Mediation of herbivore-natural enemy interactions by *Neotyphodium* endophytes: The role of insect behavioural response

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Abstract

This study examined how differences in the behavioural response of two sod webworm species to *Neotyphodium* endophytes mediated their susceptibility to an entomopathogenic nematode. In choice and non-choice assays, *Parapediasia teterrella* (Zincken) clearly preferred endophyte-free tall fescue over endophyte infected tall fescue whereas *Fissicrambus mutabilis* (Clemens) displayed no clear preference for either. *P. teterrella* spent more time searching for suitable host material than *F. mutabilis* and committed to the first host encountered only 37% of the time whereas *F. mutabilis* did so 90% of the time. Increased foraging times associated with *P. teterrella* host selection translated into greater rates of infection by the nematode *Steinernema carpocapsae* (Wieser). Results demonstrate the potential importance of insect behavioural response to fungal endophytes in determining herbivore vulnerability to natural enemies and support the more general idea that the form of insect response to plant defenses may, in part, predict the outcome of insect-natural enemy interactions.

Keywords: tall fescue, sod webworm, entomopathogenic nematode, trophic interactions

Introduction

Neotyphodium endophytes are seed-borne fungal endosymbionts of many important cool-season grass species. While the fungus benefits from access to plant nutrients, infected plants may benefit from a number of ecological advantages including resistance to insect herbivory. The basis for endophyte-mediated insect resistance lies in the synthesis of a number of defensive alkaloids which may deter insect feeding (antixenosis), or reduce insect performance (antibiosis) (Braman *et al.* 2002; Bacon *et al.* 1997; Breen, 1994). Evidence from a number of studies indicates that *Neotyphodium*-mediated resistance may also influence interactions between insect herbivores and their natural enemies (Goldson *et al.* 2000; Bultman *et al.* 1997; Grewal *et al.* 1995), which may have community-wide implications (Omacini *et al.* 2000). The majority of work concerning the influence of *Neotyphodium* endophytes on higher-level trophic interactions (herbivore-natural enemy interactions) has focused on the effects endophyte-mediated defenses have on insect physiology. In theory, insect natural enemies may be adversely affected by direct exposure to endophyte-mediated toxins within the tissues of their herbivore prey/host, or benefit from the resulting prolongation of herbivore development (Faeth & Bultman 2002; Breen 1994; Popay & Rowan 1994) and weakened physiological state (Grewal *et al.* 1995; Clancy & Price 1987). Although *Neotyphodium* endophytes may also influence insect behaviour (Richmond & Shetlar, 1999, 2000), the implications of this for insect natural enemy interactions have not been explicitly examined.

The effects of *Neotyphodium* endophytes on insect-natural enemy interactions may range widely and are often difficult to predict. For example, Grewal *et al.* (1995) reported that Japanese beetle larvae (*Popillia japonica* Newman) feeding on the roots of endophyte-infected tall fescue were more susceptible to infection by entomopathogenic nematodes and suggested that reduced vigour due to starvation increased larval susceptibility to the nematode. Conversely, Richmond *et al.* (2004) demonstrated that fall armyworm larvae feeding on endophyte infected perennial ryegrass were less susceptible to entomopathogenic nematodes and suggested that endophyte-mediated toxins within the armyworm tissues reduced the virulence of the pathogenic bacteria vectored by the nematode. Other studies have demonstrated that insects feeding on endophyte infected plants may be poorer quality hosts for parasitoids (Barker & Addison 1996) and theoretical predictions based on the slow growth-high mortality hypothesis (Clancy & Price 1987) imply that prolonged developmental times associated with herbivores feeding on endophyte-infected plants (Hardy *et al.* 1986; Breen 1994; Popay & Rowan 1994) may increase herbivore susceptibility/vulnerability to natural enemies.

Compartmentalisation of endophyte-mediated effects on insect-natural enemy interactions as direct or indirect has provided some basis for predicting the outcome of interactions as either positive or negative at the third trophic level (Faeth & Bultman 2002). In general, evidence suggests that direct effects of endophytes on insect natural enemies tend to be negative for the natural enemy (Richmond *et al.* 2004; Kunkel & Grewal 2003; Faeth & Bultman 2002; Barker & Addison 1996). However, outside of one study (Grewal *et al.* 1995), indirect effects, which would include endophyte-mediated effects on insect behaviour that affect interactions with natural enemies, have received very little attention. The lack of published data in this regard has hindered the development of a broader conceptual framework for describing and predicting the range of effects *Neotyphodium* endophytes may have on interactions between insect herbivores and their natural enemies. The development of such a framework may help facilitate integration of endophyte-mediated resistance with biological controls in managed systems and clarify areas where more basic research is needed.

One of the primary needs then for understanding the full range of impacts *Neotyphodium* endophytes may have on insect-natural enemy interactions, is data describing indirect endophyte-mediated effects. Evidence suggests that herbivore behavioural response to endophyte-mediated defenses, particularly increases in movement, may deserve more attention in this regard (Richmond & Shetlar 1999, 2000). The potential risks associated with herbivore movement have been demonstrated repeatedly in the literature (Bergelson & Lawton 1988; Martson *et al.* 1978; den Boer 1971) and foraging strategies employed by insect herbivores are thought to reflect an evolutionary balance between the costs and benefits associated with foraging within a particular environment (Montllor & Bernays 1993). In a review of the relationship between toxin spatial distribution and insect adaptation to toxins, Hoy *et al.* (1998) point out that insect herbivores frequently avoid plant defensive compounds through behavioural responses including redistribution within a plant canopy and increased movement to neighbouring plants. Just how such movement may alter the relationship between predator (or parasite) and prey remains unclear.

Table 1 Cumulative percentage ± SE of sod webworm second instar larvae observed on or under the host plant material, which they were webbed to after 24 h, at the end of seven different time intervals (1 minute, 5 minutes, 10 minutes, 30 minutes, 1 hour, 12 hours or 24 hours) in choice test arenas containing endophyte infected and uninfected tall fescue.

Insect Species	1 minute	5 minutes	10 minutes	30 minutes	1 hour	12 hours	24 hours
P. teterrella	26.7±4.7a	36.7±4.7a	46.7±3.3a	60.0±4.7a	70.0±4.7a	80.0±4.7a	90.0±2.4a
F. mutabilis	60.0±47b	70.0±4.7b	96.7±3.3b	96.7±4.7b	96.7±4.7b	96.6±4.7a	96.6±2.4a

* Numbers within a column followed by the same letter are not significantly different (α=0.05)

Figure 1 Proportion of *Parapediasia teterrella* and *Fissicrambus mutabilis* second instar larvae residing on clippings taken from endophyte infected (E+) and uninfected (E-) tall fescue, or not residing on any clippings (uncommitted) after 24 hours in Petri dish choice assays. Error bars represent standard error (SE)

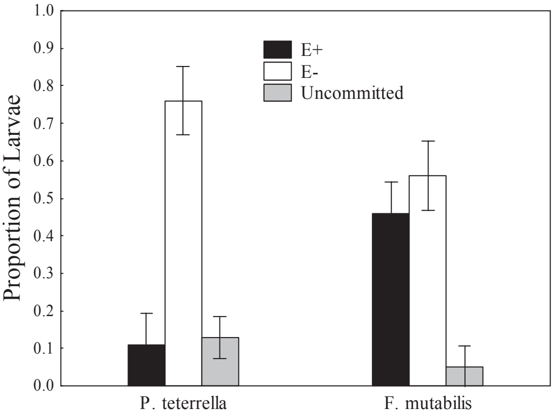
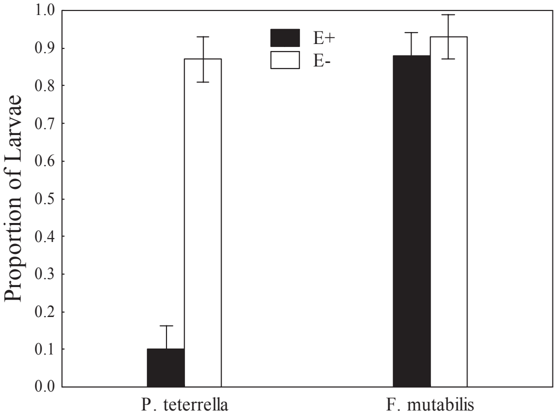


Figure 2 Proportion of *Parapediasia teterrella* and *Fissicrambus mutabilis* second instar larvae residing on clippings taken from endophyte infected (E+) or uninfected (E-) tall fescue after 24 hours in Petri dish non-choice assays. Error bars represent standard error (SE)



Sod webworms are a ubiquitous group of Lepidopteran insects in the family Crambidae which, as larvae, feed above ground on the leaf and stem tissues of many species of cool-season grasses including many of our most agronomically important turf and pasture species. Their status as pests of cereal and forage crops has been known for some time (Ainslie 1922) and their ability to damage turfgrass has been thoroughly documented (Bohart 1947; Heinrichs 1973). In temperate North America, both univoltine and multivoltine species are common. Unlike many Lepidoptera, sod webworm moths usually oviposit by scattering their eggs about the vegetation canopy while in flight (Vittum *et al.* 1999). During at least the first two larval instars, most sod webworms are "window feeders", quickly selecting and webbing themselves to a leaf blade or clipping and feeding on one side of the leaf from within the safety of the silken pocket they have constructed (Vittum *et al.* 1999). However, despite very similar life history strategies, preliminary feeding trials have indicated obvious interspecific differences in behavioural responses to endophyte infected plants (Richmond, unpublished).

Entomopathogenic nematodes are ubiquitous natural enemies of insects. These nematodes infect insects by entering through natural body openings, or by penetrating the insect cuticle directly. Once inside the insect, entomopathogenic nematodes release a lethal payload of symbiotic bacteria which generally kills the insect host in 24 to 48 h (Forst *et al.* 1997). *Steinernema carpocapsae* (Wieser) is one species of entomopathogenic nematode which has received much attention because of its potential as a biological control agent. Although host-finding behaviour varies among nematode species, *S. carpocapsae* employs a "sit and wait" or "ambush" strategy which depends heavily on movement bringing the insect host into striking distance (Gaugler 2002). As a result, *S. carpocapsae* has been used widely as a biological control against free-ranging, mobile insects such as Lepidoptera larvae.

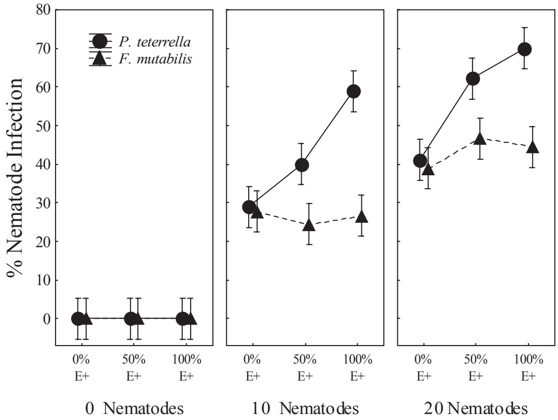
Using two different sod webworm species and the entomopathogenic nematode *S. carpocapsae*, this study examined how differing insect behavioural responses to *Neotyphodium* endophytes influences their susceptibility to a common natural enemy, *S. carpocapsae*. To accomplish this, the behavioural response of the sod webworms *Parapediasia teterrella* (Zincken) and *Fisicrambus mutabilis* (Clemens) to endophyte infected and uninfected tall fescue was characterised in detail. The hypothesis that interspecific differences in behavioural response to endophyte-mediated resistance may influence insect susceptibility to natural enemies was then tested in laboratory assays using the entomopathogenic nematode *S. carpocapsae*. Results of this study provide insight into an area of endophyte-insect-natural enemy interactions which has received very little attention and help inform a broader conceptual framework for future work and integration of endophyte-mediated resistance and biological controls.

Materials and Methods

Biological materials

Plant materials used in the present study were acquired from a greenhouse grown isoline cultured from the tall fescue cultivar

Figure 3 Percentage of *Parapediasia teterrella* and *Fisicrambus mutabilis* second instar larvae infected by the entomopathogenic nematode *Steinernema carpocapsae* after 24 hours in Petri dishes containing 100, 50, or 0% endophyte infected (E+) grass clippings and 0, 10 or 20 infective juvenile nematodes. Error bars represent standard error (SE)



“Goalkeeper”. Both endophyte infected (E+) and uninfected (E-) lines have been maintained in greenhouse culture for more than 3 years by occasional separation and sprigging of vegetative tillers. The endophyte *Neotyphodium coenophialum* (Morgan-Jones & Gams) was originally removed from the E- portion of the culture using the systemic fungicide propiconazole applied at six times the label rate and re-sprigging has been performed several times since then. Cultures are regularly monitored to ensure 100% pure E+ and E- plant material using tissue-print immunoblotting procedures (Agrinostics Ltd. Co., Watkinsville, GA, U.S.A.). Larvae of the sod webworms *Parapediasia teterrella* (Zincken) and *Fissicrambus mutabilis* (Clemens) were obtained by light trapping adult moths, identifying them to species and placing them individually into 20 ml vials to oviposit. Eggs were collected from the vials daily and placed in Petri dishes lined with moist filter paper until eclosion. On eclosion, larvae were transferred to a clean Petri dish and reared to the second instar on clippings taken from a Kentucky bluegrass lawn. The entomopathogenic nematode *S. carpocapsae* (All strain) was cultured in the laboratory using larvae of the greater wax moth, *Galleria mellonella* (L.). Infective (dauer) juvenile nematodes were collected from infected *G. mellonella* cadavers following the procedures of White (1927) and held at 10°C in large Petri dishes (15 cm diameter) in 0.5 cm of water. Nematode cultures were continually cycled through *G. mellonella* larvae (2 week intervals) to ensure ready access to healthy infective juveniles of roughly the same age.

Choice assays

Clippings (approx. 2.0 cm in length) were taken from a single genotype of greenhouse grown tall fescue plants (var. Goalkeeper) known to be infected or uninfected (tissue print immunoblotting) by *N. coenophialum*. Two clippings from each type of plant material (E+ or E-) were spaced evenly and in an alternating fashion around the inside perimeter of a plastic Petri dish (9.0 cm diameter) containing moist filter paper. The identity of each clipping (E+ or E-) was marked on the bottom of the Petri dish directly below each clipping using a permanent black marker to aid in observations. One, second instar larva of either *P. teterrella* or *F. mutabilis* was then placed in the middle of the arena and allowed 24 h to select a host. The location of each larva was then recorded as either E+, E- or uncommitted and the experiment was repeated using 100 larvae of each species Host selection criteria was satisfied if, after 24 h, larvae had webbed themselves to the leaf and begun feeding. Interspecific comparisons for each category (E+, E-, uncommitted) were made using the Mann-Whitney U-test and intraspecific comparisons for endophyte preference were made using the Sign test (Statsoft Inc. 2006)

Non-choice assays

Non-choice assays were performed using an arena similar to that used in choice assays, but only E+ or E- were offered to the insect. One, second larva of either *P. teterrella* or *F. mutabilis* was placed in the middle of the arena and allowed 24 h to commit to a host. The test was again repeated 100 times with larvae of each sod webworm species. Host selection criteria were the same as for choice assays and after 24 h the location of each larva was recorded as either committed or uncommitted. Interspecific comparisons for each grass type (E+, E-) were made using the Mann-Whitney U test and intraspecific comparisons for endophyte preference were made using the Sign test (Statsoft Inc. 2006).

Extended observation

Extended observations were made using the same experimental arenas used for the choice assays. One second instar sod webworm larva was introduced into the centre of the arena and its location was recorded at the end of seven subsequent time intervals (1 min, 5 min, 10 min, 30 min, 1 h, 12 h, and 24 h). Each replicate consisted of ten individual larvae of each species and there were three complete replicates. The cumulative proportion of larvae at the end of a given time interval which had committed to a host (i.e. the clipping to which they were webbed at 24 h after introduction) was arcsin square root transformed and data were analysed using univariate repeated-measures ANOVA with Greenhouse-Geyser-adjusted degrees of freedom. The Greenhouse-Geyser procedure adjusts the degrees of freedom for the tests based on intercorrelations among the repeated measures hypotheses (Statsoft Inc. 2006).

Endophytes and entomopathogenic nematodes

To investigate the influence of endophyte infected plant material on sod webworm susceptibility to entomopathogenic nematodes, an arena similar to the arena used for the choice assays was employed. However, three different choice scenarios were used. These included 1) four clippings taken from endophyte infected plants (100% E+), 2) two clipping from infected plants and two clippings from uninfected plants (50% E+), and 3) four clippings from uninfected plants (0% E+). Prior to adding the clippings, a pre-determined number of entomopathogenic nematodes (*Steinernema carpocapsae* Weiser) was delivered to the filter paper in the bottom of each dish using a hand-held spray bottle calibrated to deliver 0, 10 or 20 nematodes/ml. Clippings were then spaced evenly around the outside perimeter of the Petri dish on moist filter paper and, for the 50% E+ scenario, clippings were also placed in an alternating fashion so that two E+ clippings were never adjacent to each other. The identity of each clipping (E+ or E-) was marked on the bottom of the Petri dish directly under each clipping using a permanent black marker to aid in observations. One, second instar larva of either *P. teterrella* or *F. mutabilis* was then placed in the middle of the arena and allowed 24 h to select a host. After 24 h the location of each larvae was recorded as for the choice assay (E+, E- or uncommitted). Each larva was then removed from its arena using soft forceps and placed in a clean Petri dish containing moist filter paper and fresh food (E-). Larvae were then observed over the next 5 days for mortality and dead larvae were immediately placed on small White's traps (White 1927) for 14 days to confirm nematode infection as the cause of death. Ten larvae of each species subject to each of the nine experimental treatments (three clipping combinations x three nematodes densities) was considered a replicate and each treatment was replicated three times in each of three trials for a total of 90 insects of each species per treatment. The mean proportions of infected larvae for each trial were arcsin square root transformed and analysed using three-way factorial analysis of variance. A separate slopes model was then used to describe inter-specific differences in the relationship between nematode infection and endophyte infection (%E+) at each nematode density (Statsoft Inc. 2006).

Results

Choice assays

In choice assays, *P. teterrrella* displayed a stronger preference for E- plant material with *P. teterrella* larvae committing to E- more frequently (Z=2.57; P=0.01) and committing to E+ less frequently than *F. mutabilis* (Z=-4.28; P<0.0001) (Fig. 1). The frequency of uncommitted larvae did not differ significantly between sod webworm species (Z=0.98; P=0.33). *P. teterrella* also committed to E- more frequently than it did to E+ (Z=6.86; P<0.00001), whereas *F. mutabilis* committed to either E+ or E- with roughly equal frequency (Z=0.86; P=0.39).

Non-choice assays

In non-choice assays, *P. teterrella* committed to E+ less frequently than *F. mutabilis* (Z=-9.04; P<0.000001), whereas both species committed to E- with roughly equal frequency (Fig. 2). While *P. teterrella* committed to E- more frequently than it did to E+ (Z=8.18; P<0.000001), *F. mutabilis* committed to E+ and E- with equal frequency (Z=1.34; P=0.18).

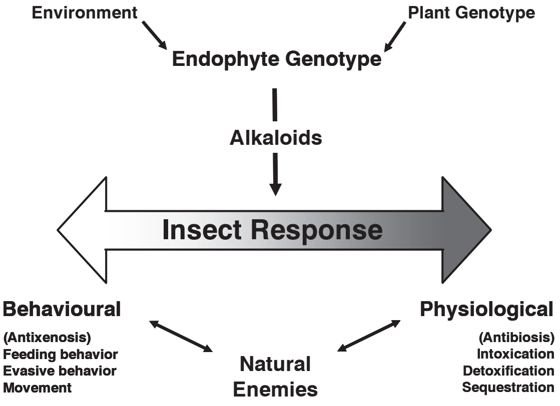
Extended observations

Extended observations indicated that, when both E+ and E- clippings were present, *P. teterrella* spent significantly more time searching for an acceptable host than *F. mutabilis* (time x species interaction F=9.52; df (adj.)=2.21, 8.85; P=0.00002). A larger proportion of *P. teterrella* larvae remained uncommitted through the first hour after being introduced into the choice arena compared to *F. mutabilis*. On average, greater than 95% of *F. mutabilis* larvae committed to a host within 10 minutes of being introduced into the choice arena, whereas only 47% of *P. teterrella* larvae had committed within the same amount of time (Table 1). In general, both species spent the majority of time either searching for a suitable host, or sampling the plant material they encountered and very little time was spent resting away from the plant material.

Endophytes and entomopathogenic nematodes

The proportion of sod webworm larvae infected by the entomopathogenic nematode *S. carpocapsae* was a function of sod webworm species, the percentage of endophyte infected clippings in the arena, and the density of entomopathogenic nematodes (three way interaction F=4.78; df=4, 36; P=0.003) (Fig. 3). Therefore, a separate slopes model was employed to describe interspecific (sod webworm species) differences in the relationship between nematode infection and the percentage of endophyte infected clippings at each nematode density. No sod webworms of either species became infected by nematodes when nematode density was zero (Fig. 3a), and nematode infection did not differ between sod webworm species when only E- clippings were present in the arena, regardless of nematode density (for nematode densities of 10 and 20 respectively: F=0.02; df=1, 14; P=0.88 and F=0.53; df=1, 14; P=0.48). However, when 50% E+ or 100% E+ clippings were presented to the larvae, nematode infection rates differed significantly between species at nematode densities of 10 (F=16.77; df=2, 14; P=0.0002) and 20 (F=24.66; df=2, 14; P=0.00003) nematodes per Petri dish (Figs. 3b and c). Under these nematode densities, infection rates in *P. teterrella* increased as the percentage of endophyte infected plant material increased (t=5.79; P=0.00005; r^2=0.78 and t=6.90; P=0.000007; r^2=0.83 respectively) whereas, infection rates for *F. mutabilis* were relatively unaffected by the percentage of E+ clippings (for nematode densities of 10 and 20 respectively: t=-0.21; P=0.83 and t=1.33; P=0.21).

Figure 4 A conceptual model of *Neotyphodium*-insect-natural enemy interactions using insect response (behavioral vs physiological) to endophyte-mediated defensive compounds as the primary predictor of effects at the third trophic level. In this model, plant genotype and other environmental variables (drought, fertility, temperature, etc…) mediate the strength of alkaloid expression whereas endophyte genotype mediates the types of alkaloids expressed. Insect response may vary from primarily behavioural to primarily physiological depending on the degree of behavioural and physiological adaptation to the alkaloids being expressed. Effects at the third trophic level (natural enemies) will vary with the form of insect response to endophyte-mediated alkaloids.



Discussion

The question of how herbivore behaviour, particularly movement, influences the dynamics of predator-prey interactions is not new (Rilling *et al.* 1959; Herzog & Burghardt 1974). Insect behaviour may either increase or decrease the probability of an encounter with a potentially dangerous predator, parasite, or pathogen. For instance, activities associated with insect feeding, such as leaf rolling, provide visual cues which are relatively easily detected by avian predators (Heinrich & Collins 1983) whereas cryptic positioning on parts of the host-plant, which provide the least contrast between the insect and plant (Greene 1989), may allow herbivores to go unnoticed by natural enemies even at relatively short distances. Movement, however, is inherently risky for herbivores (Montllor & Bernays 1993; Bergelson & Lawton 1988; Martson *et al.* 1978; den Boer 1971) because it increases the likelihood of encountering or being noticed by a natural enemy. In the present study, interspecific differences in sod webworm movement resulting from a preference for E- plant material translated directly into differential infection by the entomopathogenic nematode.

There are several aspects of the present study that are important for interpretation and generalisation of results. Although the chosen experimental arena was deemed necessary to facilitate observations, the author is aware of the possible shortcomings of such reductionist approaches, and a few in particular are especially worthy of further consideration. The arena used in the present study was intended to simulate a uniformly dense stand of grass containing E+ and E- plants. However, in reality the density of grass swards may vary widely depending on management and intended use. While turfgrasses tend to be very uniform, pastures and other semi-natural swards usually contain mixtures of grasses and forbs (Stuedemann & Seman 2005) and the spatial distribution and density of endophyte infected plants may vary considerably in these systems (Saikkonen *et al.* 1998). Individual plants are likely to be larger and more robust in semi-natural swards which can also affect plant density (Lush 1990). Differences in uniformity and density could potentially alter the spatial dynamics of insect movement between suitable and unsuitable host-plants or patches with unknown consequences on insect-natural enemy interactions.

Characteristics of the natural enemy may also determine the degree to which insect response mediates insect-natural enemy interactions. In particular, the influence of reactive distance (i.e. the distance at which a natural enemy perceives prey) (Stamp & Wilkens 1993) and foraging strategy (Koppenhöfer & Kaya 1996) of natural enemies has been previously demonstrated. Although the reactive distance of *S. carpocapsae* is not specifically known, its ambushing strategy implies that a potential host must contact or pass very close to the infective juvenile nematode in order to be attacked and infected. This strategy lies in contrast to other entomopathogenic nematodes, such as *Heterorhabditis bacteriophora*, which displays a cruising strategy in which infective juvenile nematodes actively search for hosts. These differences in foraging strategies among entomopathogenic nematode species form, in large part, the basis for their efficacy and commercial use against specific insect pests in the field. While ambushers are most effective against more mobile insects such as lepidopteran larvae, cruisers are usually most effective against less mobile, soil-inhabiting insects such as scarab larvae (Gaugler 2002). In a study by Koppenhöfer and Fuzy (2003), efficacy of the cruising nematode *H. bacteriophora* against several white grub species feeding on endophyte infected grasses was only marginally enhanced and results were highly variable. Furthermore, it is unclear weather or not endophyte infection actually altered white grub behaviour in this study since results of Grewal et al. (1995) implicate a weakened physiological state as the main reason underlying enhanced nematode susceptibility of white grubs feeding on the roots of endophyte infected grasses. Just how insect behavioural responses to endophyte infection influence their vulnerability to natural enemies which use cruising or other alternative foraging strategies is unclear and worthy of further investigation.

The particular life stage of the insects used in the current study was chosen as a matter of compromise. Second instar sod webworms were used in the study for two important reasons. First instars, although the logical choice for such a study, are too small to accurately determine nematode infection without microscopic dissection of each individual larva. White's traps provide a very dependable method for determining nematode induced mortality by facilitating the collection infective juveniles from the host cadaver. However, the host insect must be large enough to sustain the reproductive cycle of the nematode and serve as an adequate resource for the production of a reasonable number of infective juvenile nematodes. Second instar sod webworm behaviour and feeding habits are similar to those of first instar larvae (Vittum *et al.* 1999; Richmond, personal observation), but second instars provide enough of a resource for nematode reproduction to accurately determine nematode infection as the cause of death.

The types and concentrations of alkaloids present in E+ plants is likely to be the most important determinant of insect response and there are several factors which may influence variation in the expression of these alkaloids. While the particular types of alkaloids produced in E+ plants are mainly a function of the fungal strain or genotype (Popay & Bonos 2005), concentrations of alkaloids present in plant tissues may vary with plant genotype (Hiatt & Hill 1997; Rowan & Latch 1994; Roylance *et al.* 1994), plant tissue type (Lane *et al.* 2000; Ball *et al.* 1997; Siegel & Bus, 1996), temperature (Breen 1992), soil fertility (Arechavaleta *et al.* 1992; Belesky *et al.* 1988), soil moisture (Bultman & Bell 2003), and season (Ball *et al.* 1995; Belesky *et al.* 1987). Furthermore, individual alkaloids may vary with respect to their activity against insect herbivores. Ergot alkaloids (particularly ergovaline), loline alkaloids, and lolitrem B may be toxic and/or deterrent to many insects (Popay & Bonos 2005; Wilkinson *et al.* 2000; Popay & Lane 2000; Siegel & Bush 1996), but apparently have little or no direct impact on others (Richmond *et al.* 2004; Kunkel & Grewal, 2003). Although the alkaloid peramine does not have major effects on insect herbivores, it is a strong deterrent to Argentine stem weevil and has been implicated as a marker enabling this insect to avoid other more potent endophyte-mediated toxins (Rowan & Latch 1994). Because different alkaloids may elicit different insect responses, variability in alkaloid expression may play an important role in mediating interactions between insect herbivores and their natural enemies. Further work will be necessary to determine which alkaloid(s) are responsible for the strong behavioural response displayed by *P. teterrella*.

In theory, insect response to toxins may vary across a continuum from primarily physiological to primarily behavioural based on the degree of genetic and behavioural adaptation (Hoy *et al.* 1998) and insect response to endophyte-mediated toxins should be no exception in this regard. In their review of insect adaptation to toxin spatial heterogeneity, Hoy *et al.* (1998) suggest the need for more attention to insect behaviour in studies aimed at evaluating plant-protective toxins. The current study reinforces this viewpoint, and illustrates that natural enemies could potentially benefit from plant defenses which elicit primarily behavioural responses from insects. However, natural enemies may benefit from toxin-mediated changes in insect behaviour other than increased movement. In a study by Koppenhöfer *et al.* (2000) scarab larvae exposed to sublethal doses of imidacloprid were more susceptible to entomopathogenic nematodes. The authors concluded that sluggishness and lack of normal grooming and evasive behaviors made the larvae more vulnerable to nematode infection. Therefore, insect response to endophyte infection may serve as an important platform in the framework describing multitrophic interactions involving *Neotyphodium* endophytes, insects and their natural enemies.

The current study provides the first evidence of a linkage between insect behavioural response to *Neotyphodium* endophytes and its role in mediating susceptibility to a natural enemy. No doubt, much more work using a broader range of insect herbivores and natural enemies will be necessary to determine how general these findings are. However, when combined with the findings of related studies, the present study creates a more comprehensive picture of endophyte-insect-natural enemy interactions. The preponderance of studies regarding the influence of *Neotyphodium* endophytes on herbivore-natural enemy interactions have been concerned primarily with the effects endophyte-mediated defenses have on insect physiology. Although natural enemies may be adversely affected by exposure to endophyte-mediated toxins within the tissues of their prey/host, they may also or benefit from the resulting prolongation of herbivore development (Faeth & Bultman 2002; Breen 1994; Popay & Rowan 1994) and weakened physiological state (Grewal *et al.* 1995; Clancy & Price 1987) resulting from ingestion of endophyte-mediated alkaloids. Although the ability of *Neotyphodium* endophytes to influence insect behaviour has been previously demonstrated (Richmond & Shetlar 1999, 2000), this is the first study which clearly demonstrates how endophyte-mediated affects on insect behaviour may alter insect susceptibility to natural enemies. The model in Figure 4 represents a synthesis of insect responses to endophyte-mediated defenses and uses the form of response as the linchpin for describing/predicting effects at the third trophic level. This model may serve as a predictive framework for integrating endophyte-mediated resistance with biological control in managed systems such as turfgrass and help identify and frame future questions surrounding endophyte effects on insect-natural enemy interactions.

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