Taylor & Francis
Taylor & Francis Group

Biocontrol Science and Technology

ISSN: 0958-3157 (Print) 1360-0478 (Online) Journal homepage: http://www.tandfonline.com/loi/cbst20

Parameters affecting plant defense pathway mediated recruitment of entomopathogenic nematodes

Camila Cramer Filgueiras, Denis S. Willett, Ramom Vasconcelos Pereira, Paulo Henrique de Siqueira Sabino, Alcides Moino Junior, Martin Pareja & Donald W. Dickson

To cite this article: Camila Cramer Filgueiras, Denis S. Willett, Ramom Vasconcelos Pereira, Paulo Henrique de Siqueira Sabino, Alcides Moino Junior, Martin Pareja & Donald W. Dickson (2017) Parameters affecting plant defense pathway mediated recruitment of entomopathogenic nematodes, Biocontrol Science and Technology, 27:7, 833-843, DOI: 10.1080/09583157.2017.1349874

To link to this article: http://dx.doi.org/10.1080/09583157.2017.1349874

Published online: 11 Jul 2017.

Submit your article to this journal

Article views: 35

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=cbst20

Download by: [UNICAMP] **Date:** 15 August 2017, At: 10:29

BIOCONTROL SCIENCE AND TECHNOLOGY, 2017
VOL. 27, NO. 7, 833–843
https://doi.org/10.1080/09583157.2017.1349874

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Check for updates

Parameters affecting plant defense pathway mediated recruitment of entomopathogenic nematodes

Camila Cramer Filgueiras[a], Denis S. Willett[b], Ramom Vasconcelos Pereira[a], Paulo Henrique de Siqueira Sabino[a], Alcides Moino Junior[a], Martin Pareja[c] and Donald W. Dickson[d]

[a]Department of Entomology, Federal University of Lavras, Lavras, Brazil; [b]Agricultural Research Service, United States Department of Agriculture, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, USA; [c]Biology Institute, State University of Campinas – UNICAMP, Animal Biology Department, Campinas, Brazil; [d]Entomology and Nematology Department, University of Florida, Gainesville, FL, USA

Downloaded by [UNICAMP] at 10:29 15 August 2017

ABSTRACT
Entomopathogenic nematodes are natural enemies and effective biological control agents of subterranean insect herbivores. Interactions between herbivores, plants, and entomopathogenic nematodes are mediated by plant defense pathways. These pathways can induce release of volatiles and recruit entomopathogenic nematodes. Stimulation of these plant defense pathways for induced defense against belowground herbivory may enhance biological control in the field. Knowledge of the factors affecting entomopathogenic nematode behaviour belowground is needed to effectively implement such strategies. To that end, we explore the effect of elicitor, elicitor dose, mechanical damage, and entomopathogenic nematode release distance on recruitment of entomopathogenic nematode infective juveniles to corn seedlings. Increasing doses of methyl jasmonate and methyl salicylate elicitors recruited more entomopathogenic nematodes as did mechanical damage. Recruitment of entomopathogenic nematodes was higher at greater release distances. These results suggest entomopathogenic nematodes are highly tuned to plant status and present a strategy for enhancing biological control using elicitor-stimulated recruitment of entomopathogenic nematodes.

ARTICLE HISTORY
Received 14 December 2016
Revised 26 June 2017
Accepted 29 June 2017

KEYWORDS
Elicitors; salicylic acid; jasmonic acid; entomopathogenic nematodes; plant–insect interactions

1. Introduction

Entomopathogenic nematodes are natural enemies of belowground insect herbivores that have shown promise for biological control in subterranean and cryptic environments (Gaugler & Kaya, 1990). Applications of entomopathogenic nematodes for biocontrol is effective in a variety of cropping systems and new strategies are being developed to extend the abilities of entomopathogenic nematodes to control previously inaccessible pests. Entomopathogenic nematodes have been effective control agents for diptera in mushroom houses (Kaya & Gaugler, 1993), for mole crickets and scarab larvae in turf (Koppenhöfer & Fuzy, 2009; Parkman & Smart Jr, 1996), for weevil herbivores in citrus

CONTACT Camila Cramer Filgueiras ✉ camilacramer@gmail.com

© 2017 Informa UK Limited, trading as Taylor & Francis Group

Downloaded by [UNICAMP] at 10:29 15 August 2017

(McCoy, Shapiro, Duncan, & Nguyen, 2000; Stuart, Shapiro-Ilan, James, Nguyen & McCoy, 2004), and for corn rootworm *Diabrotica virgifera virgifera* larvae in corn (Journey & Ostlie, 2000; Toepfer, Peters, Ehlers, & Kuhlmann, 2008), among others (Georgis et al., 2006; Lacey et al., 2015; Lacey & Georgis, 2012). Extending and enhancing the effectivenes of entomopathogenic nematodes of biological control agents has long been a goal and various means of augmentation including plant genetic manipulation and nematode encapsulation have been pursued (Degenhardt et al., 2009; Hiltpold, Hibbard, French, & Turlings, 2012).

Plant volatiles are critical to developing new strategies for enhancing biological control with entomopathogenic nematodes (Turlings, Hiltpold, & Rasmann, 2012; Turlings & Ton, 2006). These strategies appropriate existing communication systems in plants that recruit entomopathogenic nematodes to sites of belowground herbivory. In citrus, for example, herbivory by larvae of the weevil *Diaprepes abbreviatus* releases the herbivore induced plant volatile pregeijerene which recruits a wide range of entomopathogenic nematodes (Ali et al., 2012; Ali, Alborn, & Stelinski, 2010, 2011). Similarly, herbivory by weevil larvae on white cedar *Thuja occidentalis* recruits the entomopathogenic nematode *Heterorhabditis megidis* (Van Tol et al., 2001). In corn, herbivory by larvae of the western corn rootworm *Diabrotica virgifera virgifera* results in the release of *E-β* caryophyllene which recruits *H. megidis* (Rasmann et al., 2005).

Recruitment of entomopathogenic nematodes belowground is mediated by plant defense pathways which regulate plant responses to herbivory and pathogen infection (Bezemer & van Dam, 2005; Bezemer, Wagenaar, Van Dam, & Wäckers, 2003). While induction of these pathways can regulate release of plant volatiles belowground as described above, stimulation of these pathways in the absence of herbivory can also produce effects belowground, potentially regulating responses to pest-pathogen complexes (Filgueiras et al., 2016a). Indeed, induction of plant defense pathways aboveground can influence recruitment of natural enemies belowground and vice versa (Rasmann & Turlings, 2007). The two most prominent pathways likely to mediate recruitment of entomopathogenic nematodes belowground are the jasmonic acid and salicylic acid pathways. The jasmonic acid pathway is thought to be stimulated mainly by herbivory and often results in upregulation of plant defenses targeting herbivorous pests (Thaler, Humphrey, & Whiteman, 2012). In contrast, the salicylic acid pathway is thought to be stimulated by and mediate resistance to biotrophic pathogen infection (Glazebrook, 2005). Additionally, both pathways have been implicated in the recruitment of natural enemies aboveground (Thaler, 1999b; van Poecke & Dicke, 2002) and recent work suggests they could be acting similarly belowground (Filgueiras et al., 2016b).

The role these pathways play in mediating interactions between plants, herbivores, and natural enemies has prompted exploration of the possibility of applying plant defense elicitors for induction of plant resistance in the field. Results of this strategy aboveground have been promising. Stimulation of the jasmonic acid pathway in tomatoes reduces aboveground herbivory with no detrimental effects on yield (Thaler, 1999a) and is associated with reduction in abundance of many herbivores (Thaler, Stout, Karban, & Duffey, 2001). Similarly, induced resistance through stimulation of the salicylic acid pathway can reduce bacterial infection (Thaler, Fidantsef, Duffey, & Bostock, 1999). Development of practical applications of elictors of induced defense for recruitment of

Downloaded by [UNICAMP] at 10:29 15 August 2017

entomopathogenic nematodes belowground is being explored and will rely upon knowledge of parameters affecting nematode behaviour and application efficacy.

To explore these parameters, we use a corn model system involving the larval herbivore *Diabrotica speciosa*, a polyphagous and ubiquitous pest of corn in South America (Metcalf, Krysan, & Miller, 2012; Walsh, 2003), and the entomopathogenic nematode *Heterorhabditis amazonensis*, a natural enemy of *D. speciosa* with potential for biocontrol (Figure 1) (Andaló, Nguyen, & Moino, 2006; Santos, Moino, Moreira, & Olinda, 2011). The potential for using elicitor stimulated induced defenses in this system has been raised (Filgueiras et al., 2016b); here we explore the effect of elicitor, elicitor dose, plant damage, and distance on recruitment of entomopathogenic nematodes belowground.

2. Materials and methods

To explore methods of using elicitors of plant defense to augment biological control of larval *D. speciosa* using entomopathogenic nematodes, we evaluated distance of nematode release and doses of elicitors as possible factors affecting efficacy of entomopathogenic nematode recruitment and control. Elicitors were applied foliarly while nematode response and infection were evaluated belowground in sand filled arenas.

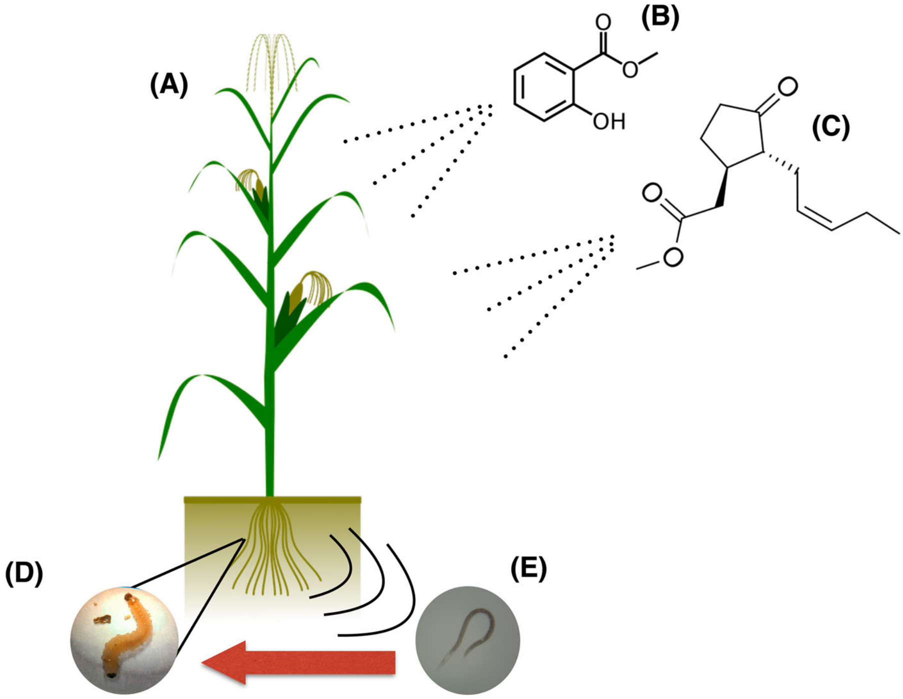

Figure 1. Model system involving corn (A) to which plant defense elicitors Methyl Salicylate (B) and Methyl Jasmonate (C) were applied. In the field, stimulation of plant defense pathways mediates interactions between larval *Diabrotica speciosa* (D) and its natural enemy the entomopathogenic nematode *Heterorhabditis amazonensis* (E).

2.1. Organisms

Maize seedlings were germinated in moist vermiculite and used after cultivation for 20 days in organic substrate. While under cultivation, maize seedlings were kept in a growth chamber at 25 ± 2.0°C with a 12:12 light:dark cycle. A maize line TC1507 in widespread use throughout Brazil expressing the Herculex I gene (Dow AgroSciences, Pioneer Hi-Bred International), was used in all experiments. *H. amazonenensis* entomopathogenic nematodes from cultures maintained at the Federal University of Lavras were reared in larvae of the greater wax moth *Galleria mellonela* and infective juveniles collected using white traps (Kaya & Stock, 1997; White, 1927). Collected nematodes were used in experiments within a week of collection. *Galleria* larvae used for rearing entomopathogenic nematodes were likewise reared in the laboratory on artificial diet (Dutky, Thompson, & Cantwell, 1962; Parra, 1986).

2.2. Elicitor preparations

Methyl jasmonate (Sigma Aldrich, 95% purity) and methyl salicylate (Sigma Aldrich, 99% purity) elicitors were applied foliarly to corn seedlings in Tween20 (at 0.1 mL/L) and ethanol (at 2.5 mL/L) solutions using 30 ml calibrated sprays while ensuring homogeneous uniform application as in (Filgueiras et al., 2016a, 2016b). Control plants received spray solutions without elicitors, i.e. only Tween20-ethanol solutions. Applications were prevented from entering the soil using an aluminum foil barrier.

2.3. Dose response

To evaluate the effect of different elicitor doses on recruitment of the entomopathogenic nematode *H. amazonensis* eight-choice olfactometers filled with washed autoclaved sand adjusted to 10% moisture by volume were used. Eight-choice olfactometers were constructed from 30 cm diameter plastic containers into which eight 4 cm diameter PVC elbows were inserted (Filgueiras et al., 2016b). Each elbow received one corn seedling which was allowed 72 hours to acclimate to the olfactometer environment prior to receiving elicitor treatment. Methyl salicylate was applied in solutions prepared as described above such that seedlings received either 0, 65, 130, or 260 μL total compound. Similarly, methyl jasmonate was applied in solutions prepared as described above such that seedlings received either 0, 109, 218, or 436 μL. Doses were calculated based on previous work (Filgueiras et al., 2016a) because they provided a biologic response. Treatments were arranged in alternating opposition around the eight-choice olfactometer. Forty-eight hours following treatment application, 2500 *H. amazonensis* infective juveniles were released into the centre of each olfactometer. After allowing 24 hours for nematodes to respond, olfactometers were disassembled and nematodes collected from the elbows via Baermann funnels and counted. Ten replications (i.e. olfactometers) for each elicitor with different nematode batches were conducted across all four doses.

2.4. Elicitor comparison

To determine if nematodes were more responsive to stimulation of either the jasmonic acid or salicylic acid pathway, contrasts between plants treated with methyl jasmonate

Downloaded by [UNICAMP] at 10:29 15 August 2017

Downloaded by [UNICAMP] at 10:29 15 August 2017

(at 130 μL) and methyl salicylate (218 μL) were conducted as above in eight-choice sand filled olfactometers observing the same schedule for seedling planting, application of elicitor treatments, and collection of nematodes. In this case, instead of evaluating effects versus untreated corn seedlings, nematode response to methyl jasmonate treated plants versus methyl salicylate treated plants was evaluated in both healthy and mechanically damaged plants. Mechanically damaged plants received foliar damage with a scalpel using a template to replicate feeding by adult *D. speciosa* with 3 cm^2 total leaf area removed. Four replications (i.e. olfactometers) of each treatment combination were conducted with different nematode batches.

2.5. Distance assays

The effect of distance on recruitment of entomopathogenic nematodes to elicitor treated and mechanically damaged corn plants was evaluated in 20.5 cm by 20.5 cm by 75 cm rectangular planters. Planters were filled with autoclaved sand adjusted to 10% moisture by volume; a single corn seedling was placed at one end of the planter and allowed 72 hours to acclimate to their environment. Following the application period, corn seedlings received elicitor and damage treatment. Corn seedlings either received a control spray containing no elicitors, 65 μL of methyl salicylate, or 109 μL of methyl jasmonate prepared as described above. Damage treatment consisted of undamaged control plants or plants receiving mechanical foliar damage with a scalpel mimicking herbivory by adult *D. speciosa*. Forty-eight hours after corn seedlings received treatment, 3000 entomopathogenic nematode *H. amazonensis* infective juveniles were released either 30 or 60 cm from the corn seedling. After allowing 24 hours for response, nematodes were extracted from the root system of the seedlings using Baermann funnels and counted. Three replications of each factor combination were conducted with different nematode batches.

2.6. Statistical analysis

Linear models and analysis of variance were used to evaluate the effect of elicitor dose on entomopathogenic nematode *H. amazonensis* infective juvenile response in eight-choice olfactometers. Infective juvenile response was summed across treatment within a given olfactometer to remove effects of aggregation. Summed responses to treatments were converted to percentages to evaluate additional attraction to increasing dose. Models were chosen based on model significance, examining lack of fit, and ANOVA results. Responses and residuals were examined by visual inspection of diagnostic plots, Shapiro–Wilk's test, and Levene's test to ensure conformance with assumptions of normality and homoscedasticity.

Nematode response to methyl jasmonate versus methyl salicylate treated corn seedlings was first summed to obviate effects of nematode aggregation per olfactometer, then evaluated with paired *t*-tests after ensuring adherence to assumptions of normality and homoscedasticity.

The effect of distance on recruitment of the entomopathogenic nematode *H. amazonensis* to damaged and elicitor treated corn seedlings was evaluated with analysis of variance (ANOVA) by using Distance, Damage, and Elicitor to explain nematode response following interrogation with visual inspection of quantile-quantile plots,

Downloaded by [UNICAMP] at 10:29 15 August 2017

Shapiro–Wilk's test, and Levene's test to confirm adherence to assumptions of normality and homoscedasticity.

All data were compiled in Microsoft Excel 2011 then analysed in R version 3.2.2 (R Core Team, 2015) using the RStudio version 0.99.484 (RStudio Team, 2015) development environment. The following packages were used to facilitate analysis above and beyond base R functionality: *dplyr* (Wickham & Francois, 2015) and *tidyr* (Wickham, 2015) for data management and summary statistics, *ggplot2* (Wickham, 2009) for graphics capabilities, *xlsx* (Dragulescu, 2014) for interface with Microsoft Excel, and *car* (Fox & Weisberg, 2011) for ANOVA analysis.

3. Results

3.1. Dose response

Methyl jasmonate dose significantly (F=16.173, df = 4, 6, P=0.002) explained 85.8% of observed variation in *H. amazonensis* infective juvenile response to methyl jasmonate treated seedlings. Similarly, methyl salicylate dose significantly (F=11.367, df=4,6, P = 0.006) explained 80.5% of observed variation in *H. amazonensis* infective juvenile response in eight-choice olfactometers. Additional infective juvenile attraction increased by 2.4 ± 2.9% with increasing doses of methyl jasmonate, then declined at the highest doses (Figure 2). Increasing doses of methyl salicylate increased nematode attraction by, on average, 12.6 ± 5.1% (Figure 2).

3.2. Elicitor comparison

Following mechanical damage, methyl jasmonate treated plants recruited significantly (t=4.7, df = 3, P=0.017) more *H. amazonensis* entomopathogenic nematodes than did methyl salicylate treated plants. This preference was not observed in the undamaged plants (t=–0.82, df = 3, P=0.47).

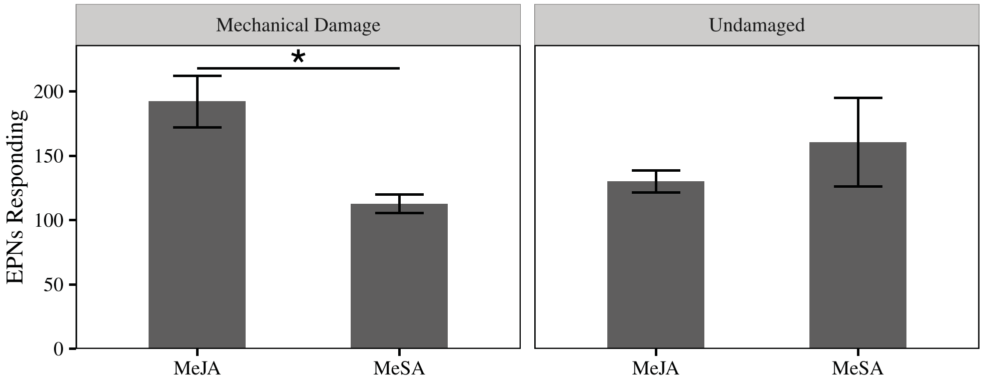

Figure 2. Additional *H. amazonensis* infective juvenile attraction to increasing doses of elicitors applied to corn seedlings. Nematode attraction represents the per cent additional infective juveniles responding to that treatment over controls. Lines and shaded region denote mean response and standard error, respectively.

Downloaded by [UNICAMP] at 10:29 15 August 2017

3.3. Distance assays

Distance and damage treatments significantly (F=11.95, df = 1, 23, P=0.002 for distance, F=10.77, df = 1, 23, P=0.003 for damage) explained observed *H. amazonensis* response (Figure 3). Elicitor treatment did not show significant effects (F=0.64, df = 2, 23, P=0.535). An average of 6.21 ± 1.8 more infective juveniles recruited to the seedling when released at 60 cm distance as compared to 30 cm. An average of 5.9 ± 1.8 more infective juveniles recruited toward mechanically damaged corn seedlings as compared to undamaged negative controls.

4. Discussion

Increasing doses of elicitors on aboveground foliage of corn seedlings recruit increasingly more entomopathogenic nematodes belowground. This effect reverses at the highest doses for methyl jasmonate, but is consistent for tested doses of methyl salicylate (Figure 2). In addition, stimulation of the jasmonic acid pathway seems to be attractive in the presence of mechanical damage (Figure 4). These results emphasise the role of plant defense pathways in mediating belowground recruitment of entomopathogenic nematodes and suggest that increased induction of plant defense pathways could potentially augment control. The differential appeal of methyl jasmonate versus methyl salicylate application on mechanically damaged versus undamaged plants suggests that entomopathogenic nematodes can closely monitor differences in plant health, above and beyond recognition of herbivore presence as observed previously in corn and citrus (Ali et al., 2010; Rasmann et al., 2005).

The significant effect of damage in distance assays seems to confirm this observation. Mechanically damaged plants recruited more entomopathogenic nematodes versus undamaged seedlings (Figure 3). In addition to suggesting a broad role for plant signaling belowground, differential recruitment to mechanically damaged seedlings also may present opportunities for adaptive control in the field. Because mechanically damaged seedlings in which the jasmonic defense pathway is stimulated are more attractive to entomopathogenic nematodes, applications of elicitors for induced defense could be tailored

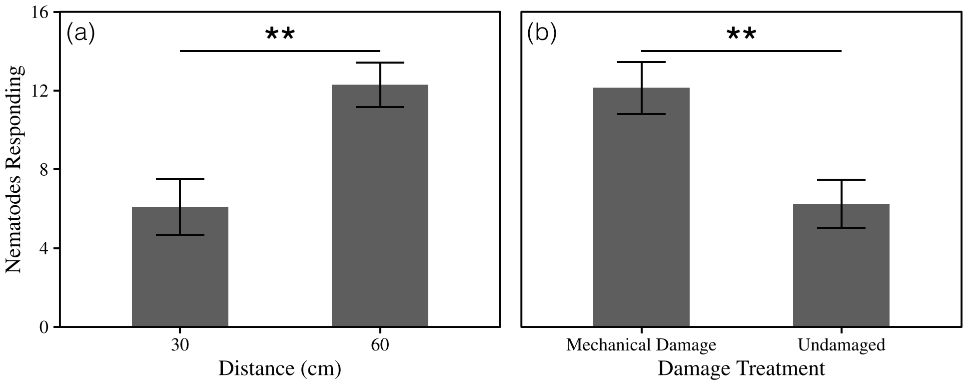

Figure 3. Effect of distance and damage treatment on *H. amazonensis* response. Bars and error bars denote mean preference and standard error respectively. ** indicates significance at $P < 0.01$.

Downloaded by [UNICAMP] at 10:29 15 August 2017

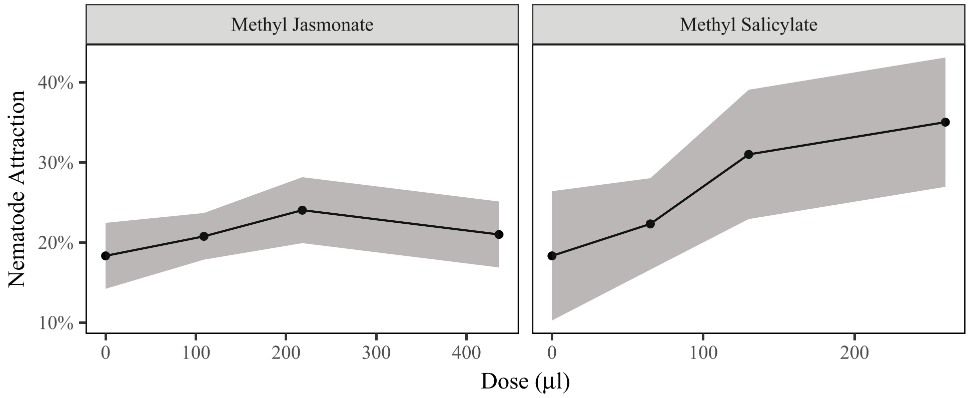

Figure 4. Entomopathogenic nematode *H. amazonensis* infective juvenile response to methyl jasmonate (MeJA) versus methyl salicylate (MeSA) treated corn seedlings. Bars and error bars denote mean preference and standard error respectively. * indicates significance at $P<0.05$.

for plant health status. Mechanically damaged or physiologically stressed plants could benefit from methyl jasmonate treatment to enhance biological control of root herbivores.

Distance of release also affects recruitment of entomopathogenic nematodes belowground. Interestingly, nematodes released farther away from elicitor treated seedlings responded in greater numbers. While this may seem counterintuitive, and that greater response is expected closer to the plant, it may be that different signals are at play. Maize is known to use simple rapidly diffusing signals to communicate in the rhizosphere (Hiltpold & Turlings, 2008). Additionally, the media in which the nematodes and plants are interacting may act as a source or sink of organic volatiles (Penuelas et al., 2014). Closer to the plant, the number and variety of volatiles released by the plant is likely higher; diffusion and adsorption rates of many plant volatiles are variable and higher concentrations of volatiles in specific blends may to be present closer in to the plant (Hiltpold & Turlings, 2008). Conversely, the number of volatiles permeating and traveling through the pore space up to 60 cm is likely to be much lower and limited to smaller, more easily diffusible volatiles (Hiltpold & Turlings, 2008). These results could suggest that nematodes are responding to specific cues at distance and, in the presence of additional components closer to the plant, orientation is complicated by the presence of additional compounds.

The variable effect of distance on nematode recruitment presents opportunities for further exploration of the basic factors affecting attraction to volatile signals belowground. Soil texture and composition, for example, are likely to influence diffusion of volatiles and recruitment of entomopathogenic nematodes belowground. In sand, our results suggest that exogenous applications of entomopathogenic nematodes for biological control can be made some distance away from the target area and still have an effect. This long-distance travel of nematodes in response to elicitor treated corn seedlings presents a novel strategy for augmentation biological control of subterranean root herbivores; entomopathogenic nematodes can be applied in a non-specific manner then called in to areas of herbivory through targeted application of plant defense elicitors. While this strategy remains to be tested in the field, elicitor induced defense could hold promise for enhancing biological control in the belowground environment.

Downloaded by [UNICAMP] at 10:29 15 August 2017

Acknowledgements

Maria Eduarda C.F.R Silva assisted and provided invaluable support for all aspects of the project.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) program and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) provided financial support for the project through studentships and grants to CCF. MP was supported by a FAEPEX-PAPDIC grant from UNICAMP, and CNPq project 474449/2012-2.

References

Ali, J. G., Alborn, H. T., Campos-Herrera, R., Kaplan, F., Duncan, L. W., Rodriguez-Saona, C., … Stelinski, L. L. (2012). Subterranean, herbivore-induced plant volatile increases biological control activity of multiple beneficial nematode species in distinct habitats. *PLoS One*, *7*(6), e38146.

Ali, J. G., Alborn, H. T., & Stelinski, L. L. (2010). Subterranean herbivore-induced volatiles released by citrus roots upon feeding by diaprepes abbreviatus recruit entomopathogenic nematodes. *Journal of Chemical Ecology*, *36*(4), 361–368.

Ali, J. G., Alborn, H. T., & Stelinski, L. L. (2011). Constitutive and induced subterranean plant volatiles attract both entomopathogenic and plant parasitic nematodes. *Journal of Ecology*, *99*(1), 26–35.

Andaló, V., Nguyen, K. B., & Moino, A. (2006). *Heterorhabditis amazonensis* n.sp.(rhabditida: Heterorhabditidae) from Amazonas, Brazil. *Nematology*, *8*(6), 853–867.

Bezemer, T. M., & van Dam, N. M. (2005). Linking aboveground and belowground interactions via induced plant defenses. *Trends in Ecology & Evolution*, *20*(11), 617–624.

Bezemer, T., Wagenaar, R., Van Dam, N., & Wäckers, F. (2003). Interactions between above-and belowground insect herbivores as mediated by the plant defense system. *Oikos*, *101*(3), 555–562.

Degenhardt, J., Hiltpold, I., Köllner, T. G., Frey, M., Gierl, A., Gershenzon, J., … Turlings, T. C. (2009). Restoring a maize root signal that attracts insect-killing nematodes to control a major pest. *Proceedings of the National Academy of Sciences*, *106*(32), 13213–13218.

Dragulescu, A. A. (2014). *xlsx: Read, write, format Excel 2007 and Excel 97/2000/XP/2003 files*. R Package Version 0.5.7. Retrieved from http://CRAN.R-project.org/package=xlsx

Dutky, S. R., Thompson, J. W., & Cantwell, G. E. (1962). A technique for mass rearing the greater wax moth (lepidoptera: Galleriidae). *Proceedings of the Entomological Society of Washington*, *64*(1), 56–58.

Filgueiras, C. C., Willett, D. S., Junior, A. M., Pareja, M., El Borai, F., Stelinski, L. L., & Duncan, L. W. (2016a). Stimulation of the salicylic acid pathway aboveground recruits entomopathogenic nematodes belowground. *PloS one*, *11*(5), e0154712.

Filgueiras, C. C., Willett, D. S., Pereira, R. V., Junior, A. M., Pareja, M., & L. W. Duncan (2016b). *Eliciting maize defense pathways aboveground attracts belowground biocontrol agents*. Scientific Reports 6.

Fox, J., & Weisberg, S. (2011). *An R companion to applied regression* (2nd ed.). Thousand Oaks, CA: Sage. Retrieved from http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Gaugler, R. & Kaya, H. K. (1990). *Entomopathogenic nematodes in biological control*. Boca Raton: CRC Press.

Downloaded by [UNICAMP] at 10:29 15 August 2017

Georgis, R., Koppenhöfer, A., Lacey, L., Bélair, G., Duncan, L., Grewal, P., … Van Tol, R. (2006). Successes and failures in the use of parasitic nematodes for pest control. *Biological Control*, *38* (1), 103–123.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology*, *43*, 205–227.

Hiltpold, I., Hibbard, B. E., French, B. W., & Turlings, T. C. (2012). Capsules containing entomopathogenic nematodes as a trojan horse approach to control the western corn rootworm. *Plant and Soil*, *358*(1–2), 11–25.

Hiltpold, I., & Turlings, T. C. (2008). Belowground chemical signaling in maize: When simplicity rhymes with efficiency. *Journal of Chemical Ecology*, *34*(5), 628–635.

Journey, A., & Ostlie, K. (2000). Biological control of the western corn rootworm (coleoptera: Chrysomelidae) using the entomopathogenic nematode, *Steinernema carpocapsae*. *Environmental Entomology*, *29*(4), 822–831.

Kaya, H. K., & Gaugler, R. (1993). Entomopathogenic nematodes. *Annual Review of Entomology*, *38* (1), 181–206.

Kaya, H. K., & Stock, S. (1997). Techniques in insect nematology. *Manual of Techniques in Insect Pathology*, *1*, 281–324.

Koppenhöfer, A. M., & Fuzy, E. M. (2009). Long-term effects and persistence of *Steinernema scarabaei* applied for suppression of anomala orientalis (coleoptera: Scarabaeidae). *Biological Control*, *48*(1), 63–72.

Lacey, L., Grzywacz, D., Shapiro-Ilan, D., Frutos, R., Brownbridge, M., & Goettel, M. (2015). Insect pathogens as biological control agents: Back to the future. *Journal of Invertebrate Pathology*, *132*, 1–41.

Lacey, L. A., & Georgis, R. (2012). Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. *Journal of Nematology*, *44*(2), 218–225.

McCoy, C. W., Shapiro, D. I., Duncan, L. W., & Nguyen, K. (2000). Entomopathogenic nematodes and other natural enemies as mortality factors for larvae of *Diaprepes abbreviatus* (coleoptera: Curculionidae). *Biological Control*, *19*(2), 182–190.

Metcalf, R., Krysan, J. L., & Miller, T. A. (2012). *Methods for the study of pest Diabrotica*. New York, NY: Springer Science & Business Media.

Parkman, J., & Smart Jr, G. (1996). Entomopathogenic nematodes, a case study: Introduction of *Steinernema scapterisci* in florida. *Biocontrol Science and Technology*, *6*(3), 413–420.

Parra, J. (1986). Criaç ao de insetos para estudos com patógenos. In S. B. Alves (Ed.), *Controle microbiano de insetos* (pp. 348–373). Piracicaba, Sao Paulo: Manole.

Peñuelas, J., Asensio, D., Tholl, D., Wenke, K., Rosenkranz, M., Piechulla, B., & Schnitzler, J. (2014). Biogenic volatile emissions from the soil. *Plant, Cell & Environment*, *37*(8), 1866–1891.

R Core Team. (2015). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Rasmann, S., Köllner, T. G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., … Turlings, T. C. (2005). Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature*, *434*(7034), 732–737.

Rasmann, S., & Turlings, T. C. (2007). Simultaneous feeding by aboveground and belowground herbivores attenuates plant-mediated attraction of their respective natural enemies. *Ecology Letters*, *10*(10), 926–936.

RStudio Team (2015). *RStudio: Integrated development environment for R. RStudio*. Boston, MA. Retrieved from http://www.rstudio.com/

Santos, V., Moino, Junior, Moreira, C. C., & Olinda, R. A. d. (2011). Virulence of entomopathogenic nematodes (rhabditida: Steinernematidae and heterorhabditidae) for the control of *Diabrotica speciosa* germar (coleoptera: chrysomelidae). *Ciência e Agrotecnologia*, *35*(6), 1149–1156.

Stuart, R. J., Shapiro-Ilan, D. I., James, R. R., Nguyen, K. B., & McCoy, C. W. (2004). Virulence of new and mixed strains of the entomopathogenic nematode *Steinernema riobrave* to larvae of the citrus root weevil *Diaprepes abbreviatus*. *Biological Control*, *30*(2), 439–445.

Thaler, J. S. (1999a). Induced resistance in agricultural crops: Effects of jasmonic acid on herbivory and yield in tomato plants. *Environmental Entomology*, *28*(1), 30–37.

Thaler, J. S. (1999b). Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature*, *399*(6737), 686–688.

Thaler, J. S., Fidantsef, A. L., Duffey, S. S., & Bostock, R. M. (1999). Trade-offs in plant defense against pathogens and herbivores: A field demonstration of chemical elicitors of induced resistance. *Journal of Chemical Ecology*, *25*(7), 1597–1609.

Thaler, J. S., Humphrey, P. T., & Whiteman, N. K. (2012). Evolution of jasmonate and salicylate signal crosstalk. *Trends in Plant Science*, *17*(5), 260–270.

Thaler, J. S., Stout, M. J., Karban, R., & Duffey, S. S. (2001). Jasmonate-mediated induced plant resistance affects a community of herbivores. *Ecological Entomology*, *26*(3), 312–324.

Toepfer, S., Peters, A., Ehlers, R.-U., & Kuhlmann, U. (2008). Comparative assessment of the efficacy of entomopathogenic nematode species at reducing western corn rootworm larvae and root damage in maize. *Journal of Applied Entomology*, *132*(5), 337–348.

Turlings, T. C., Hiltpold, I., & Rasmann, S. (2012). The importance of root-produced volatiles as foraging cues for entomopathogenic nematodes. *Plant and Soil*, *358*(1–2), 51–60.

Turlings, T. C., & Ton, J. (2006). Exploiting scents of distress: The prospect of manipulating herbivore-induced plant odours to enhance the control of agricultural pests. *Current Opinion in Plant Biology*, *9*(4), 421–427.

van Poecke, R. M., & Dicke, M. (2002). Induced parasitoid attraction by *Arabidopsis thaliana*: Involvement of the octadecanoid and the salicylic acid pathway. *Journal of Experimental Botany*, *53*(375), 1793–1799.

Van Tol, R. W., Van Der Sommen, A. T., Boff, M. I., Van Bezooijen, J., Sabelis, M. W., & Smits, P. H. (2001). Plants protect their roots by alerting the enemies of grubs. *Ecology Letters*, *4*(4), 292–294.

Walsh, G. C. (2003). Host range and reproductive traits of *Diabrotica speciosa* (germar) and *Diabrotica viridula* (f.)(coleoptera: Chrysomelidae), two species of south american pest rootworms, with notes on other species of diabroticina. *Environmental Entomology*, *32*(2), 276–285.

White, G. (1927). A method for obtaining infective nematode larvae from cultures. *Science*, *66* (1709), 302–303.

Wickham, H. (2009). *ggplot2: Elegant graphics for data analysis*. New York, NY: Springer. Retrieved from http://had.co.nz/ggplot2/book

Wickham, H. (2015). *tidyr: Easily Tidy Data with 'spread()' and 'gather()' functions*. R package version 0.3.0. Retrieved from http://CRAN.R-project.org/package=tidyr

Wickham, H., & Francois, R. (2015). *dplyr: A grammar of data manipulation*. R package version 0.4.3. Retrieved from http://CRAN.R-project.org/package=dplyr

Downloaded by [UNICAMP] at 10:29 15 August 2017