced release of SOS-signals from plant roots attracting the entomopatho-

Table 1 Olfactory response of entomopathogenic nematodes

Odour source (+)	Odour source (-)	% Nematodes to (+) [#]	Results
Control	Control	50.7	49, 54, 46, 54, 54, 53, 50, 53
Vine weevil larvae	Control	72.5*	63, 73, 82, 67, 91, 66
<i>Thuja</i> roots	Control	88.7*	90, 87, 84, 99, 69, 87, 91
<i>Thuja</i> roots + vine weevil larvae	Vine weevil larvae	80.6*	78, 87, 80, 80, 81, 80
<i>Thuja</i> roots + vine weevil larvae	<i>Thuja</i> roots	82.6*	94, 74, 78, 75, 81, 85
Weevil-damaged <i>Thuja</i> roots	Mechanically-damaged <i>Thuja</i> roots	79.7*	71, 80, 84, 82
Weevil-damaged <i>Thuja</i> roots	Undamaged <i>Thuja</i> roots	73.1*	73, 68, 82, 74

Regression analysis was performed on logit transformed data with the Genstat 5 computer program. Values followed by an asterisk (*) indicate statistically significant preferences for a particular odour at the 5% level.

[#]Percentage nematodes to (+) are predicted values from the regression analysis and are thus not presenting the exact average values of the range results shown in the table.

genic nematodes in the direction of the odour source. Whether the attractants released in response to weevil damage are *de novo* synthesized, produced in larger amounts or in different relative amounts needs further study. Short-range attraction to fluids exuded from plant roots has been shown earlier (Bird & Bird 1986; Choo *et al.* 1989; Lei *et al.* 1992), but long-range attraction by means of SOS signalling of plants is an entirely new result. This method of host habitat location will be vital to the searching nematode larvae because they are exclusively designed to search, not to feed, they have limited energy reserves and experience host scarcity in the soil. A mutualistic interaction between plants and entomopathogens in the soil is therefore to be expected and this may well provide new insight into how plants protect their roots against grubs and how biological control of soil pests may be achieved (Elliot *et al.* 2000).

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BIOSKETCH

Rob van Tol studies plant–weevil–parasite interactions in the soil and the implications for pest control strategies in different agricultural systems. He is currently working on the identification of attractants of the vine weevil and development of a monitoring system for this pest in ornamentals.

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