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Entomopathogenic nematodes induce systemic resistance in tomato against *Spodoptera exigua*, *Bemisia tabaci* and *Pseudomonas syringae*

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HIGHLIGHTS

- Soil application of EPNs induces systemic resistance in tomato which lasts for 7–10 day.
- EPN-induced resistance reduce the development of beet armyworms on tomato.
- EPN-induced resistance delays the egg hatch of sweetpotato whitefly on tomato.
- EPN-induced resistance reduces disease spot caused by *Pseudomonas syringae* on tomato.
- EPN-induced resistance has no fitness cost to tomato during vegetative growth stage.

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ABSTRACT

Entomopathogenic nematodes (EPNs) *Steinernema* and *Heterorhabditis* are important biocontrol agents for soil-inhabiting insect pests in many high-value cropping systems. EPNs have also been shown to be antagonistic to plant-parasitic nematodes but the mechanisms are poorly understood. It has been recently discovered that soil application of EPNs can induce components of systemic resistance in hosta and Arabidopsis plants. Here, we hypothesized that EPN-induced systemic resistance is of broad spectrum with activity against chewing insects, sucking insects, and bacterial pathogens. We tested this hypothesis by comparing the development of beet armyworm *Spodoptera exigua*, sweetpotato whitefly *Bemisia tabaci*, and bacterial pathogen *Pseudomonas syringae* on EPN-treated and control tomato plants. *Steinernema carpocapsae*-infected waxworm cadavers were applied to the soil around tomato plants in pots whereas the control plants received freeze-killed waxworms. EPN-induced defense responses were evaluated at 3, 7 and 15 days after treatment (DAT). We observed that the EPN-treatment had significant negative impact on all three organisms on tomato leaves 3 or 7 DAT, but not 15 DAT. Treatment with EPNs delayed immature beet armyworms from reaching the next developmental stage, impaired whitefly egg hatch, and reduced lesion formation of the bacterial pathogen on the leaves. These results confirm the hypothesis that soil application of EPNs can result in a broad-spectrum systemic induced resistance in tomato plants. While the evolutionary significance of this phenomenon is not yet understood, the findings suggest that soil applications of EPNs can provide benefits beyond the target insect control by boosting general plant immunity.

1. Introduction

Increasing concerns about the risks of large-scale application of chemical pesticides have generated strong impetus for the development of alternative methods for plant protection. One of the ideal alternatives is biological control that involves the use of natural enemies for managing pests and mitigating their negative effects on plant health. Among the wide range of natural enemies, entomopathogenic nematodes (EPNs), Heterorhabditidae and Steinernematidae, serve as important biocontrol agents of soil dwelling insect pests (Grewal et al., 2005). EPNs have a mutualistic association with entomopathogenic bacteria: *Heterorhabditis* with *Photorhabdus* and *Steinernema* with *Xenorhabdus*. The partnership with the bacteria enables the EPNs to exploit a diverse array of insects as hosts (Grewal, 2012).

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Besides acting as an attractive biocontrol agent against target insect pests, EPNs may carry additional benefits in agriculture. Earlier studies have recorded reductions in populations of a variety of plant parasitic nematodes following the soil application of EPNs (Bird and Bird, 1986; Grewal et al., 1997; Ishibashi and Kondo, 1986; Lewis and Grewal, 2005; Somasekhar et al., 2002). Recent studies have revealed another intriguing potential benefit of EPN applications to the soil by which EPNs can boost plant immunity. Jagdale and Grewal (2008) made an interesting observation that soil application of *Steinernema carpocapsae* reduced multiplication of the foliar nematode *Aphelenchoides fragariae* in hosta leaves suggesting the possibility of induced systemic resistance. Further studies revealed that soil application of *S. carpocapsae* infective juveniles and their symbiotic bacteria *Xenorhabdus nematophilus* activated the production of key defense enzymes and hormones in hosta and *Arabidopsis thaliana* leaves, and induced the expression of a plant defense protein promoter *PR1* gene in *A. thaliana* (Jagdale et al., 2009). They suggested that the elusive antagonistic effect of EPNs on plant-parasitic nematodes may be at least partially attributed to this EPN-induced plant defense response (Jagdale et al., 2009).

In plants, induced systemic defense responses are regulated by a network of interconnecting signal transduction pathways in which the hormonal signals salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play a major role, and other hormones such as brassinosteroids and abscisic acid can also be involved (Audenaert et al., 2002; Nakashita et al., 2003; Ton and Mauch-Mani, 2004). SA, JA, and ET pathways can be elicited by exposure to pathogenic and non-pathogenic organisms, as well as certain chemicals or herbivore damage. Activation of the signaling pathways leads to the systemic expression of a broad-spectrum and long-lasting increased resistance that can affect herbivorous insects, fungi, bacteria and viruses (Karban et al., 1987; Pieterse et al., 2014). As a widespread phenomenon in plants, induced resistance is being intensively studied with respect to the underlying signaling pathways and for its potential use in plant protection.

In this study, we hypothesized that EPN-induced systemic resistance is broad spectrum with activity against chewing insect, sucking insects and bacterial pathogen. We tested this hypothesis by determining the development of a generalist chewing pest, beet armyworm (*Spodoptera exigua*), a generalist sucking pest, sweetpotato whitefly (*Bemisia tabaci*), and a bacterial pathogen *Pseudomonas syringae* pv tomato on EPN-treated and control tomato plants at 3, 7, and 15 days after treatment (DAT). We also estimated fitness cost to tomato plant biomass for maintaining the EPN-induced systemic resistance in the absence of pests and pathogens. Tomato was chosen as a model for this investigation due to its economic and dietary value and the availability of the full genome sequence (Tomato Genome Consortium, 2012) so that molecular mechanisms of the EPN-induced systemic resistance could be explored in the future.

2. Materials and methods

2.1. Sources of plants, nematodes, insects and bacteria

Seeds of tomato *Solanum lycopersicum* variety 'Moneymaker' were sown in autoclaved peat-based growing medium (Premier Pro-Mix BX, Premier Horticulture, Red Hill, PA) in plastic cube-trays in a growth chamber at 25 °C with a photoperiod of 13 h darkness and 11 h light. When seedlings reached 2–3 leaf stage, they were individually transplanted into 15-cm diameter pots containing the autoclaved growing medium and moved to a greenhouse with temperature set between 2527 °C and 27 °C with a photoperiod of 13 h darkness and 11 h light. In addition to watering, the plants were provided with Miracle-Gro (N-P-K; 18-18-21) synthetic water-soluble granules at regular intervals throughout the experiment. The infective juveniles of *S. carpocapsae* All strain were produced in the last-instar wax moth *Galleria mellonella* at 25 °C using methods described by Kaya and Stock (1997). Nematode-infected *G. mellonella* cadavers were obtained by exposing each last-instar larva to approximately 100 *S. carpocapsae* infective juveniles. To ensure that the nematodes would be delivered into the soil, *G. mellonella* larvae 13 days post infection at which the infective juveniles just started emerging from the cadavers were used in all experiments to treat tomato rhizospheres.

The colonies of sweetpotato whitefly, *B. tabaci*, were maintained in a greenhouse. The eggs of beet armyworm (BAW), *S. exigua*, were purchased from Benzon Research Inc. (Carlisle, PA) and incubated 48 h at 28 °C for hatching into 1st-instar larvae before beginning the bioassays. Bacteria *P. syringae* pv tomato was received from Dr. Sally Miller of The Ohio State University and were subcultured in Luria–Bertani broth at 28 °C for all experiments.

2.2. Experimental design and treatments

For all experiments described below tomato plants were grown in the greenhouse and were treated with *S. carpocapsae*-infected *G. mellonella* cadavers at 4–5 leaf stage. Three *S. carpocapsae*-infected *G. mellonella* cadavers were buried around the roots 5 cm below the surface in each pot containing a single tomato plant. The control plants were treated with freeze-killed *G. mellonella* larvae which were free of the EPNs and their microbial associates. The plants were labeled and arranged in a randomized complete block design with 1 meter distance from each other to avoid any interference of volatiles (Holopainen and Blande, 2012). The whitefly, BAW, and *P. syringae* trials were each repeated at 3, 7 and 15 DAT with the insect cadavers on new sets of plants; plant fitness costs were evaluated at 15 DAT. The entire experiment was repeated using two sets of 20 plants (10 control plus 10 treated) for the whitefly or BAW and two sets of 10 plants (5 control plus 5 treated) for the *P. syringae* experiment or fitness cost assessment at each time point.

2.3. Beet armyworm tests

Tomato leaf bioassays were conducted to test the effectiveness of EPN-induced systemic resistance against the chewing insect BAW. From each EPN-treated or control plant, one leaflet from each of the two youngest fully-expanded leaves was removed and placed individually in a Petri dish with moist filter paper. Thirteen 1st-instar BAW larvae were carefully transferred onto each leaflet using a fine paint brush and placed in the growth chamber at 25 °C with a photoperiod of 10-h-darkness and 14-h-light. After 24 h, the numbers of larvae were adjusted to 10 per leaflet to start the experiment with the same numbers of larvae in each replication to account for any larval mortality during transfer of the neonate larvae. Survival and instar development were recorded daily for 6 days. Effects of the treatment on the larval development were compared using a mean instar index, which was calculated as $\Sigma[(n_i)(i)]/\Sigma n_i$, where "*i*" represents instar and "*n*" means the numbers of instars (Banke, 1970).

2.4. Whitefly tests

The effectiveness of the EPN-induced resistance in tomato against the sweetpotato whitefly, *B. tabaci*, was tested directly on the plants. For each plant, five adult female whiteflies were released in a plastic clip-cage, which was attached to one leaflet on each of two youngest fully expanded leaves. After 48 h, the clip-cages and whiteflies were removed, and the number of eggs