OVERWINTERING BEHAVIOR OF THE ENTOMOPATHOGENIC NEMATODES *STEINERNEMA SCARABAEI* AND *HETERORHABDITIS BACTERIOPHORA* (RHABDITIDA: STEINERNEMATIDAE AND HETERORHABDITIDAE) AND THEIR WHITE GRUB HOSTS (COLEOPTERA: SCARABAEIDAE)

by

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ABSTRACT OF THE THESIS

OVERWINTERING BEHAVIOR OF THE ENTOMOPATHOGENIC NEMATODES *STEINERNEMA SCARABAEI* AND *HETERORHABDITIS BACTERIOPHORA* (RHABDITIDA: STEINERNEMATIDAE AND HETERORHABDITIDAE) AND THEIR WHITE GRUB HOSTS (COLEOPTERA: SCARABAEIDAE)

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Entomopathogenic nematodes (EPN) *Steinernema scarabaei* and *Heterorhabditis bacteriophora,* species endemic to New Jersey turfgrass habitats, have great potential as biological control agents of various white grub pest species. EPN have potential for long-term white grub suppression, but to more reliably achieve this, a better understanding is necessary of the nematodes' survival mechanisms expresses during harsh seasonal conditions. Infective juveniles (IJ) may employ similar vertical migratory patterns as other soil-inhabiting animals during harsh seasonal conditions. To determine the existence of vertical relocation as an overwintering mechanism we investigated the vertical

distribution of *S. scarabaei* and *H. bacteriophora* relative to (1) fluctuating soil temperature and (2) changing vertical position of two white grub hosts, Japanese Beetle (*Popillia* japonica) and oriental beetle (*Anomala orientalis*), during the late fall, winter and early spring season. The vertical distributions of white grub and IJ populations were monitored every 14-18 days in established turf plots (3-4 years old) from mid-October to the first week of May in two consecutive years (2006-2007 and 2007- 2008). The vertical distribution of *S. scarabaei*, *H. bacteriophora,* and *Steinernema carpocapsae* did not change from the fall to the spring season. Soil temperature did not appear to influence IJ vertical distribution. The vertical distribution of both white grub species changed with temperature during fall and spring but not in winter. Overwintering *S. scarabaei* and *H. bacteriophora* IJs were only recovered in the soil. There was no evidence for successful in-host survival or latent infection in endemic white grub populations. Although the relationship between temperature and vertical distribution for EPN and their white grub hosts differed, a considerable degree of spatial and temporal overlap was observed between respective EPN and host populations. Overwintering EPN appear to survive primarily or exclusively as IJs in the soil and presumably employ various physiological and biochemical mechanisms as survival strategies during seasonally cold and freezing conditions.

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DEDICATION

I dedicate this thesis to the memory of my Grandma Ruth *Z”L*, who passed away at the beginning of my graduate studies.

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INTRODUCTION

Entomopathogenic nematodes (EPN) in the families Steinernematidae and Heterorhabditidae (Order: Rhabditida) are soil-inhabiting obligate insect parasites. These lethal pathogens have been recovered from soils throughout the world with their distribution strongly determined by the availability of susceptible insect hosts (Hominick 2002). The only EPN stage capable of surviving outside of hosts is the infective juvenile (IJ). The sole functions of the IJ are to disperse, survive harsh environmental conditions, and locate a host (Grewal et al. 2006). Each of these behaviors contributes to distinct spatial and temporal distributions of IJ populations. Unlike the relationship between movement and host seeking, the relationship between movement and survival is far from resolved.

EPN have received considerable attention for their biological control potential, yet various ecological and commercial production challenges have limited their implementation as a competitive short-term biopesticide (Grewal et al. 2005, Shapiro-Ilan et al. 2002). In light of these limitations, long-term control strategies such as innoculative releases in combination with conservation biological control may offer more feasible alternatives for EPN implementation (Parkman 1996, Koppenhöfer and Fuzy 2009, Lewis et al. 1998). A better understanding of how endemic EPN species naturally survive prolonged periods of harsh conditions will be crucial for enhancing the long-term performance of EPN as a biological control agent.

The IJ's ability to overcome various physical (e.g., thermal extremes) and biological (e.g., insect hosts) challenges (Wharton 2004) is paramount for the long-term persistence of an EPN meta-population (Stuart et al. 2006). According to Wharton (2004), parasitic nematodes may employ four possible survival mechanisms including: (1) a migratory response, (2) acclimation, (3) ecological synchronization, and (4) dormancy. EPN endemic to temperate climates should possess distinct overwintering strategies that allow them to survive prolonged periods of cold during the winter while retaining the ability to infect when thermally favorable conditions return in the spring and summer (Bornstein-Forst et al. 2005). Previous authors investigating EPN persistence and survival (García del Pino and Palomo 1996, Susurluk and Ehlers 2008) have speculated that IJs may employ similar vertical migratory patterns as other soil-inhabiting animals to avoid harsh thermal or moisture conditions. From this, the questions at hand are whether the vertical position of IJs changes as seasonal soil temperatures change and whether this vertical migration pattern is correlated with that of their natural insect host(s).

According to Wallace (1958), three general phases of movement are associated with the IJ: emergence from a host, movement in the soil towards a new host, and host invasion. During the IJ's transit phase, various biotic or abiotic soil factors will interact with innate behavioral tendencies resulting in distinct spatiotemporal distributions (Lewis et al. 1992, 2006, Campbell et al. 1996). Soil, the natural habitat of EPN, is a dynamic system in a continual state of flux (Kaya 1990) perpetuated by changes in weather, vegetation and soil

management (Barbercheck 1992). Changes in the physical and chemical parameters of the soil strongly influence movement (Portillo-Aguilar et al. 1999, Alumai et al. 2006, Bakonyi et al. 2007) since the actual site of movement is the water-film, which coats the pore spaces of the soil matrix (Wallace 1958, Gaugler and Bilgrami 2004). Although soil factors such as moisture, texture and temperature strongly mediate IJ movement (Stuart et al. 2006), the positions of edaphic insect hosts, whose movements are influenced by similar abiotic soil conditions, may play a more influential role in the occurrence and persistence of an EPN population (Glazer et al. 1996).

The vertical migration of various white grub species in the soil is often associated with seasonal changes in soil temperature (Villani and Wright 1990, Villani et al. 1999). The Japanese Beetle (*Popillia japonica* Newman) and the oriental beetle (*Anomala orientalis* Waterhouse), two common turfgrass insect pests of the northeastern United States, display seasonally distinct vertical movement patterns in the fall and spring (Potter 1998, Villani et al. 1999). The downward movement of *P. japonica* and *A. orientalis* in the fall begins when soil temperatures within the first 5 cm drop to or below 15 °C (Vittum et al. 1999). Downward movement continues until soil temperature at the 5 cm depth reaches 10 °C (Vittum et al. 1999). In the following spring, *P. japonica* and *A. orientalis* begin their upward movement towards the root zone when soil temperatures within the first 5 – 8 cm rises above 10 °C (Vittum et al. 1999). In addition to soil temperature, age and developmental stage appear to play important roles in the seasonal locomotion patterns of overwintering *P. japonica* (Villani and Nyrop

1991). Understanding larval behaviors of economically important scarab species is not only beneficial in developing an ecologically fine-tuned life history table but also in predicting the degree of spatial and temporal overlap a biological control agent will share with its target host over an extended period of time.

The main function of the IJ is to locate a suitable host so it can develop and reproduce (Lewis et al. 1992). Characterization of IJ foraging strategy can be based on (1) how the IJs moves through their environment as well as (2) response to stimuli (Lewis et al. 2006). IJ foraging strategies range between the extremes of sit-and-wait (ambushers) to widely searching (cruisers) (Lewis et al. 2002, Lewis et al. 2006). Ambusher foragers such as *Steinernema carpocapsae* (Weiser) are more likely to contact highly mobile hosts on or near the surface, resulting in a shallow vertical distribution, whereas cruise foragers such as *Heterorhabditis bacteriophora* Poinar and *Steinernema scarabaei* Stock & Koppenhöfer are better adapted to finding more sedentary hosts throughout the soil profile, resulting in a wider vertical distribution. In the absence of significant host stimuli, cruisers may display prolonged periods of movement while ambushers may be completely sedentary until various short-range cues (e.g., physical contact) or long-range cues (e.g., CO_2) are detected (Lewis et al. 2002). Following the detection of stimuli, IJ spatial distribution will change due to adjustments in its movement patterns, increasing its probability of locating a suitable insect host.

Upon entering the host's body cavity, the IJ releases symbiotically associated bacteria, which it carries in its intestinal tract. Bacteria and

nematodes combine to overcome the host's immune response and kill it, typically within 1-3 days. The bacteria propagate and protect the cadaver from colonization by other microorganisms. The nematodes develop through one to three generations, feeding on the bacteria and host tissues metabolized by the bacteria. Depleting food resources in the host cadaver lead to the development of a new cohort of IJs that emerge back into the soil and return to a searching mode to find a new host.

Although IJ distribution is primarily driven by their foraging strategies, fluctuating abiotic soil factors can affect the rate and degree of dispersal (Campbell et al. 1996, 1998). Temperature is an environmental factor of great significance to the behavioral and biological actions of an IJ (Rio and Cameroon 2000, Ferguson et al. 1995, Grewal et al. 1994). The thermal range of IJ activity varies widely by species and appears to be independent of geographic origin (Grewal et al. 1994). Within a single species, host seeking, host penetration and developmental recovery possess distinct yet overlapping thermal ranges. (Grewal et al. 2006). Although soil moisture is probably the most important factor affecting IJ dispersal (Wallace 1958, Glazer et al. 1996, Koppenhöfer and Fuzy 2007), temperature may have considerable influence on IJ orientation (Dusenbery 1989) as well as on the degree of movement following host cue reception (Csontos 2002, Susurluk 2008). Soil temperature follows a fluctuating gradient influenced by changes in moisture which in turn is influenced by meteorological events and soil management techniques (Mail 1930).

The extent of influence soil temperature has on IJ locomotion is not clearly understood. Microhabitats that possess favorable characteristics for energy use, reproduction or survival may be associated with distinct thermal gradients that could stimulate the IJ to orient and move towards them (Wharton 2004). Under highly artificial conditions (e.g., gel-based medium), *Meloidogyne incognita* (Kofoid and White) has been observed to change its spatial distribution relative to fluctuating thermal gradients (Dusenbery 1988). By contrast, in a sand based arena, the horizontal and vertical distribution of *Steinernema glaseri* (Steiner) and *H. bacteriophora* did not change relative to fluctuating thermal gradients characteristic of their natural habitat (Robinson 1994). According to Susurluk (2008) IJ vertical movement decreases with temperature. Similarly, Csontos (2002) reported IJ horizontal movement to decrease with temperature but suspected changes in moisture, mediated by temperature change, to be equally important for locomotion and resultant spatial distribution.

Although agar and sand based media offer logistically simple arenas to characterize basic nematode movement, the ecological simplicity of a laboratory assay limits extrapolation of observations made to predict movement patterns under field conditions (Spence et al. 2008). Not surprisingly, the relationship between IJ vertical movement and soil temperature under field conditions is even less well understood. In temperate climates, the vertical position of select cruiser species did not appear to change as soil temperature fluctuated during the spring, summer or fall season (Campbell et al. 1996, Půža and Mráček 2005). In a semi-arid region, IJ vertical distribution did change across the entire year within

a 30 cm range relative to fluctuating soil conditions such as temperature (Glazer et al. 1996, García del Pino and Palomo 1997). García del Pino and Palomo (1997) offered no specific mechanism for this seasonally distinct distribution, but Glazer et al. (1996) suspected that fluctuating soil moisture, a function of seasonally specific temperature and precipitation, drove this apparent avoidance behavior. Despite the fact that the winter period may be of limited significance to the dynamics (e.g., infection and recycling) of an EPN population (Půža and Mráček 2005), examining the existence of seasonal migration may help us understand IJ overwintering ecology and epizootiology in the upcoming season (Bale 1991). Hitherto, no observations exist on IJ vertical movement patterns among an endemic white grub host population during the winter season in a temperate climate.

Quantifying behavioral movement patterns of an IJ in a natural soil system is difficult (Kaya 1990). In the Northeastern United States, a number of endemic EPN species have been recovered either using soil sample baiting by targeting IJ populations (Stuart and Gaugler 1994, Campbell et al. 1996) or from naturally infected hosts (Stock and Koppenhöfer 2003). Various soil baiting techniques (e.g., Bedding and Akhurst 1975, Koppenhöfer et al. 1998, Spiridonov et al. 2007) used to characterize IJ distribution along a vertical profile may be flawed owing to inherent phased infectivity or induced periods of non-infectivity (Bohan and Hominick 1996, Campbell et al. 1999). Direct extraction using Baermann funnels, an alternative to soil baiting (Spiridonov et al. 2007), is too time consuming for the extensive sampling effort required to properly estimate a

changing distribution and also requires excellent nematode identification expertise. In addition to measuring the frequency and occurrence of IJs in the soil through soil baiting (Campbell et al. 1996, Koppenhöfer et al. 1998), indirect measurement of natural infection events may also provide insight into the alternative behavioral avoidance strategies employed by IJs during seasonally low temperature conditions.

Previous work, conducted exclusively under laboratory conditions, indicates physiological and behavioral adaptations in IJs that allow them to survive inside an insect host during low temperature conditions (Brown et al. 2002, Lewis and Shapiro-Ilan 2002, Serwe-Rodriguez et al. 2004, Parsa et al. 2006). Associated with this survival strategy is a phenomenon referred to as "latent infection" (Brown et al. 2002, Parsa et al. 2006). As soil temperature decreases, IJ infectivity decreases to a point at which host penetration will not occur (Grewal et al. 1994, Koppenhöfer and Fuzy 2003). If the IJ enters the host before this point is reached but temperatures continue to decrease, the infection process may be arrested, and the IJ remains within the live host and retains the ability to resume infection once suitable thermal conditions return (Brown et al. 2002, Parsa et al. 2006). However, in-host survival and the existence of latent infection among natural parasite-host populations have not been validated under field conditions.

Two main concerns exist regarding the performance of a biological control agent: its ability to establish itself and to reduce the target pest population and its short- and long-term persistence through fluctuating environmental conditions

(Bale 1991, Tanada and Kaya 1993). In a purely ecological sense, the end-user is attempting to create an epizootic event, i.e., an elevated incidence of disease within a host population. Epizootics involving EPN can occur naturally or after augmentative or inundative release of IJs into preferably dense host populations. Outside the waxing and waning periods of an epizootic, if the pathogen population persists the disease will persist in an enzootic state, i.e., at a lower but more stable incidence level (Tanada and Kaya 1993). Overall, the occurrence of an epizootic event or the perpetuation of an enzootic state are dictated by the spatial and temporal dynamics of the pathogen and host as well as the degree of pathogen transmission among hosts (Tanada and Kaya 1993).

If repeated epizootic events are necessary for long-term pest control, a permanent enzootic state is required. In a temperate climate, seasonal changes can be drastic and may directly or indirectly influence the temporal and spatial behavioral patterns of applied or endemic EPN (Glazer et al. 1996, García del Pino and Palomo 1997) as well as target insect hosts (Villani and Wright 1990). During late fall, winter, and early spring nematode parasites and larval hosts may display similar or discordant spatial distributions as part of their individual overwintering strategies prior to or during unfavorable environmental conditions. Identifying the timing and pattern of seasonal movements, and how they influence the frequency and occurrence of disease, may shed light on how an enzootic state is maintained.

The turfgrass habitat in the temperate climate of the northeastern United States has been a popular ecosystem in which to study the effects of

environmental and host conditions on the spatial and temporal dynamics of endemic and applied EPN species (Stuart and Gaugler 1994, Campbell et al. 1995, 1996, 1998, McGraw and Koppenhöfer 2009). *Steinernema scarabaei* and *H. bacteriophora*, two species endemic to central New Jersey, are commonly found naturally infecting the larval stages of *P. japonica* and *A. orientalis* (Campbell et al. 1995, 1996, Stock and Koppenhöfer 2003, D.E.E. and A.M.K., *personal observations*). A number of behavioral and pathological traits have been identified that help explain this natural association and the nematodes' performance as biological control agents (Campbell et al. 1998, Koppenhöfer and Fuzy 2003, An and Grewal 2005, Li et al. 2007). Although previous studies suggest that *P. japonica* and *A. orientalis* populations can be controlled over multiple generations by laboratory reared *H. bacteriophora* and *S. scarabaei* (Klein and Georgis 1992, Koppenhöfer and Fuzy 2009) we still do not know how these two EPN species survive harsh seasonal conditions and retain the ability to recycle the following spring season.

The objective of this study was primarily to investigate behavioral overwintering strategies, in particular vertical relocation, of *S. scarabaei* and *H. bacteriophora* during late fall, winter, and early spring. Two ecological variables of particular interest were changing soil temperatures and the vertical movement patterns of two common insect hosts during this period. The seasonal vertical movement pattern of *S. scarabaei* has never been examined; vertical movement patterns of *H. bacteriophora* and *S. carpocapsae* have been studied only during warmer seasonal periods. We also included the latter two species in our study to

facilitate a direct comparison with previous studies of seasonal change in nematode vertical position that used slightly different techniques. Three questions were addressed: 1) does the vertical position of *S. scarabaei*, *S. carpocapsae*, and *H. bacteriophora* change from late fall to spring, 2) is IJ vertical position affected by fluctuating soil temperature, and 3) is IJ vertical position correlated with white grub vertical position during this period?

METHODS AND MATERIALS

Nematodes

Heterorhabditis bacteriophora was reared in wax moth larvae, *Galleria mellonella* L. For the 2006/2007 season, *H. bacteriophora* (GPS11 strain) was used that had been cultured on *G. mellonella* for at least 4 years (3-4 rearing rounds per year). For the 2007/2008 season, two fresh field isolates of *H. bacteriophora* were used that had been baited with *G. mellonella* larvae in the preceding August from soil samples taken within the vicinity of the two turf plots intended for field trials. For the isolation, 5 samples were taken from each site with an Oakfield sampler (2 cm diam $\times$ 10 cm depth), pooled and mixed, and 10 samples of 50 g soil baited with 5 *G. mellonella* larvae each. *H. bacteriophora* infections were identified by the characteristic maroon-red cadaver coloration of infected *G. mellonella* and the morphology of infective juveniles (IJs) and first generation hermaphrodites. The isolates used consisted of progeny pooled from 10 – 15 originally infected cadavers. Since two nematode sample sites were

used in 2007/2008, two *H. bacteriophora* isolates were recovered, amplified one time in *G. mellonella*, and reapplied to the respective site.

For both field trials, *S. scarabaei* (AMK001 strain) was used that has been maintained in *P. japonica* and *A. orientalis* larvae since 2001 (two rearing rounds per year) because its production in wax moth larvae is too unreliable even though its virulence to third-instar *A. orientalis* is not affected by rearing in wax moth larvae (Koppenhöfer and Fuzy 2003).

All IJs were reared at room temperature (21–24 °C), then harvested from White traps and stored in tap water at 8 °C following complete emergence (cadaver exhaustion) for no more than 7 days before field application (Kaya and Stock 1997).

Study site

Two study sites were used, one at the Rutgers University Plant Science Research Station (Adelphia, NJ), the other at the Rutgers Horticulture Farm II (East Brunswick, NJ). Both sites possessed preexisting nematode and white grub populations. The Adelphia site had separate plots in each year, both in tall fescue (*Festuca arundinacea* Schreb*)* fields that had been planted 2.5 years before the start of the study. Mowing height was 33 mm and the thatch layer was 2 mm thick. The East Brunswick site was used both seasons for white grub sampling and once in the second season for nematode measurements. The plot consisted of a weedy low-maintenance grass area with approximately 60% grass [mixture of Kentucky bluegrass, *Poa pratensis* L.; fine fescues, *Festuca* spp.; tall fescue; crabgrass, *Digitaria sanguinalis* (Scop)], 20% clover, *Trifolium repens* L.,

15% plantain, *Plantago* spp., and 5% other weeds with a mowing height of 80 mm and a thatch layer of around 2 mm thickness. All plots received standard fertilizer applications and irrigation to maintain plant health.

At Adelphia, for the 2006/2007 season, the vertical soil profile (30 cm deep) was characterized as a loam at 0–4 cm (48% sand, 36% silt, 16% clay; 5.2% organic matter, pH 5.2), a sandy clay loam at 4–10 cm (60% sand, 18% silt, 22% clay; 2.5% organic matter, pH 5.4), a loam at 10–20 cm (41% sand, 37% silt, 22% clay; 1.6% organic matter, pH 6.0), and a loam at 20–30 cm (40% sand, 36% silt, 24% clay; 2.6% organic matter, pH 6.0). For the 2007/2008 season, the vertical soil profile (30 cm deep) was characterized as a sandy loam at 0-4 cm (63% sand, 21% silt, 16% clay; 5.4% organic matter, pH 5.5), a sandy loam at 4–10 cm (63% sand, 23% silt, 15% clay; 1.9% organic matter, pH 5.7), a sandy loam at 10–20 cm (60% sand, 21% silt, 20% clay; 1.8% organic matter, pH 6.0), and a sandy loam at 20–30 cm (61% sand, 22% silt, 18% clay; 1.5% organic matter, pH 6.0).

The vertical soil profile for the East Brunswick site was characterized as a loam at 0–4 cm (47% sand, 40% silt, 13% clay; 3.2% organic matter, pH 4.4), a loam at 4–10 cm (50% sand, 35% silt, 14% clay; 3.6% organic matter, pH 5.5), a loam at 10–20 cm (47% sand, 35% clay, 18% clay; 1.8% organic matter, pH 6.0), and a loam at 20–30 cm (43% sand, 35% silt, 22% clay; 1.5% organic matter, pH 6.0).

Temperature measurements

Soil temperature was measured by six t-type copper constantan thermocouples (1.0 mm diameter; Omega Engineering, Stamford, CT, USA). Prior to installation, each thermocouple wire end was fitted with a waterproof protective plastic tip and filled with slow drying nonconductive cement. Two independent temperature probes were installed at three depths (5, 15, 25 cm) below the soil surface approximately 10 feet from the sample plots. Air temperature was measured within the immediate vicinity of the sample plots by a standard meteorological sensor.

Temperature was recorded at 30 min intervals at both sites in both seasons using a data logger (CR10X, Campbell Sci., Logan, UT, USA). Soil and air temperature recording began well in advance of nematode field applications. Soil temperature was recorded at 30 min intervals at each depth. The minimum, maximum, and mean soil temperature per day was calculated for the three measured depths. Since soil temperature among the three depths (5, 15, 25 cm) varied by $\leq$1.0 °C, temperature measurements across the three depths were averaged into one daily value and then averaged 5 days prior to each sample date to simplify correlations between nematode and white grub data.

Nematode trials

Experimental areas for nematode observation (43.2 m^2) consisted of 12 independent plots measuring 91.5 cm x 91.5 cm and separated from each other by 91.5 cm buffers. Treatments were arranged in a randomized complete block design with 3 treatments x 4 replicates. Treatments consisted of *S. scarabaei* or

H. bacteriophora applied at a rate of 2.5 billion IJs/ha and an untreated control. Nematodes were applied in 3.5 liter of water per plot (3.5 mm) using a watering can. Controls were treated with water only. In the first season, nematodes were applied on October 28, 2006 (DOY 301) at Adelphia only. In the second season, nematodes were applied on October 2 and 4 (DOY 275 and 277) at Horticulture Farm II and Adelphia, respectively. In both seasons, applications were made when mean daily soil temperature at the 5 cm depth was 20 $\pm$ 1 °C and air temperature was 22 $\pm$ 1 °C. Applications were made at this time since this is when natural epizootic events at the soil root zone are known to occur. The experimental areas received overhead irrigation throughout the growing season as necessary to maintain plant health (i.e., up to 25 mm per week in the absence of rain fall).

To determine the vertical distribution of the nematodes, a 30 cm soil core (2 cm diameter) was taken using an Oakfield sampler with the upper surface of the thatch/soil interface as a reference. The core was subdivided into four sections (0–4, 4–10, 10–20, 20–30 cm). At each sampling event, four cores were taken per replicate plot and respective sections pooled per plot. Immediately prior to the nematode application, soil samples were taken to establish a baseline measurement. Following nematode application, soil samples were taken every 14 to 18 days until the soil temperature reached 15 °C at the 5 cm depth in the following spring. Soil samples were transported back to the laboratory in non-airtight plastic bags and were either baited immediately or kept at 9 °C for no more than 24 hours before baiting.

Nematode presence was measured by baiting 60 g sub-samples (approx. 60 ml) from each soil depth with 10 wax moth larvae at room temperature (21–24 °C) (Koppenhöfer et al. 1998). Every 3 days, dead wax larvae were replaced with fresh larvae to maintain the 10 total wax moth larvae per baiting dish. Soil was baited until no infection was observed for two consecutive baiting rounds. Cadavers were dissected and digested following Mauléon et al. (1993) to determine the number of established nematodes. Water was added to the soil samples as necessary to maintain moderate soil moisture (approximately 10-15% w/w) for optimal nematode activity.

White grub sampling

Change in vertical distribution was determined by sampling with a standard size golf hole cutter (diameter 10.5 cm) to 35 cm depth. The soil core was carefully inspected in 2 cm sections starting from the top of the core demarked by the thatch/soil interface. Sampling occurred every 14 to 18 days starting the same day as the nematode application in a location known to have white grub populations and neighboring the nematode experiments. Upon finding a white grub, the depth location was recorded and the larva identified to species in the field using a 10 X hand lens (Baush and Lashomb ®). When the soil was frozen, a 30 cm $\times$ 30 cm hole, 35 cm deep was excavated using a pitchfork. The mean depth of recovered third-instar *P. japonica* and *A. orientalis* was calculated for each sample date.

Recovered larvae were brought back to the lab, rinsed with tap water and stored in 24 well plates with moist pasteurized soil at room temperature and

inspected daily for signs of nematode infection. If nematode infection was suspected during the 14-day observational period, based on a characteristic cadaver coloration, subjects were incubated on White traps at 23 ±1 °C. If emergence did not occur after 30 days, cadavers were dissected to search for any nematodes in them. If nematodes were found, nematode species based on morphology as well as the age, sex, and number of established nematodes per host were determined.

Data analysis

The total number of nematodes recovered was recorded for each depth within each replicate and converted to percent recovery (i.e., 100% implies that at least one nematode of each species was recovered from each sample depth and treatment block per sampling event) to estimate sampling efficacy. Percent recovery was analyzed using a chi-square analysis. Nematode data were converted into a depth index value for each recovered species to obtain the average vertical position of nematodes across the 30 cm sample. The depth index value was calculated by first multiplying the total number of recovered nematodes per depth section by the mean depth of each respective sample section (2, 7, 15, 25 cm) producing four values ($I_{d\ 0\text{-}4}$, $I_{d\ 4\text{-}10}$, $I_{d\ 10\text{-}20}$, $I_{d\ 20\text{-}30}$). The four new values ($I_{d\ x}$) were summed and divided by the total number of nematodes recovered across the four sample depths to obtain an averaged indexed value (I_b) for each replicate (Equation 1). The depth index value data were analyzed using a repeated measure ANOVA (split-plot design) with a

General Linear Model (SAS Institute 1996). If different species were recovered in the experiments, they were included as factors in the analysis.

White grub data was also analyzed using a repeated measure ANOVA with a GLM model. Since neither site nor site x species interaction had any effect on vertical position, data was pooled by site with species as the class variable. For both nematode and grub data, means were separated using a Fisher's protected LSD (α= 0.05) (SAS Institute 1996). The relationships between nematode position, white grub position, and soil temperature were described using regression analysis (SAS Institute 1996).

Equation 1.

$(I_{d\,x}) = \sum$(total number nematodes per depth) **x (**Mean depth of sample).

Index value for nematodes per depth $(I_{d\,x})$ = Number of nematodes per depth section **x** average depth of the respective depth section (i.e., 2, 7, 15, and 25 cm respectively).

$(I_b) = \sum (I_{d\,0\text{-}4}, I_{d\,4\text{-}10}, I_{d\,10\text{-}20}, I_{d\,20\text{-}30})$ / Total # nematodes from 0–30 cm

Index value per block $(I_b) = \sum (I_{d\,0\text{-}4, 4\text{-}10, 10\text{-}20, 20\text{-}30})$ / Total number of nematodes in all depth sections combined (i.e., 0–30 cm).

RESULTS

Nematode vertical movement

2006 – 2007 Season

Steinernema scarabaei and *S. carpocapsae* were not recovered from the experiment but for *H. bacteriophora,* recovery was 90 – 100% throughout the season (Figure 1). The depth index value for *H. bacteriophora* changed significantly relative to time in the first season (October 25, 2006 to May 4, 2007) ($F_{14,179}$ = 2.80; P = 0.0011). At the beginning of the season, the depth index value was 12 cm. A significant change from the 10 – 20 cm region towards the 20 – 30 cm depth was detected in February (DOY 54) (Figure 2). We suspect this observation to be the result of a sampling error attributed to frozen soil samples from the 0–4, 4–10 and partial upper half of the 10–20 cm region. Frozen soil samples that thawed in the laboratory permanently lost their soil structure potentially interfering with host locating and penetration by any IJs contained in the samples. Excluding this February (DOY 54) sampling event, the depth index value of *H. bacteriophora* did not change significantly over time and ranged between 8 and 18 cm (Figure 2).

Soil temperature was found to be a significant predictor of *H. bacteriophora* depth index value (t = 19.66; df = 1,169; P = 0.0003) ($Y_{H.\ bacteriophora}$ = 15.062 – 0.3624x; r^2 = 0.0654) (Figure 3). Soil temperature did not decrease in October and November but rather increased slightly in the latter part of the fall. Only until the beginning of December did soil temperature begin to decrease (Figure 2). Maximum soil temperatures reached 14 °C and minimum soil

temperature reached 0.5 °C. Rainfall was very high throughout the fall and the first half of the winter season. Heavy snow accumulation (12.5 cm) occurred in February (Figure 2). In relation to host position, only *P. japonica* was found to be a significant predictor of *H. bacteriophora* depth index value (t = 20.34; df = 1, 169; P = 0.0009) ($Y_{H.\ bacteriophora} = 6.79 - 0.211x_{P.\ japonica}$; r^2 = 0.0574) (Figure 3).

2007 – 2008 Season

In the second season all three species (*H. bacteriophora, S. scarabaei, S. carpocapsae)* were recovered. Percent recovery changed and differed by species over the course of the entire sampling season at Adelphia [χ^2 (3, N = 15) = 66.96; P = 0.0001] and Horticulture Farm II [χ^2 (3, N = 15) = 82.66 P = 0.0001] (Figure 1). At the Adelphia study site, the depth index values of *H. bacteriophora*, *S. scarabaei,* and *S. carpocapsae* did not change significantly from October to April ($F_{3, 160}$ = 3.78; P = 0.1096). The depth index value ranged between 8 to 16 cm for *H. bacteriophora*, 2 to 24 cm *S. scarabaei*, and 4 to 21 cm for *S. carpocapsae* (Figure 4). At Horticulture Farm II, the depth index value for *H. bacteriophora*, *S. scarabaei,* and *S. carpocapsae* did not change significantly from October to April ($F_{3,157}$ = 0.31; P = 0.60). The depth index value averaged by sampling date ranged between 8 to 15 cm for *H. bacteriophora*, 5 to 14 cm for *S. scarabaei*, and 2 to 24 cm for *S. carpocapsae* (Figure 5).

Nematode species did not have a significant effect on depth index value during the sample season at the Adelphia and Horticulture Farm II study site

($F_{2,160}$ = 2.68; P = 0.16 and $F_{2,157}$ = 0.52; P = 0.62 respectively). Nematode treatment did not have a significant effect on depth index value during the sample season at the Adelphia and Horticulture Farm II study site ($F_{2,160}$ = 1.51; P = 0.31 and $F_{2,157}$ = 0.40; P = 0.68, respectively). Since there was no significant interaction detected between nematode species and treatment at Adelphia and Horticulture Farm II sites (α = 0.05), data was pooled to a single depth index value for each sample. A significant relationship was detected between soil temperature and depth index value from October to April at Adelphia (t = 18.36; df = 1, 221; P = 0.0007) (Y = 12.198 – 0.2224x; r^2 = 0.0511) (Figure 6) but not at Horticulture Farm II (t = 14.68; df = 1,219; P = 0.83) (Y = 10.26 + 0.014x; r^2 = -0.0043) (Figure 7). Maximum soil temperatures reached 20 ±1°C and minimum soil temperature of 1 ±1°C. Rain and snow fall was considerably less in the 2006/2007 season (Figure 2, Figure 8).

Nematode – host vertical relationship

The same pooled depth index values were also compared to the vertical position of *P. japonica* and *A. orientalis.* At Adelphia, the relationship between nematode and host position was significant for *P. japonica* (t = 7.64; df = 1,221; P = 0.0026) ($Y_{nematode}$ = 7.515 + 0.3281x $_{P.\ japonica}$; r^2 = 0.0402) and *A. orientalis* (t = 11.51; df = 1,221; P = 0.0087) ($Y_{nematode}$ = 7.938 + 0.20897X $_{A.\ orientalis}$; r^2 = 0.0307) (Figure 6). At Horticulture Farm II, the relationship was significant for *A. orientalis* only (t = 11.51; df = 1,219; P = 0.0374) ($Y_{nematode}$ = 12.501 – 0.184x $_{A.\ orientalis}$; r^2 = 0.0152) (Figure 7).

Nematode position and recovery via field cadaver

Nematode presence was also detected inside field recovered white grubs. In the first season on January 1, 2007, one *P. japonica* third-instar cadaver was recovered at 8.75 cm depth displaying an orange-red coloration indicative of an *H. bacteriophora* infection. On March 21, 2007 one *A. orientalis* third-instar cadaver was recovered at 12.5 cm depth displaying a greenish-yellow coloration indicative of an *S. scarabaei* infection. Both cadavers were White trapped and incubated at 23 ±1 °C. We were unsuccessful in obtaining progeny but found adults in both cadavers. Infected white grubs were only recovered from Adelphia.

In the second season on October 14, 2007 two third-instar *P. japonica* and two third-instar *A. orientalis* cadavers with signs of nematode infection were recovered. The *P. japonica* samples were detected at the 2.5 cm and 5.5 cm depths with one displaying a red-maroon coloration characteristic for *H. bacteriophora* infection and the other the yellowish-green *S. scarabaei* infection coloration. The two *A. orientalis* cadavers were recovered from 1.25 cm and 2.5 cm depths and both displayed *H. bacteriophora* infection coloration. All cadavers were White trapped and incubated at 23 ±1 °C. We were only able to obtain progeny from the *H. bacteriophora* infections. The *S. scarabaei* infected cadavers did not produce progeny but did contain adult nematodes. Infected white grubs were only recovered from Adelphia.

White grub vertical position

White grub vertical position over time

Site had no significant effect on white grub vertical position, and data from both sites were combined into one data set for each season. A significant interaction between species and time on vertical position was detected in the first ($F_{13,518}$ = 2.96; P = 0.0003) and second ($F_{14,403}$ = 6.20; P = 0.0001) season.

At the beginning of the 2006/2007 experiment (October 25, 2006), *P. japonica* and *A. orientalis* were detected at the 4 – 6 cm depth, slightly below the expected root–feeding depth. A change in the vertical position was detected on October 27, 2006 (300 DOY) for *P. japonica* and on November 3, 2006 (DOY 307) for *A. orientalis* (Figure 9). In 2007/2008, fall measurements were taken earlier (October 2, 2007) detecting *P. japonica* and *A. orientalis* at the 1–3 cm depth within the root-feeding zone. A significant change in the vertical position was detected on October 31, 2007 (304 DOY) for *P. japonica* and on November 27, 2007 (331 DOY) for *A. orientalis* (Figure 10). In both seasons, the final overwintering position for both species was below the frost zone (Figure 2 and Figure 8) and the average overwintering position for *A. orientalis* (12 ±1 cm) was deeper than for *P. japonica* (10 ±1 cm). The vertical position for either species did not change during the winter months (Figure 9 and Figure 10). Upward movement of *P. japonica* and *A. orientalis* in 2006/2007 occurred on May 4, 2007 (124 DOY) for both species (Figure 9). In 2007/2008, *A. orientalis* began its upward movement on March 30, 2008 (175 DOY) followed by *P. japonica* on April 13, 2008 (189 DOY) (Figure 10). In 2006/2007, white grub upward

movement was detected with soil temperature above 10 °C (Figure 9). In 2007/2008, *Anomala orientalis* upward movement was detected when soil temperatures was at or below 10 °C while *P. japonica* started when soil temperature was above 10 °C (Figure 10).

White grub vertical position and temperature change

White grub and soil temperature data from both seasons (2006/2007 and 2007/2008) were combined since we failed to reject the null hypothesis of equal variance using Levene's test for equality of variance ($\alpha = 0.05$). A significant linear relationship between white grub vertical position and soil temperature from the fall to spring season (October to May) was detected for *P. japonica* (t = -10.73; df = 1, 587; P = 0.0001) ($Y_{PJ\ depth}$ = 11.38 – 0.3051x; r^2 = 0.1624) and *A. orientalis* (t = -15.33; df = 1, 416; P = 0.0001) ($Y_{AO\ depth}$ = 17.28 – 0.7056x; r^2 = 0.3594) (Figure 11). A significant relationship between grub depth and soil temperature was not detected during the winter season (November to March) for *P. japonica* (t = -1.11; df = 1, 374; P = 0.2693) ($Y_{PJ\ depth}$ = 9.79 + 0.0065x ; r^2 = 0.0006) or *A. orientalis* (t = -1.04; df = 1, 211; P = 0.2973) ($Y_{AO\ depth}$ = 15.0 – 0.1427x ; r^2 = 0.0004) (Figure 12).

DISCUSSION

Our study, the first to investigate EPN distribution during the cooler part of the year, did not reveal a distinct change in the vertical distribution of three EPN species from fall through mid-spring. Active physical relocation did not appear to be a prevalent component in the overwintering strategy of two cruiser EPN

species common to a temperate turfgrass habitat. Nevertheless, the free-living IJ stage appeared to be able to persist through extended periods of freezing conditions**.** In-host survival at low temperature, previously suggested as a potential overwintering strategy but only investigated under laboratory conditions (Brown et al. 2002), did not appear to be a common or viable survival strategy for EPNs under field conditions in our study system. Therefore, populations appeared to survive primarily or exclusively as IJs in the soil, presumably using various physiological and biochemical mechanisms not investigated.

The vertical position of *S. scarabaei, H. bacteriophora*, and *S. carpocapsae* did not change with fluctuating environmental and host conditions. The absence of a distinct vertical distribution pattern has been observed during warmer seasonal periods using daily (Campbell et al. 1996), weekly (Duncan and McCoy 1996), or monthly (Půža and Mráček 2005) sampling intervals. *Heterorhabditis bacteriophora,* well known as a cruiser forager (e.g., Campbell et al. 1996), and *S. scarabaei*, also a cruiser forager (Koppenhöfer and Fuzy 2003), did not display a seasonally distinct vertical movement pattern from fall to spring. *Steinernema carpocapsae*, an ambush forager (e.g., Lewis et al. 1992), was also consistently recovered across a wide vertical range similar to that of *H. bacteriophora* and *S. scarabaei.* Despite possessing inherently different foraging behaviors, continuous recovery of multiple nematode species in a single habitat indicates a state of coexistence (Spiridonov et al. 2007).

Differences in foraging strategy, host preference, and virulence facilitate coexistence (Koppenhöfer and Kaya 1996). Although *S. scarabaei* is more virulent than *H. bacteriophora* against *P. japonica* and *A. orientalis (*Koppenhöfer and Fuzy 2006), other insect species including sod webworms, cutworms, and billbugs may represent suitable alternative host resources for *H. bacteriophora* and *S. carpocapsae* in our turfgrass system (Koppenhöfer and Fuzy 2009). During cool periods, decreasing soil temperature may uniformly reduce species-specific foraging behaviors and infectivity (Rio and Cameron 2000) resulting in co-persisting meta-populations. In the absence of a suitable host, internal lipid energy reserves determine IJ survivorship and the ability to regain infectivity when environmental conditions improve (Glazer et al. 2002, Fitters and Griffin 2004).

Endemic and laboratory reared *S. scarabaei* and *H. bacteriophora* can persist through prolonged periods of cold and freezing conditions and still retain their ability to infect once warmer conditions return. Koppenhöfer and Fuzy (2009) observed persistence of initially released *S. scarabaei* and endemic *H. bacteriophora* populations across three to four consecutive seasons, although with greatly fluctuating densities. Unlike previous field studies (Ferguson et al. 1995, Campbell et al. 1996, Glazer et al. 1996), our sampling predominantly occurred when soil temperatures were too low to allow recycling (Grewal et al. 1995, Koppenhöfer and Fuzy 2003). In the absence of recycling, some sort of long-term survival mechanism may have been expressed in the IJ. We have no explanation for the absence of *S. scarabaei* in our samples during the first

experiment. However, *S. scarabaei* was recovered throughout the second season. Although there is no definitive explanation for changes in percent recovery over the course of the sampling season, it is highly unlikely that increased recovery at various sampling dates was due to newly emerging IJs.

Prolonged periods of low temperature along with associated changes in the physical properties of soil can reduce the occurrence of infection. In response to sudden harsh environmental conditions, a facultative dormancy response or quiescence condition would increase the persistence of free-living IJs (Grewal et al. 2006). In a quiescent state, metabolic rates are decreased to conserve limited internal energy resources (Glazer 2002). A form of quiescence established during prolonged periods of low temperature, classified as thermobiosis (Barrett 1991), can initiate the synthesis of cryoprotectants including trehalose (Grewal et al. 2006). Once favorable conditions return, IJs should recover from this quiescent state, although some may still remain inactive (Fan and Hominick 1991). Observed changes in percent recovery among the *H. bacteriophora* populations may have represented the existence of a “temporary non-infectious state” (Campbell et al. 1999), a condition related to the “phased-infectivity hypothesis” of Hominick and Reid (1990). Conversely, the two *Steinernema* populations may have expressed an “inducible non-infectious” state, regulated in part by various endogenous or exogenous factors (Campbell et al. 1999).

Although temperature has been implicated as an important factor influencing IJ activity (Rio and Cameron 2000; see also Grewal et al. 1994,

Csontos (2002) Koppenhöfer and Fuzy 2003, Parsa et al. 2006), soil temperature appeared to have little effect on the vertical position of nematodes from fall to the early spring. At constant incubation, low temperature can decrease the degree of horizontal (Csontos 2002) and vertical (Susurluk 2007) movement in the presence of a suitable host. Under laboratory conditions Robinson (1994) reported that *H. bacteriophora* and *S. glaseri* exhibit erratic movements and weak spatial responses to fluctuating soil temperature gradients typical of its natural habitat. The absence of a thermotactic response behavior may provide a mechanistic explanation for the absence of changes in vertical distribution as temperature changes

Seasonally distinct spatial movement patterns among nematodes in temperate climates may not be as apparent as in behaviorally similar species in semi-arid climates (Glazer et al. 1996, García del Pino and Palomo 1997). The severity and type of environmental change may dictate the occurrence and expression of active avoidance strategies such as vertical migration (García del Pino and Palomo 1997). Soil moisture and pore size strongly affect nematode vertical movement (Wallace 1958). In the temperate climate of New Jersey, nematode locomotion may decrease during the cooler part of the year due to increased waterlogging of soil pores; a probable result of frequent precipitation combined with diminished evaporation during this period. Although decreasing soil temperature may diminish the metabolic rate that fuels IJ locomotion (Yeats 2005), moisture, which is a mediating component of water film surface tension (Lewis et al. 2006), may have a more significant retardant effect on locomotion.

Our results on vertical distribution of third-instar *P. japonica* and *A. orientalis* from fall to spring, concur with those of Vittum et al. (1999) and Fleming (1972). Downward movement in the fall may be a behavioral strategy to evade freezing conditions rather than cold temperature since vertical soil temperature uniformly changed across the entire sample region. Slight differences in soil moisture can initiate frost development (Matsuoko 1998). White grubs were found below but also at the 8 – 10 cm frost line, suggesting that an environmental cue other than soil temperature may have initiated this seasonally distinct downward vertical movement. The creation of a sheltered microhabitat or "grub cave" (Vittum et al. 1999) was often observed among overwintering *P. japonica* and *A. orientalis*. Jagged ice nucleating points surrounding the exterior of the "cave" appeared to regulate ice formation and prevent ice crystals from penetrating the inner surface (DEE *personal observation)*. Upward movement in spring coincided with increasing temperature, a trend previously observed under controlled experimental conditions (Villani and Nyrop 1991.

Developmental age may play a subtle yet important role in the spatial distribution of *P. japonica* (Villani and Nyrop 1999). The absence of a significant response to temperature during the winter period could have been primarily dictated by age and time of year (Villani and Nyrop 1991) and then by fluctuating soil temperature conditions. Statistically, soil temperature during the fall and spring explained only a small degree of variability associated with vertical position. Other factors including age and moisture could have also contributed to this seasonal movement pattern. The ecological and behavioral components

mediating *A. orientalis* vertical movement may not be exactly the same as those of *P. japonica*. Third-instar European chafers, *Rhizotrogus majalis*, another white grub species common in turfgrass in the Northeast, does not exhibit a similar age dependent vertical response as *P. japonica* (Villani and Nyrop 1991). Although the seasonal vertical movements of *P. japonica* and *A. orientalis* occurred roughly at the same time, experimental examination under controlled conditions as conducted by Villani and Nyrop (1991) would be necessary to resolve any subtle behavioral differences.

In spite of the weak relationship between nematode and white grub vertical distributions, nematodes and white grubs distribution overlapped throughout most of the sampling period. Overlap between host and pathogen is essential for a persisting enzootic state (Tanada and Kaya 1993) as well as a functioning biological control system (Ferguson et al. 1995). Presence, abundance (Glazer et al. 1996, Půža and Mráček 1983**),** and aggregation (Mráček and Bečvář 2000) of hosts strongly influence nematode orientation and distribution. Short range and long range stimuli in the form of host $CO_{2,}$ fecal waste and gut fluids positively influence IJ host detection (Lewis et al. 1992, Wang and Gaugler 1998, Koppenhöfer and Fuzy 2009). Upon detection of a suitable host cue, the wide ranging IJ searching behavior may change to a localized search pattern characterized by decreased rate in movement and frequent changes in direction (Lewis et al. 2006). Through a decreased rate of movement, the foraging IJ is presumed to increase its chance of encountering or remaining in close proximity to a suitable host (Lewis et al. 1992). Under field

conditions, decreasing soil temperature may diminish the IJ's ability to respond to host CO_2 volatile cues (Barbercheck and Duncan 2004), possibly hampering its ability to locate a suitable host. Furthermore, behavioral avoidance of a pathogen by a host (Villani and Nyrop 1991) may be reduced during harsh fluctuating soil conditions. Nevertheless, overlap may still occur under harsh conditions after an extended period of time if similar behavioral preferences for microhabitat havens are expressed (Glazer et al. 1996 and Gouge et al. 2000).

Steinernema scarabaei and *H. bacteriophora* did not appear to exploit larvae of *P. japonica* and *A. orientalis* as protective havens during extended periods of cold and freezing conditions. In-host survival was not observed across two consecutive seasons despite overlapping endemic populations of nematodes and white grubs. Of the few recovered cadavers that displayed signs of nematode infection during the winter period (December to February), none produced nematode progeny following re-warming at laboratory conditions. Bornstein-Forst et al. (2005) similarly reported a significant reduction in developmental performance among nematodes exposed to incrementally decreasing temperature while inside wax moth larva. Development and emergence was observed only among *H. bacteriophora*-infected white grub cadavers recovered in the warmer period of the fall. *Steinernema scarabaei* failed to develop or emerge following similar ecological and seasonal conditions. Low temperature survival is known to differ by species (Brown and Gaugler 1998, Brown et al. 2002).

Latent infection was not observed among field recovered *P. japonica* and *A. orientalis*. To our knowledge, this is the first time the existence of latent infection or in-host survival at low temperature has been studied in overlapping endemic populations. Our observations suggest that latent infection is not a viable low temperature survival mechanism for *S. scarabaei* and *H. bacteriophora* naturally persisting among susceptible *P. japonica* and *A. orientalis* white grub hosts. Limited laboratory observations using second- and third-instar *P. japonica* as hosts also failed to support the possibility of latent infection of *H. bacteriophora* or *S. scarabaei* as an overwintering mechanism (D.E.E. *unpublished data*). Host type may dictate the viability of in-host survival at low temperatures (Dick 1983).

Infective juveniles can actively respond to environmental extremes if appropriate physical and chemical stimuli exist (Grewal et al. 2006). Quiescent response mechanisms including anhydrobiosis and thermobiosis, which involve the production of stress-induced cryoprotectants such as trehalose, enable the free-living infective juvenile to osmoregulate and maintain the structural integrity of the cellular membrane at low temperature (Grewal and Jagdale 2002). The host cadaver (at least for wax moth larvae) may also provide some degree of protection for infective juveniles and other developmental stages from freezing conditions (Lewis and Shapiro-Ilan 2002, Brown et al. 2002, Parsa et al. 2006, Serwe-Rodriguez 2004). Severe drying conditions inside desiccating cadavers may stimulate the biosynthesis of compounds that offer significant “cross-

protection" against extreme thermal conditions (Serwe-Rodriguez 2004). How widespread nematode survival in desiccated host cadavers may be is unknown.

Moisture conditions leading up to an osmotic threshold necessary to induce a thermal cross–protective response may not exist within this specific EPN–white grub host system. Soil conditions conducive to this physiological survival mechanism may be more prevalent near the soil surface where soil moisture can change more rapidly. Overwintering *P. japonica* and *A. orientalis* as well as respective nematode-infected cadavers were found in very cold microclimates that had relatively constant moisture conditions. Of the few cadavers recovered, none were even partially desiccated, suggesting this specific microclimate may have prevented the establishment of a cross–protective response (Serwe-Rodriguez 2004). The occurrence and degree of osmotic stress may depend on the physical scale of the immediate microclimate (e.g., host cadaver, soil pore). The induction of an anhydrobiotic/thermobiotic cross–protective response, as reported by Grewal and Jadgdale (2002), may occur more readily in free-living IJs that are surrounded by transient water films in the soil than in IJs inside host cadavers which retain moisture much better (Koppenhöfer et al. 1996). Experimental examination along the lines of Serwe-Rodriguez (2004), with the consideration of realistic seasonal field conditions, may aid in resolving the question whether live white grub hosts and cadavers play a role in IJ low temperature survival.

Bibliography

Alumai, A., Grewal, P.S., Hoy, C.W., Willoughboy, D.A. 2006. Factors affecting the natural occurrence of entomopathogenic nematodes in turfgrass. Biological Control. 36(3), 368-374.

Bakonyi, G., Nagy, P., Kovács-Láng, E., Kovács, E., Barabás, S., Répási, V., Seres, A. 2007. Soil nematode community structure as affected by temperature and moisture in a temperate semiarid shrubland. Applied Soil Ecology. 37, 31-40.

Barbercheck, M.E. 1992. Effect of soil physical factors on biological control agents of soil insect pests. Florida Entomologist. 75(4), 539-548.

Barbercheck, M.E., Duncan, L. 2004. Abiotic factors. In: Gaugler, R., Bilgrami, A.L. (Eds.), Nematode Behavior. ©CAB International, Oxfordshire, UK, 309-322 pp.

Barrett, J. 1991. Anhydrobiotic nematodes. Agricultural Zoology Reviews 4, 161-176

Bornstein-Forst, S., Kiger, H., Rector, A. 2005. Impacts of fluctuating temperature on the development and infectivity of entomopathogenic nematode *Steinernema carpocapsae* A10. Journal of Invertebrate Pathology 88, 147-153

Brown, I.M., Gaugler, R. 1998. Survival of steinernematid nematodes exposed to freezing. Journal of Thermal 23, 75-80.

Brown, I.M., Lovett, B.J., Grewal, P.S. 2002. Latent infection: a low temperature survival strategy in steinernematid nematodes. Journal of Thermal Biology. 27, 531-539.

Campbell, J.F., Lewis, E., Yoder, F. 1996. Entomopathogenic nematode (Heterorhabditidae and Steinernematidae) spatial distribution in turfgrass. Parasitology. 113, 473-482.

Campbell, J.F., Orza, G. Yoder, F., Lewis, E., Gaugler, R. 1998. Spatial and temporal distribution of endemic and released entomopathogenic nematode populations in turfgrass. Entomologia Experimentalis et Applicata 86, 1-11.

Campbell, J.F., Koppenhöfer, A.M., Kaya, H.K., Chinnasri, B. 1999. Are there temporarily non-infectious dauer stages in entomopathogenic nematode populations: a test of the phased infectivity hypothesis. Parastiology. 118, 499-508

Csontos, A.S. 2002. Lateral movement of the entomopathogenic nematodes *Steinernema glaseri* and *Heterorhabditis bacteriophora* in sand at different temperatures in response to host seeking. Biocontrol Science and Technology. 12, 137-139.

Dick, T.A. 1983. Infectivity of isolates *Trichinella* and the ability of an arctic isolate to survive freezing temperatures in the raccoon, *Procyon lotor*, under experimental conditions. Journal of Wildlife Diseases. 19, 333-336.

Duncan, L.W., McCoy, C.W. 1996. Vertical distribution in soil, persistence, and efficacy against citrus root weevil (Coleoptera: Curculionidae) of two species of entomopathogenous nematodes (Rhabditida: Steinernematidae; Heterorhabditidae). Environmental Entomology. 25, 174-178.

Dusenbery, D.B. 1988. Behavioral responses of *Meloidogyne incognita* to small temperature changes. Journal of Nematology. 20, 351-355.

Dusenbery, D.B. 1989. A simple animal can use a complex stimulus pattern to find a location: nematode thermotaxis in soil. Biological Cybernetics. 60, 431-437.

Fan, X., Hominick, W.M. 1991. Effects of low storage temperature on survival and infectivity of two *Steinernema* species (Nematoda: Steinernematidae). Revue de Nématologie. 14, 407-412

Ferguson, C.S., Schroeder, P.C., Shields, E.J. 1995. Vertical distribution, persistence, and activity of entomopathogenic nematodes (Nematoda, Heterorhabditidae and Steinernematidae) in Alfalfa Snout Beetle (Coleoptera, Curculionidae) infested fields. Environmental Entomology. 24, 149-158.

Fitters, F.L., Griffin, C.T. 2004. Spontaneous and induced activity of *Heterorhabditis megidis* infective juveniles during storage. Nematology. 6, 911-917.

Fleming, W.E. 1972. Biology of the Japanese beetle. USDA Technical Bulletin 1449.

García del Pino, F.G., Palomo, A. 1997. Temporal study of natural populations of Heterorhabditid and Steinernematid nematodes in horticultural crop soils. Fundamental and Applied Nematology. 20, 473-480.

Gaugler, R., Bilgrami, A.L. 2004. Introduction and overview. In: Gaugler, R., Bilgrami, A.L. (Eds.), Nematode Behavior. ©CAB International, Oxfordshire, UK, xiv. pp.

Glazer, I., Kozodoi, E., Salame, L., Nestel, D. 1996. Spatial and temporal occurrences of natural populations of *Heterorhabditis* spp. (Nematoda: Rhabditida) in a semiarid region. Biological Control. 6, 289-294.

Glazer, I. 2002. Survival Biology. In: Gaugler, R. (Eds.) Entomopathogenic Nematology. ©CAB International, New York, NY 169-182 pp.

Gouge, D.H., Smith, K.A., Lee, L.L., Henneberry, T.J. 2000. Effect of soil depth and moisture on the vertical distribution of *Steinernema riobrave* (Nematoda: Steinernematidae). Journal of Nematology. 32, 223-228.

Grewal, P.S., Selvan, S., Gaugler, R. 1994. Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment, and reproduction. Journal of Thermal Biology. 19, 245-253.

Grewal, P.S., Jagdale, G.B. 2002. Enhanced trehalose accumulation and desiccation survival of entomopathogenic nematodes through cold preacclimation. Biocontrol Science and Technology. 12, 533-545.

Grewal, P.S., Koppenhöfer, A.M., Choo, H.Y. 2005. Lawn, turfgrass, and pasture pests. In: Grewal, P.S., Shapiro-Ilan, D.I., Ehlers, R.-U. (Eds.), Nematodes as Biocontrol Agents. CAB International, Wallingford, UK, pp. 115-146.

Grewal, P.S., Bornstein-Forst, S., Burnell, A.M., Glazer, I., Jagdale, G.B. 2006. Physiological, genetic, and molecular mechanisms of chemoreception, thermobiosis, and anhydrobiosis in entomopathogenic nematodes. Biological Control. 38, 54-65.

Hominick, W.M., Reid, A.P. 1990. Perspectives on entomopathogenic nematology. In: Gaugler, R., Kaya, H.K. (Eds.), Entomopathogenic Nematodes in Biocontrol. CRC Press, Boca Raton, Florida, 327-343 pp.

Hominick, W.M. 2002. Biogeography. In: Gaugler, R. (Eds.) Entomopathogenic Nematology. ©CAB International, New York, NY 115-137 pp.

Kaya, H.K. 1990. Soil Ecology. In: Gaugler, R., Kaya, H.K. (Eds.), Entomopathogenic Nematodes in biological control. CRC Press, Boca Raton, FL., 93 pp.

Kaya, H.K., Stock, S.P. 1997. Techniques in insect nematology. In: Lacey, L. (Ed.), Manual of Techniques in Insect Pathology. Academic Press, San Diego, CA., 281-324 pp.

Koppenhöfer, A.M., Kaya, H.K. 1996. Coexistence of two steinernematid nematode species (Rhabditida: Steinernematidae) in the presence of two host species. Applied Soil Ecology. 4, 221-230.

Koppenhöfer, A.M., Baur, M.E., Kaya, H.K. 1996. Competition between two steinernematid nematode species for an insect host at different soil depths. Journal of Parasitology. 82, 34-40.

Koppenhöfer, A.M., Campbell, J.F., Kaya, H.K., Gaugler, R. 1997. Estimation of entomopathogenic nematode density in soil using the correlation between bait insect mortality and nematode penetration. Fundamental and Applied Nematology. 21, 95–102.

Koppenhöfer, A.M., Fuzy, E.M. 2003. Ecological characterization of *Steinernema scarabaei*, a scarab-adapted entomopathogenic nematode from New Jersey. Journal of Invertebrate Pathology. 83, 139-148.

Koppenhöfer, A.M., Fuzy, E.M. 2006. Effect of soil type on infectivity and persistence of the entomopathogenic nematodes *Steinernema scarabaei, Steinernema glaseri, Heterorhabditis zealandica,* and *Heterorhabditis bacteriophora*. Journal of Invertebrate Pathology. 92, 11-22.

Koppenhöfer, A.M., Fuzy, E.M. 2007. Soil moisture effects on infectivity and persistence of the entomopathogenic nematodes *Steinernema scarabaei*, *Steinernema glaseri*, *Heterorhabditis zealandica*, and *Heterorhabditis bacteriophora*. Applied Soil Ecology. 35(1), 128-139.

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, 63-72.

Lewis, E.E., Gaugler, R., Harrison, R. 1992. Entomopathogenic nematode host finding-response to host contact cues by cruise and ambush foragers. Parasitology. 105, 309-315.

Lewis, E.E., Gaugler, R., Harrison, R. 1993. Response of crusier and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. Canadian Journal of Zoology. 71. 765-769.

Lewis, E.E., Campbell, J.F., Gaugler, R. 1998. A conservation approach to using entomopathogenic nematodes in turf and landscapes. In: Barbosa, P. (Ed.), Conservation Biological Control. Academic Press, New York, NY, pp. 235-254.

Lewis, E.E., Gaugler, R., Harrison, R. 2002. Entomopathogenic nematode host finding – response to host contact cues by cruise and ambush foragers. Parasitology. 105, 309-315.

Lewis, E.E., Shapiro-Ilan, D.I. 2002. Host cadavers protect entomopathogenic nematodes during freezing. Journal of Invertebrate Pathology. 81, 25-32.

Lewis, E.E., Campbell, J., Griffin, C., Kaya, H., Peters, A. 2006. Behavioral ecology of entomopathogenic nematodes. Biological Control. 38, 66-79.

Mail, G.A. 1930. Winter soil temperatures and their relation to subterranean insect survival. Journal of Agricultural Research. 41, 571-592.

Matsuoka, N. 1998. Soil moisture variability in relation to diurnal frost heaving on Japanese high mountain slopes. Permafrost and Periglacial Processes. 7, 139 – 151.

Mauleon, H., Briand, S., Laumond, C., Bonifassi, E. 1993. Utilisation d'enzyme digestives pour l'étude du parasitisme des Steinernematidae et Heterorhabditidae envers les larves d'insectes. Fundamental Applied Nematology. 16, 185–186.

Mráček, Z., Bečvář, S. 2000. Insect aggregation and entomopathogenic nematode occurrence. Nematology. 2, 297-301.

Parkman, J.P., Frank, J.H., Walker, T.J., Schuster, D.J. 1996. Classical biological control of *Scapteriscus spp.* (Orthoptera: Gryllotalpidae) in Florida. *Environmental Entomology.* 25, 1415-1420.

Parsa, S., Alcazar, J., Salazar, J., Kaya, H.K. 2006. An indigenous Peruvian entomopathogenic nematode for suppression of the Andean potato weevil. Biological Control. 39, 171-178.

Půža, V., Mráček, Z. 2005. Seasonal dynamics of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* as a response to abiotic factors and abundance of insect hosts. Journal of Invertebrate Pathology. 89, 116-122.

Potter, D.A. 1998. Destructive turfgrass insects, biology, diagnosis, and control. John Wiley and Sons, INC. Hoboken, New Jersey, 154 pp.

Portillo-Aguilar, C., Villani, M.G., Tauber, M.J., Tauber, C.A., Nyrop, J.P. 1999. Entomopathogenic nematode (Rhabditida : Heterorhabditidae and Steinernematidae) response to soil texture and bulk density. Environmental Entomology. 28, 1021-1035.

Rio, R.V., Cameron, A.E. 2000. *Heterorhabditis bacteriophora*: Seasonal dynamics and distribution in a stand of Sugar Maple, *Acer saccharum.* Journal of Invertebrate Pathology. 75, 36-40.

Robinson, A.F. 1994. Movement of five nematode species through sand subjected to natural temperature gradient fluctuations. Journal of Nematology. 26, 46-58.

SAS Institute, 2002. SAS 9.1 for Windows. SAS Institute, Cary, NC.

Serwe-Rodriguez, J., Sonnenberg, K., Appleman, B., Bornstein-Forst, S. 2004. Effects of host desiccation on development, survival, and infectivity of entomopathogenic nematode *Steinernema carpocapsae*. Journal of Invertebrate Pathology. 85, 175-181.

Shapiro-Ilan, D.I., Gouge, D.H., Koppenhöfer, A.M. 2002. Factors affecting commerical success: case studies in cotton, turf and citrus. In: Gaugler, R. (Ed.), Entomopathogenic Nematology. CAB International, Wallingford, pp. 333-355.

Spence, K.O., Lewis, E.E., Perry, R.N. 2008. Host-finding and invasion by entomopathogenic and plant-parasitic nematodes: evaluating the ability of laboratory bioassays to predict field results. Journal of Nematology. 40, 93-98.

Spiridonov, S.E., Moens, M., Wilson, M.J. 2007. Fine scale spatial distribution of two entomopathogenic nematodes in a grassland soil. Applied Soil Ecology. 37, 192-201.

Stock, S.P., Koppenhöfer, A.M. 2003. *Steinernema scarabaei* n. sp. (Rhabditida: Steinernematidae), a natural pathogen of scarab larvae (Coleoptera: Scarabaeidae) from New Jersey. Nematology 5, 191-204.

Stuart, R.J., Barbercheck, M.E., Grewal, P.R., Taylor, R.A.J., Hoy, C.W. 2006. Population biology of entomopathogenic nematodes: Concepts, issues, and models. Biological Control. 38, 80-102.

Stuart, R.J. Gaugler, R. 1994. Patchiness in populations of entomopathogenic nematodes. Journal of Invertebrate Pathology. 64, 39-45.

Susurluk, A., Ehlers, R.-U. 2008. Field persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora* in different crops. Biocontrol. 53, 627-641.

Tanada, Y., Kaya, H.K. 1993. Insect Pathology. Academic Press, INC. San Diego, 595-622 pp.

Villani, M.G., Wright, R.J. 1990. Environmental influences on soil macro-arthropod behavior in agricultural systems. Annual Review of Entomology. 35: 249-269.

Villani, M.G., Nyrop, J.P. 1991. Age-dependent movement patterns of Japanese Beetle and European Chafer (Coleoptera: Scarabaeidae) grubs in soil-turfgrass microcosms. Environmental Entomology. 20, 241-251.

Villani, M.G., Allee, L.L., Díaz, A., Robbins, P.S. 1999. Adaptive strategies of edaphic arthropods. Annual Review of Entomology. 44, 233-256.

Vittum, P., Villani, M.G., Tashiro, H. 1999. Turfgrass Insects of the United States and Canada. Comstock Publishing Associates. Ithaca, 205, 213 pp.

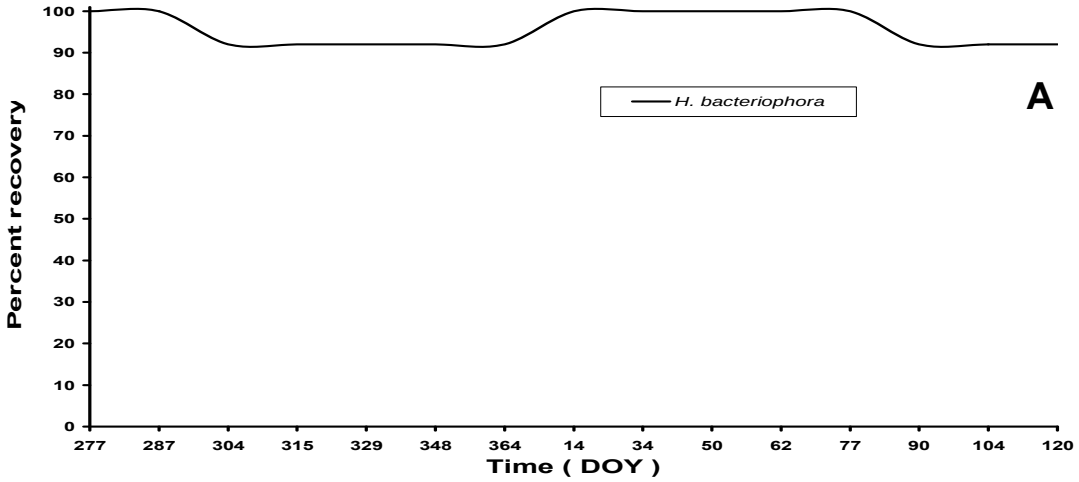
Wallace, H.R. 1958. Movement of eelworms. Annals of Applied Biology. 46, 74-85.

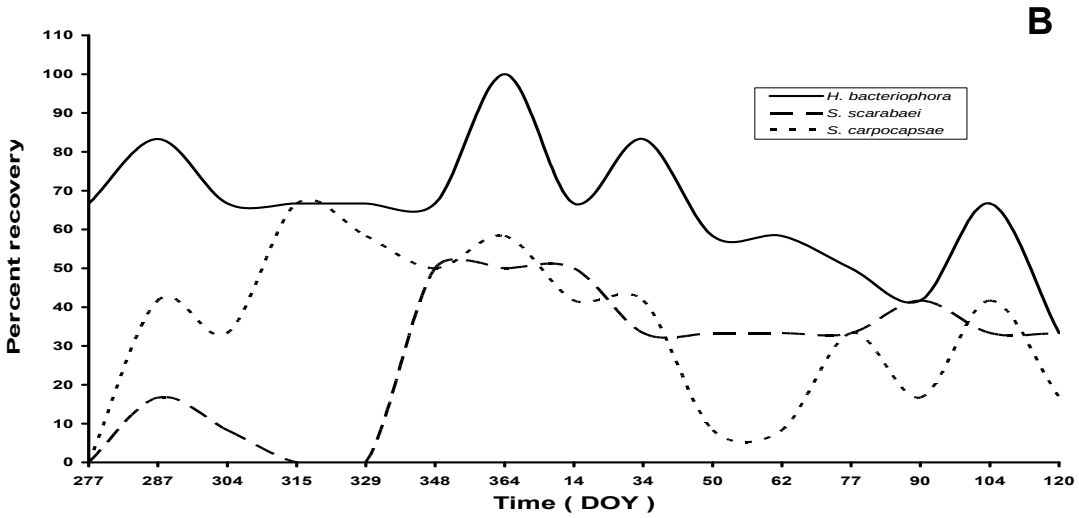
Wang, Y., Gaugler, R. 1998. Host and penetration site location by entomopathogenic nematodes against Japanese Beetle larvae. Journal of Invertebrate Pathology. 72, 313-318.

Wharton, D.A. 2004. Survival strategies. In: Gaugler, R., Bilgrami, A.L. (Eds.), Nematode Behavior. ©CAB International, Oxfordshire, UK, 372.

Yeats, G.W. 2004. Ecological and behavioral adaptations. In: Gaugler, R., Bilgrami, A.L. (Eds.), Nematode Behavior. ©CAB International, Oxfordshire, UK, 4 pp.

Figure 1. Percent recovery of nematodes across all treatment blocks during the 2006/2007 season at Adelphia for *Heterorhabditis bacteriophora* (A) and during the 2007/2008 season for *H. bacteriophora*, *Steinernema scarabaei*, and *S. carpocapsae* at Adelphia (B) and Horticulture Farm II (C). Percent recovery differed by species across the entire 2007/2008 sampling season [B: χ^2 (3, N = 15) = 66.96, $P < 0.0001$; C: χ^2 (3, N = 15) = 82.66, $P < 0.0001$].


A
H. bacteriophora
Percent recovery
Time (DOY)
0
10
20
30
40
50
60
70
80
90
100
277
287
304
315
329
348
364
14
34
50
62
77
90
104
120


B
H. bacteriophora
S. scarabaei
S. carpocapsae
Percent recovery
Time (DOY)
0
10
20
30
40
50
60
70
80
90
100
110
277
287
304
315
329
348
364
14
34
50
62
77
90
104
120

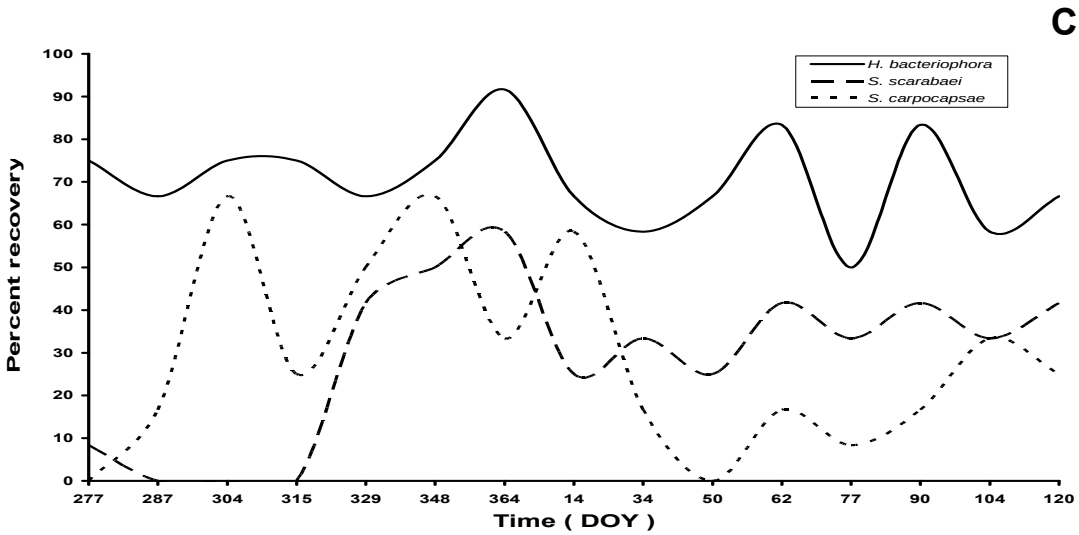
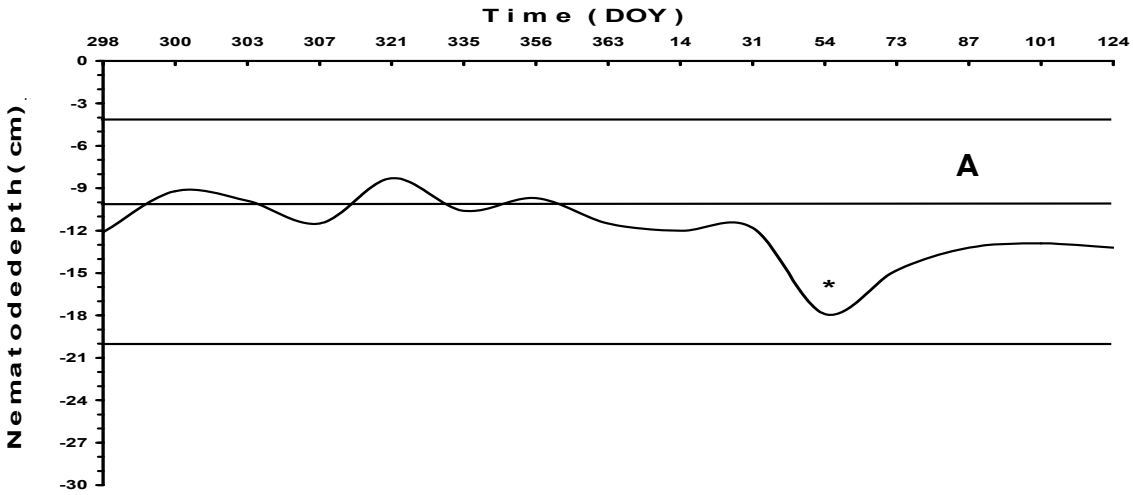
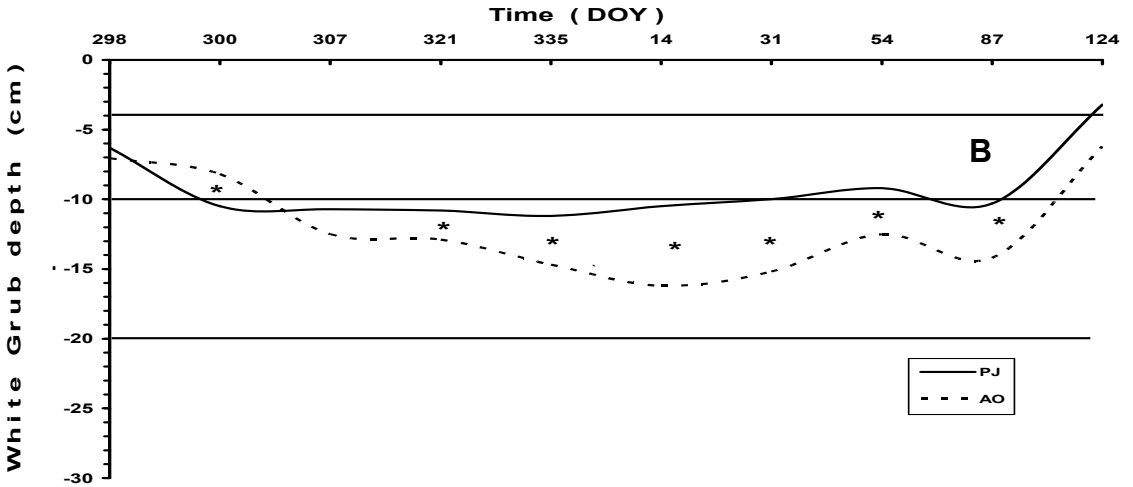

C
H. bacteriophora
S. scarabaei
S. carpocapsae
Percent recovery
Time (DOY)
0
10
20
30
40
50
60
70
80
90
100
277
287
304
315
329
348
364
14
34
50
62
77
90
104
120

Figure 2. Change in the depth index value of *Heterorhabditis. bacteriophora* (A), vertical position of *Popillia japonica* and *Anomala orienta*lis (B) and soil and meteorological conditions (C) from October 25, 2006 to May 4, 2007 at Adelphia. Nematode data represents depth index values for *H. bacteriophora* only. The asterisk indicates a significant change in depth index value. This significant point is likely due to a sampling error attributed to the difficulty of baiting soil recovered from the frost zone (A). White grub data originating from Adelphia and Horticulture Farm II are combined. Asterisks represent a significant difference in depth between *P. japonica* and *A. orientalis* ($P < 0.05$) (B). Temperature is averaged across three depths (5 cm, 15 cm, 25 cm) and the 5 days prior to each sample date.


Time (DOY)
298 300 303 307 321 335 356 363 14 31 54 73 87 101 124
0 -3 -6 -9 -12 -15 -18 -21 -24 -27 -30
Nematodedepth(cm)
A


Time (DOY)
298 300 307 321 335 14 31 54 87 124
0 -5 -10 -15 -20 -25 -30
White Grub depth (cm)
B
PJ
AO

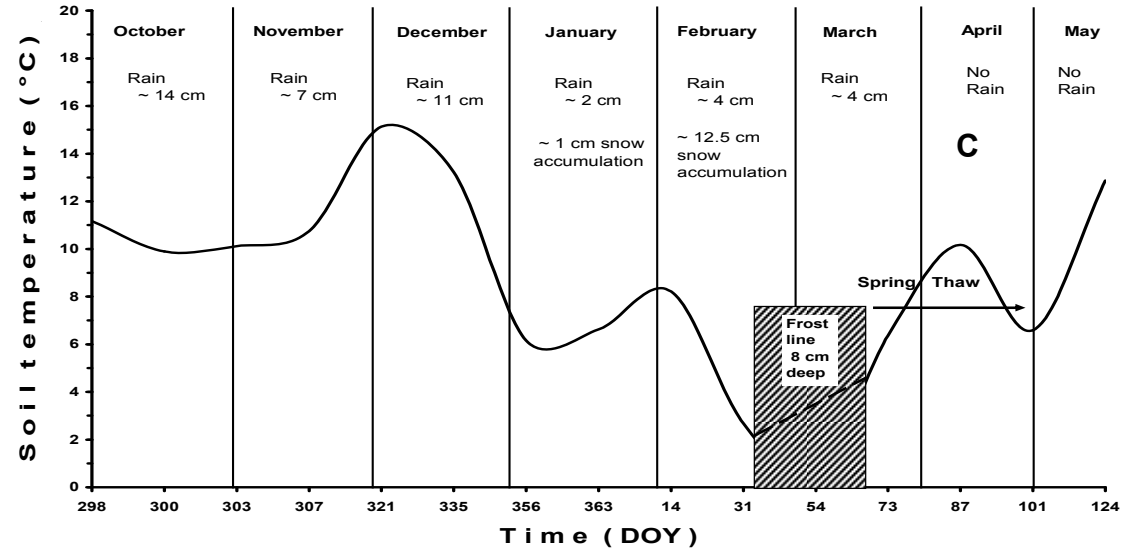
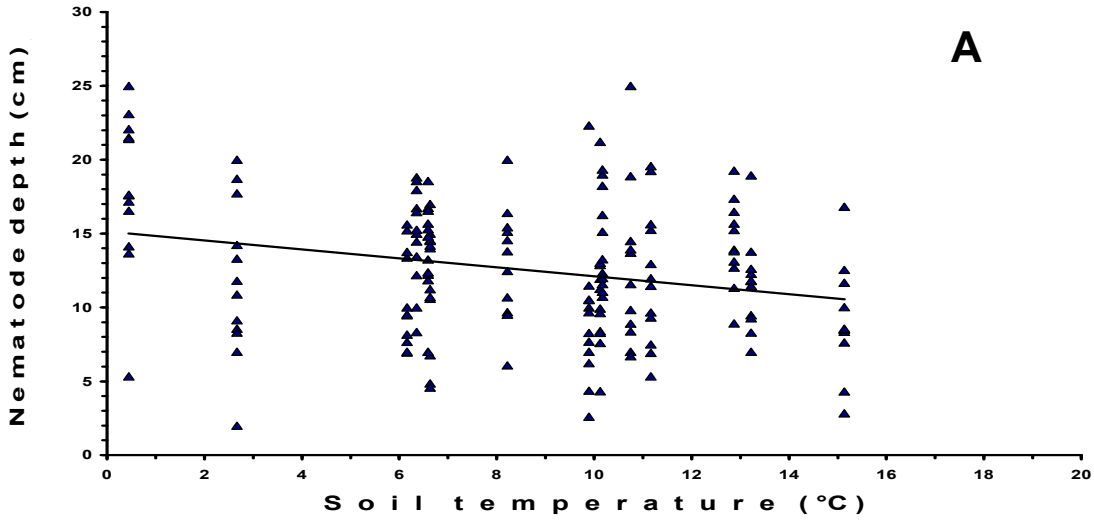
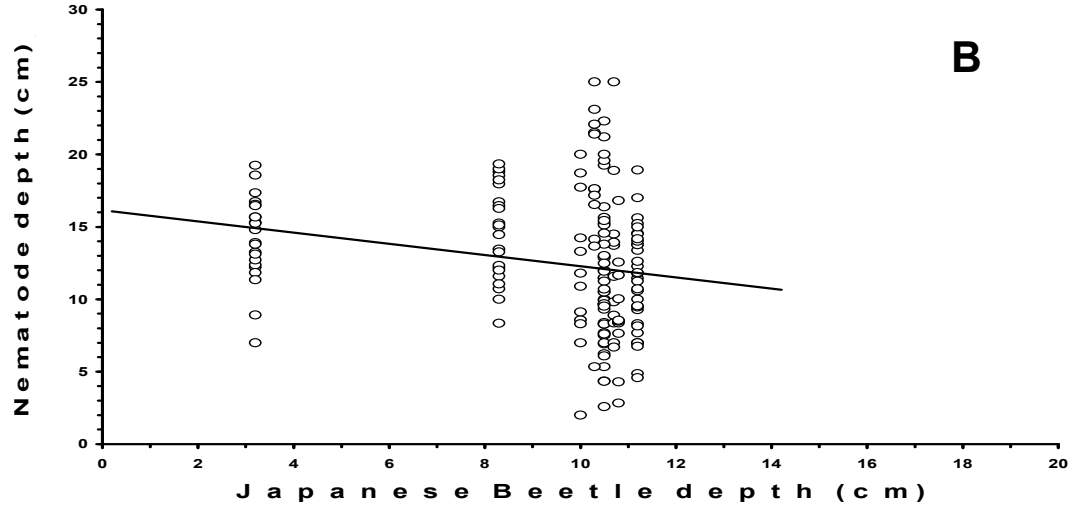

20 18 16 14 12 10 8 6 4 2 0
Soil temperature (°C)
October
Rain ~ 14 cm
November
Rain ~ 7 cm
December
Rain ~ 11 cm
January
Rain ~ 2 cm
~ 1 cm snow accumulation
February
Rain ~ 4 cm
~ 12.5 cm snow accumulation
March
Rain ~ 4 cm
April
No Rain
May
No Rain
C
Spring Thaw
Frost line 8 cm deep
298 300 303 307 321 335 356 363 14 31 54 73 87 101 124
Time (DOY)

Figure 3. The relationship of depth index value to changing soil temperature (A) and two natural hosts *Popillia japonica* (B) and *Anomala orientalis* (C) from October 28, 2006 to May 4, 2007 at Adelphia. Temperature is averaged across three depths (5 cm, 15 cm, 25 cm) and the 5 days prior to each sample date. *Heterorhabditis bacteriophora* depth index value had a significant relationship with soil temperature ($P = 0.0003$ $r^2 = 0.0654$) and *P. japonica* vertical position ($P = 0.0009$ $r^2 = 0.0574$)


A
Nematode depth (cm)
Soil temperature (°C)
0
5
10
15
20
25
30
0
2
4
6
8
10
12
14
16
18
20


B
Nematode depth (cm)
Japanese Beetle depth (cm)
0
5
10
15
20
25
30
0
2
4
6
8
10
12
14
16
18
20

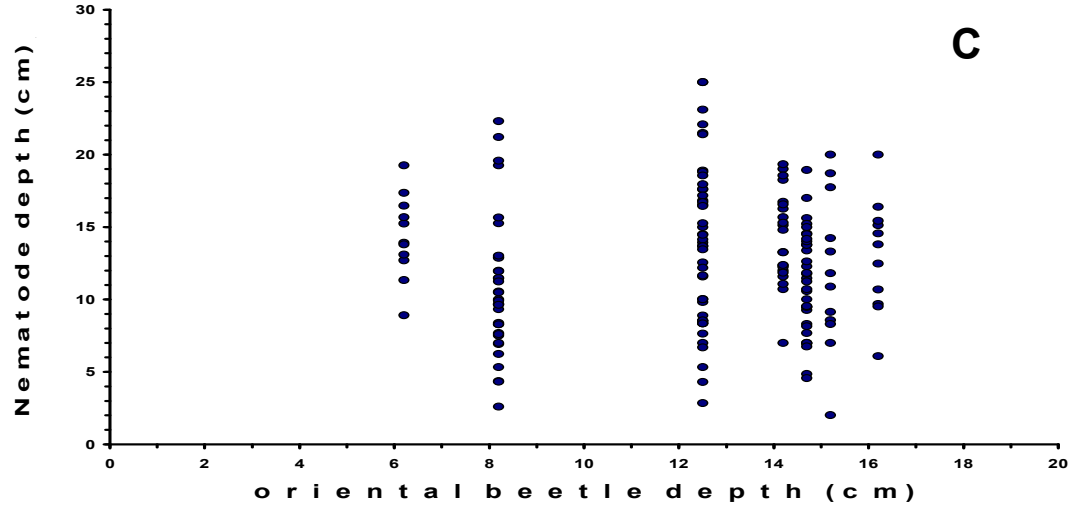

C
Nematode depth (cm)
oriental beetle depth (cm)
0
5
10
15
20
25
30
0
2
4
6
8
10
12
14
16
18
20

Figure 4. Change in depth index value from October 4, 2007 to April 29, 2008 of *Heterorhabidits bacteriophora* (A), *Steinernema scarabaei* (B), *S. carpocapsae* (C), and vertical position of *Popillia japonica* and *Anomala orientalis* (D) at Adelphia. No significant difference in vertical position was detected among or within the nematode species. White grub data are combined from both sites. Asterisks represent a significant difference in depth between *P. japonica* and *A. orientalis* ($P < 0.05$).

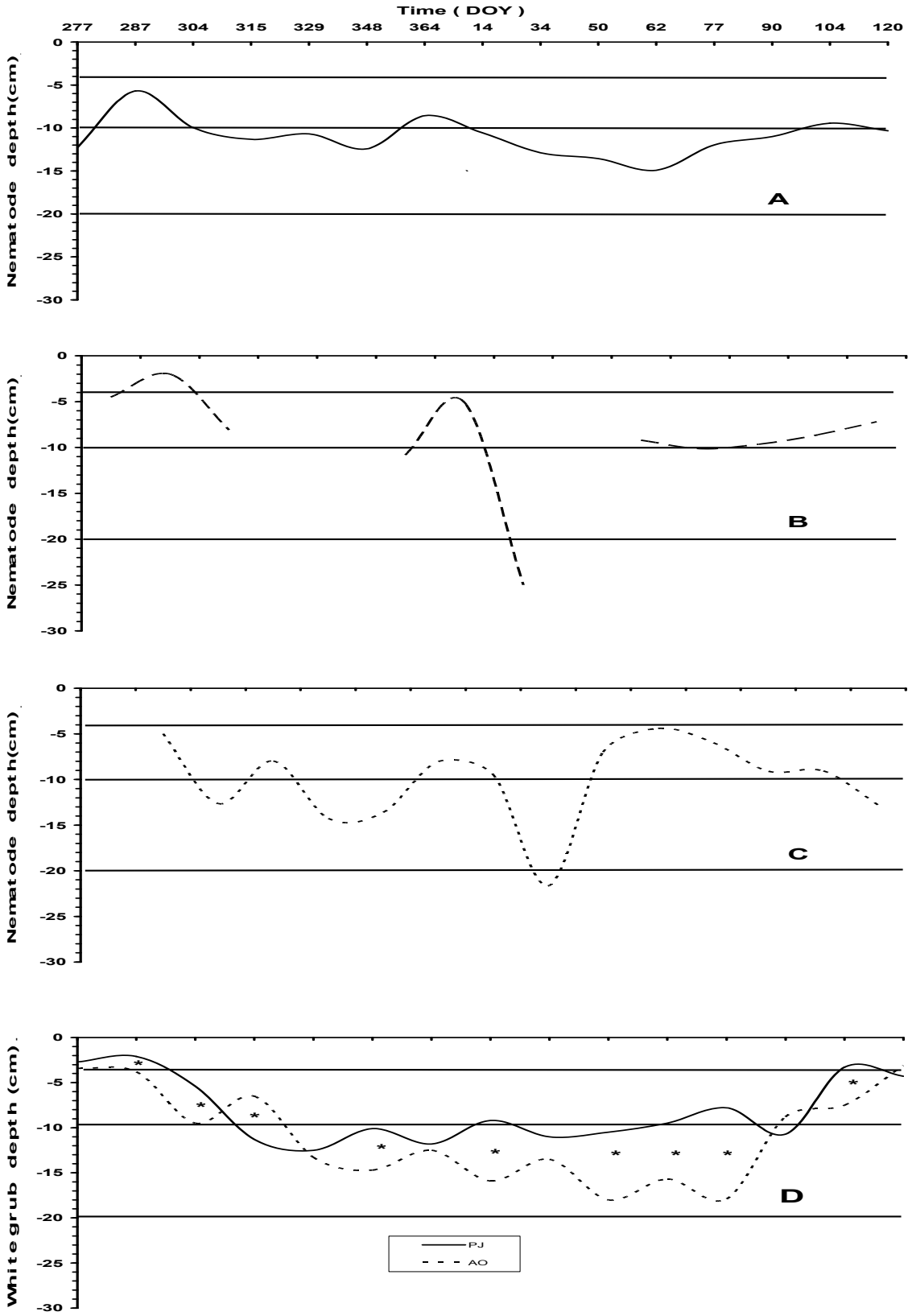

Time (DOY)
277 287 304 315 329 348 364 14 34 50 62 77 90 104 120
Nematode depth(cm)
0 -5 -10 -15 -20 -25 -30
A
B
C
White grub depth (cm)
D
PJ
AO

Figure 5. Change in depth index value from October 3, 2007 to April 28, 2008 of *Heterorhabditis bacteriophora* (A) *Steinernema scarabaei* (B), *S. carpocapsae* (C), and the vertical position of *Popillia japonica* and *Anomala orientalis* (D) at Horticulture Farm II. No significant difference in vertical position was detected among or within nematode species. White grub data from both sites are combined. Asterisks represent a significant difference in depth between *P. japonica* and *A. orientalis* ($P < 0.05$).

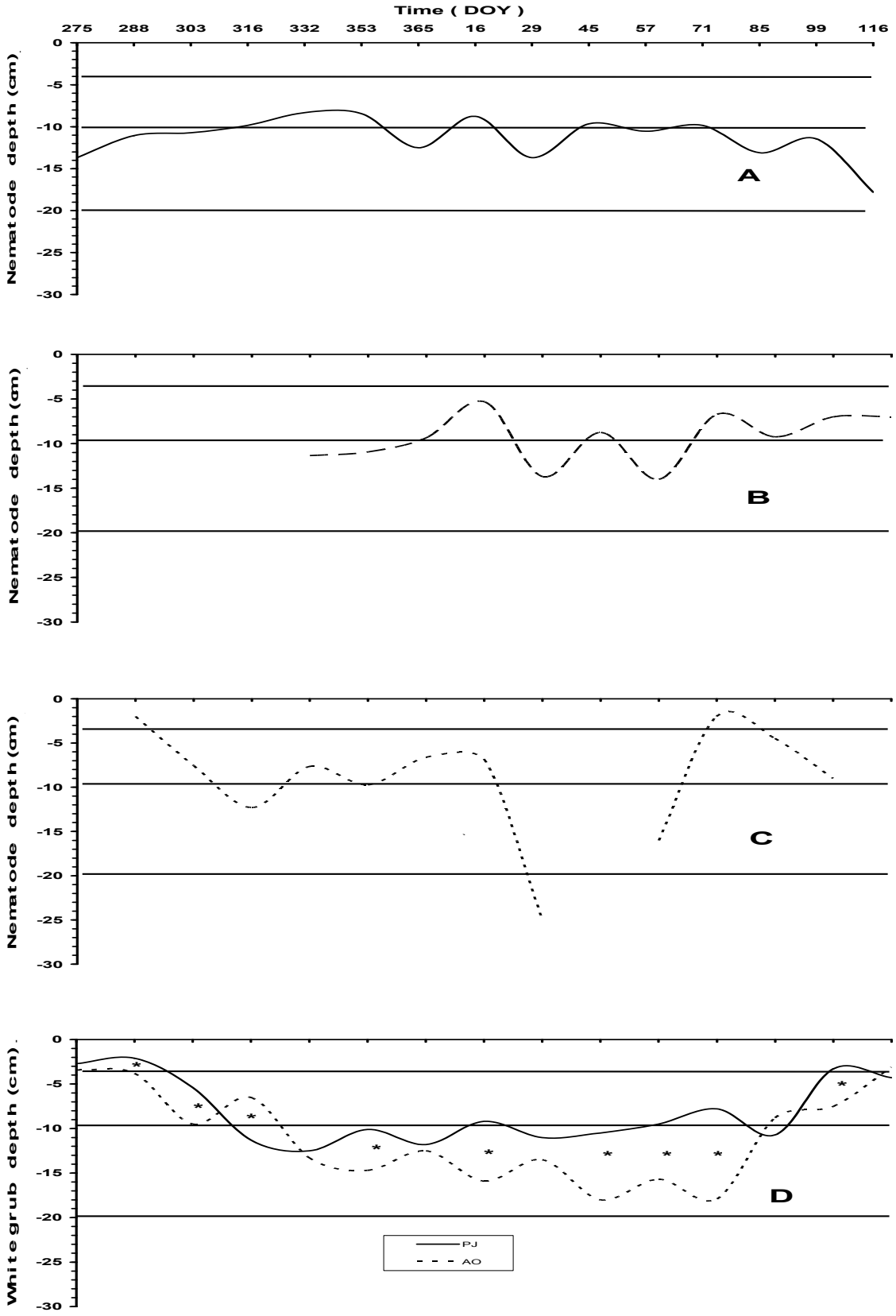

Time (DOY)
275 288 303 316 332 353 365 16 29 45 57 71 85 99 116
Nematode depth (cm)
Whitegrub depth (cm)
0 -5 -10 -15 -20 -25 -30
A
B
C
D
PJ
AO

Figure 6. The relationship of depth index value to changing soil temperature (A) and two natural hosts *Popillia japonica* (B) and *Anomala orientalis* (C) from October 4, 2007 to April 29, 2008 at Adelphia. A significant relationship was detected between depth index value and soil temperature ($P = 0.0007$ $r^2 = 0.0511$). Depth index value was also compared to the vertical position of *A. orientalis* and *P. japonica.* There was a significant relationship in vertical position between nematodes and *P. japonica* ($P = 0.0026$ $r^2 = 0.0402$) and *A. orientalis* ($P = 0.0087$ $r^2 = 0.0307$). Temperature is averaged across three depths (5 cm, 15 cm, 25 cm) and the 5 days prior to each sample date.

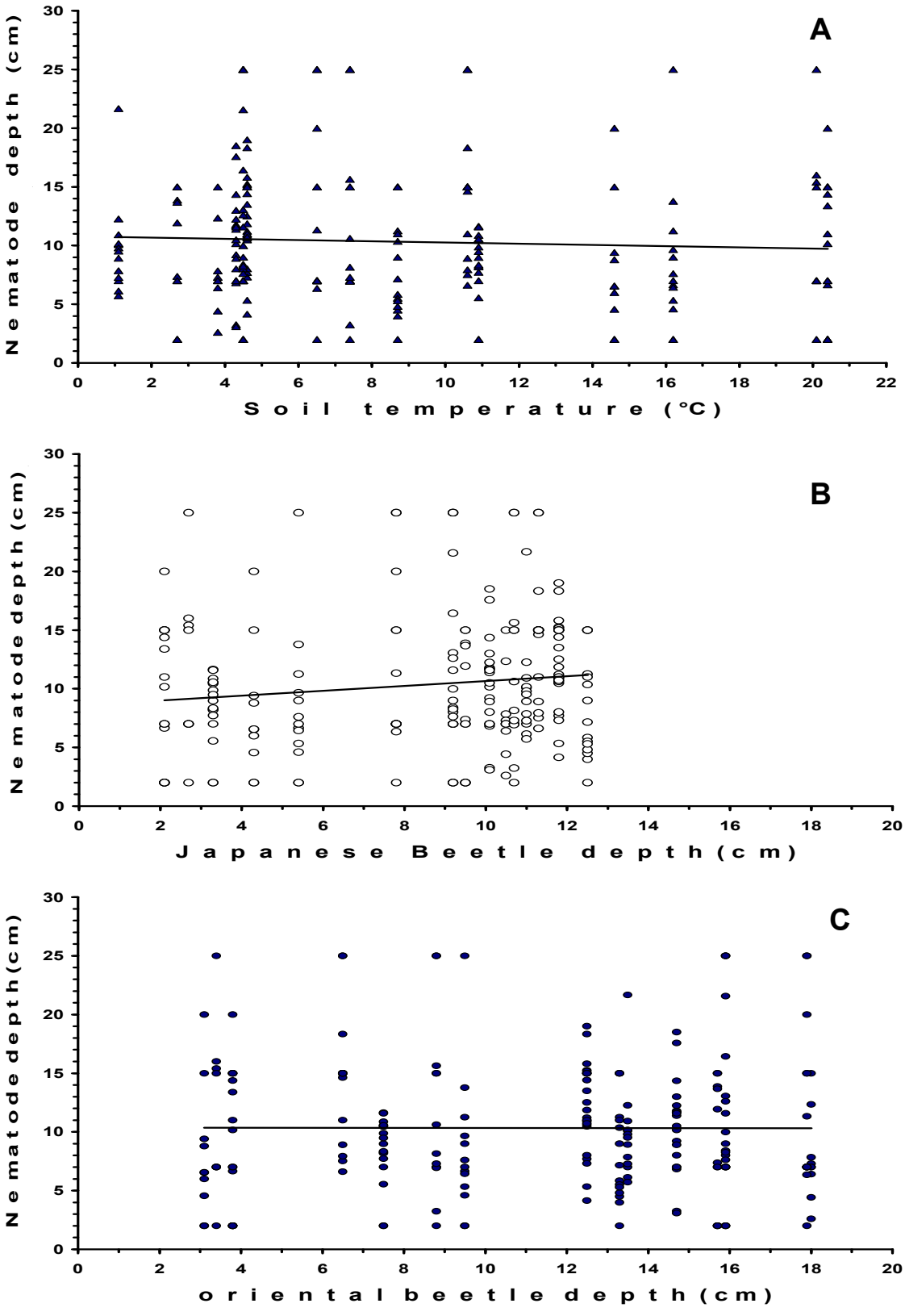
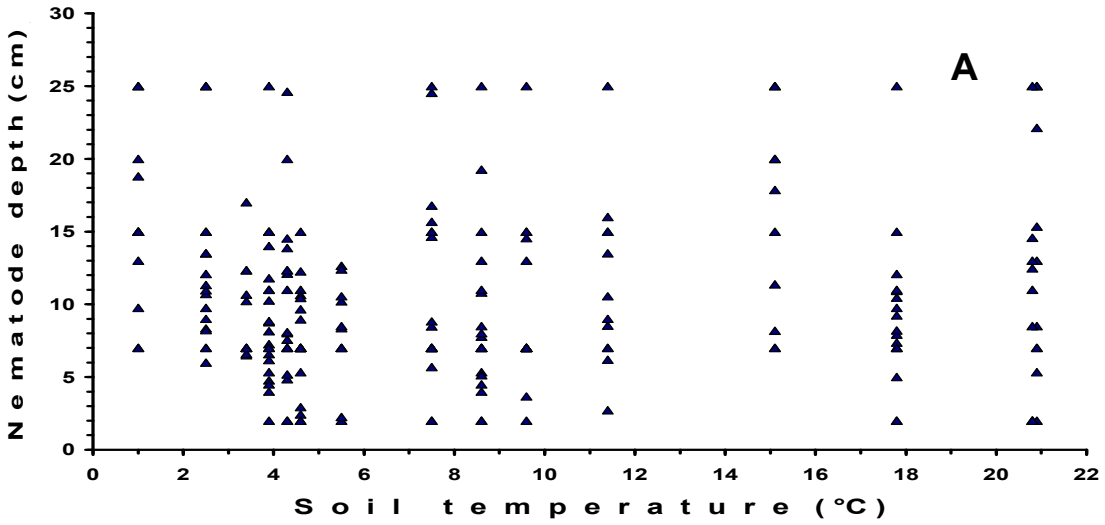
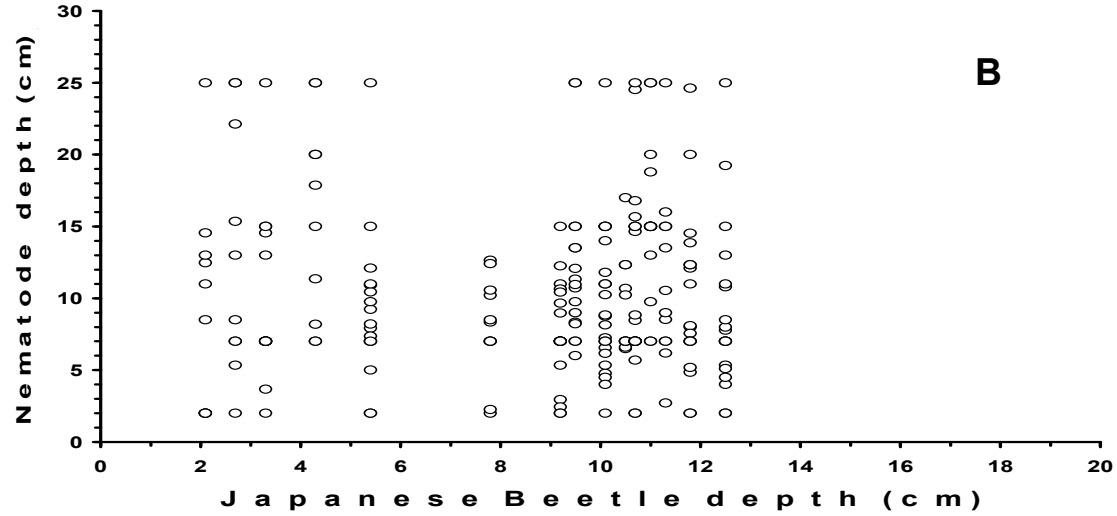

A
Nematode depth (cm)
Soil temperature (°C)
B
Nematode depth (cm)
Japanese Beetle depth (cm)
C
Nematode depth (cm)
oriental beetle depth (cm)

Figure 7. The relationship of depth index value to changing soil temperature (A) and two natural hosts *Popillia japonica* (B) and *Anomla orientalis* (C) from October 2, 2007 to April 25, 2008 at Horticulture Farm II. A significant relationship was not detected between nematode depth and soil temperature ($P = 0.83$; $r^2 = - 0.0043$). Depth index value was also compared to the vertical position of *A. orientalis* and *P. japonica.* There was a significant relationship in vertical position between nematodes and *A. orientalis* ($P = 0.0374$ $r^2 = 0.0152$) but not nematodes and *P. japonica* ($P = 0.51$; $r^2 = - 0.0026$). Temperature is averaged across three depths (5 cm, 15 cm, 25 cm) and the 5 days prior to each sample date.


A
Nematode depth (cm)
Soil temperature (°C)


B
Nematode depth (cm)
Japanese Beetle depth (cm)

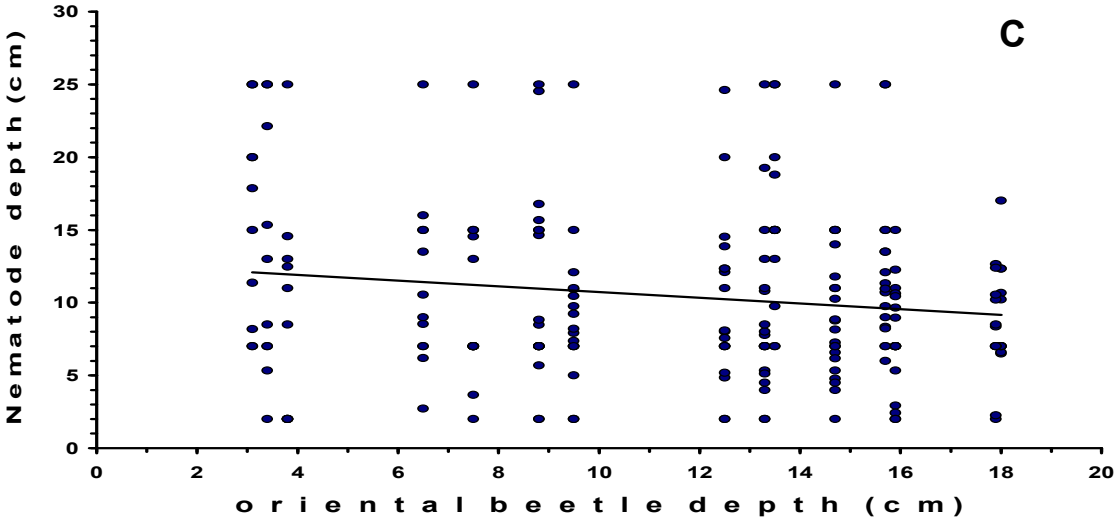
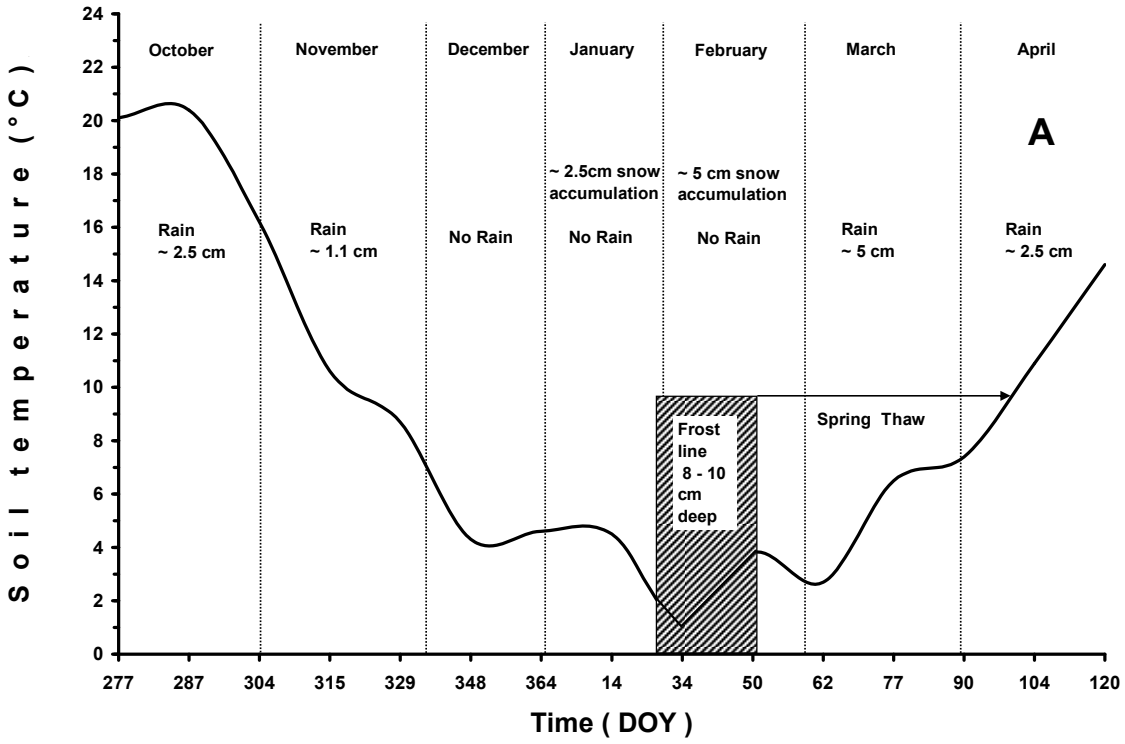

C
Nematode depth (cm)
oriental beetle depth (cm)

Figure 8. Change in soil temperature and monthly rain/snow accumulation from October 2007 to April 2008 at Adelphia (A) and Horticulture Farm II (B). Since daily average temperature did not vary significantly among the three depths, temperature was averaged across all depths. A maximum soil temperature (20.1°C, 20.9°C, respectively) was detected in the first week of October and a minimum soil temperature (1.0 ±1.0 °C) in the last week of January. From late January through mid-February the soil was frozen 8 to 10 cm depth. Temperature is averaged across three depths (5 cm, 15 cm, 25 cm) and the 5 days prior to each sample date.


Soil temperature (°C)
October
November
December
January
February
March
April
A
~ 2.5cm snow accumulation
~ 5 cm snow accumulation
Rain ~ 2.5 cm
Rain ~ 1.1 cm
No Rain
No Rain
No Rain
Rain ~ 5 cm
Rain ~ 2.5 cm
Frost line 8 - 10 cm deep
Spring Thaw
Time (DOY)

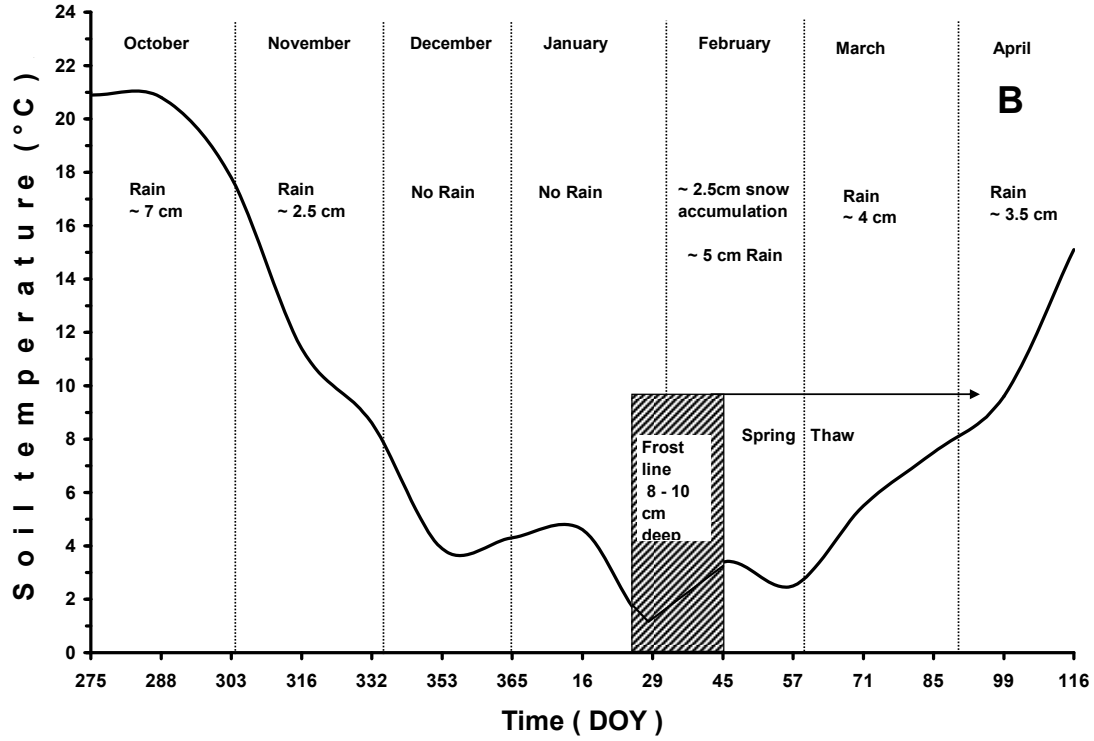

Soil temperature (°C)
October
November
December
January
February
March
April
B
Rain ~ 7 cm
Rain ~ 2.5 cm
No Rain
No Rain
~ 2.5cm snow accumulation
~ 5 cm Rain
Rain ~ 4 cm
Rain ~ 3.5 cm
Frost line 8 - 10 cm deep
Spring Thaw
Time (DOY)

Figure 9. Change in vertical distribution of *Popillia japonica* and *Anomala orientalis* relative to soil temperature from October 25, 2006 to May 4, 2007 for two sites combined (Adelphia and Horticulture Farm II). An asterisk represents a difference in depth between species on a given sampling date ($P < 0.05$). *P. japonica* and *A. orientalis* changed their vertical position over time ($F_{13,518} = 2.96$; $P = 0.0003$) but it was not possible to obtain a means separation. Temperature is averaged across three depths (5 cm, 15 cm, 25 cm) and the 5 days prior to each sample date.

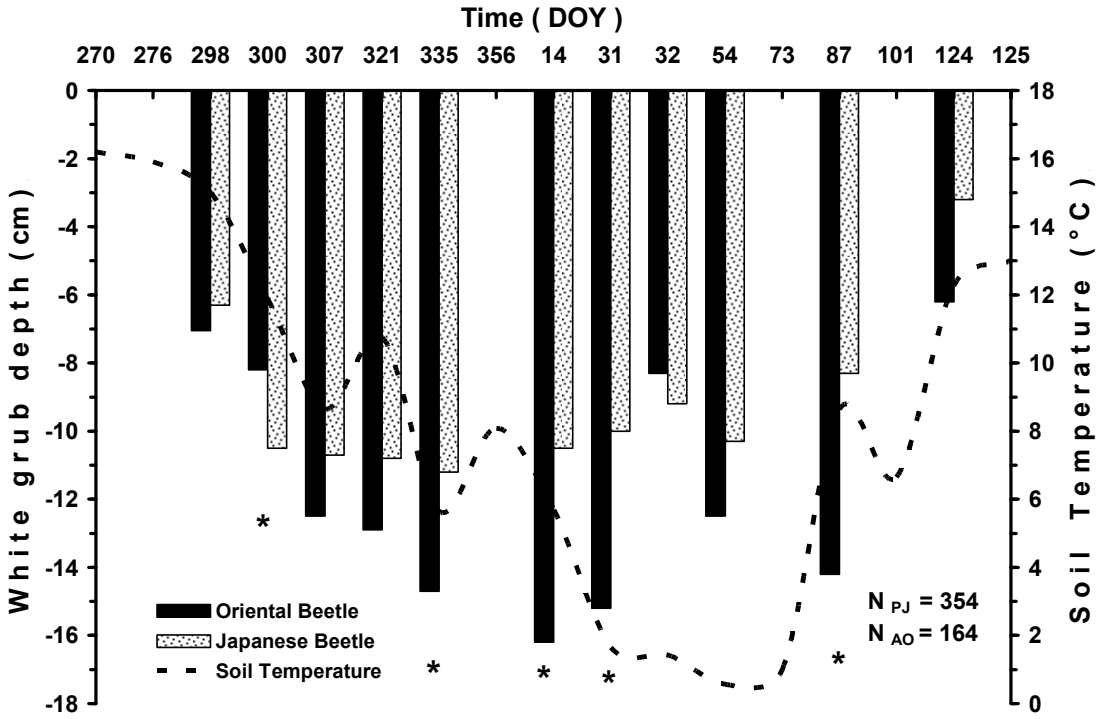

Time (DOY)
270 276 298 300 307 321 335 356 14 31 32 54 73 87 101 124 125
White grub depth (cm)
0 -2 -4 -6 -8 -10 -12 -14 -16 -18
Soil Temperature (°C)
18 16 14 12 10 8 6 4 2 0
Oriental Beetle
Japanese Beetle
Soil Temperature
N PJ = 354
N AO = 164

Figure 10. Change in vertical distribution of *Popillia japonica* and *Anomala orientalis* relative to soil temperature from October 3, 2007 to April 28, 2008 for two sites combined (Adelphia and Horticulture Farm II). An asterisk represents a difference in depth between species on a given sampling date ($P < 0.05$). *P. japonica* and *A. orientalis* changed their vertical distribution over time ($F_{14,403} = 6.20$; $P = 0.0001$). Means within each species sharing the same letter are not significantly different ($P < 0.05$). Temperature is averaged across three depths (5 cm, 15 cm, 25 cm) and the 5 days prior to each sample date.

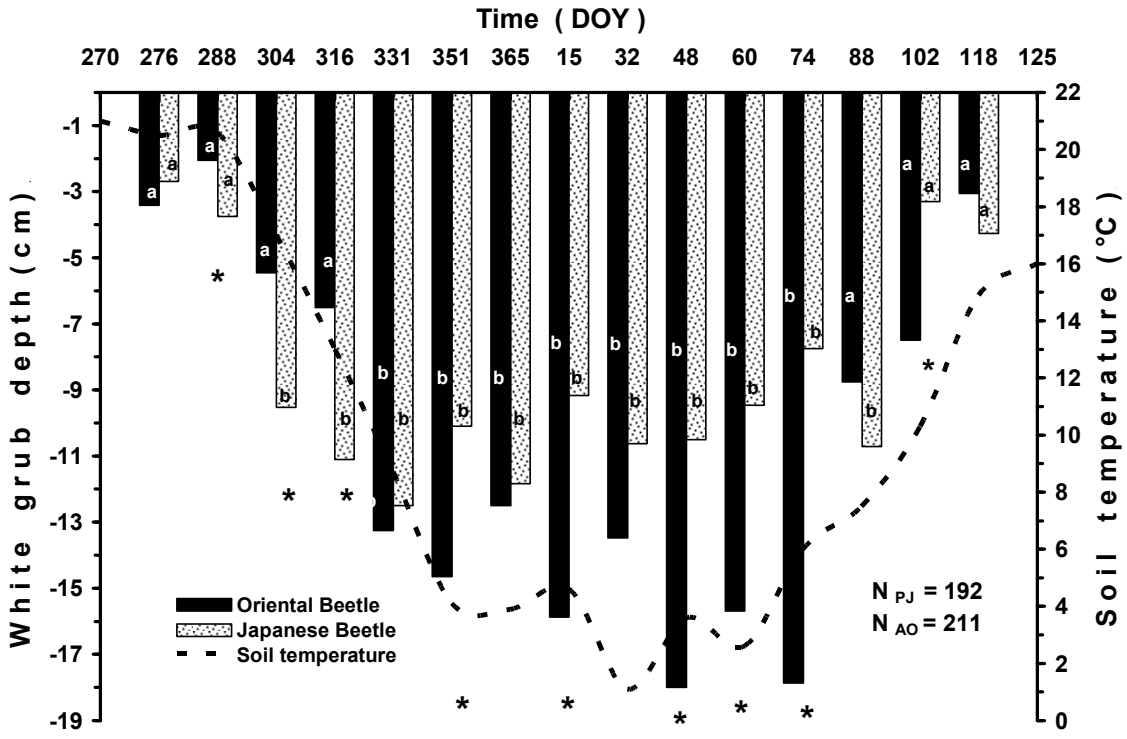

Time (DOY)
270 276 288 304 316 331 351 365 15 32 48 60 74 88 102 118 125
White grub depth (cm)
-1 -3 -5 -7 -9 -11 -13 -15 -17 -19
Soil temperature (°C)
22 20 18 16 14 12 10 8 6 4 2 0
Oriental Beetle
Japanese Beetle
Soil temperature
N PJ = 192
N AO = 211

Figure 11. Relationship between white grub depth and soil temperature from October to May of the 2006/2007 and 2007/2008 seasons combined. Linear relationships were detected for *Popillia japonica (P* = 0.0001; r^2 = 0.1624) and *Anomala orientalis* (*P* = 0.0001; r^2 = 0.3594). White grub and soil temperature data from both seasons were combined since we failed to reject the null hypothesis of equal variance using Levene's test for equality of variance (*P* = 0.05). Temperature is averaged across three depths (5 cm, 15 cm, 25 cm) and the 5 days prior to each sample date.

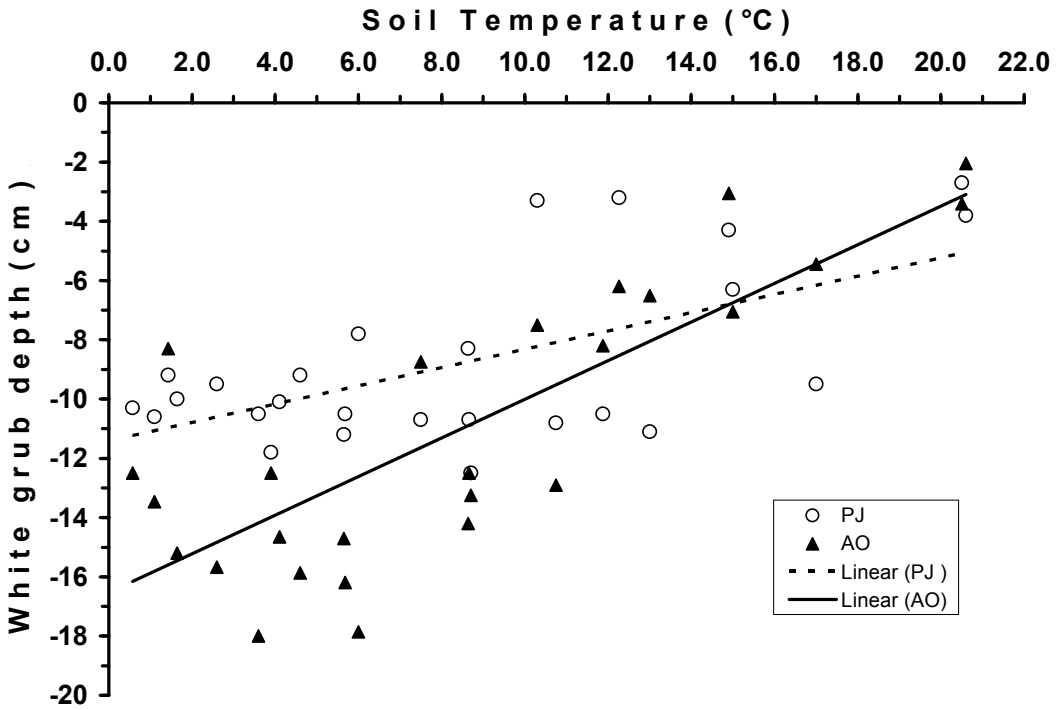
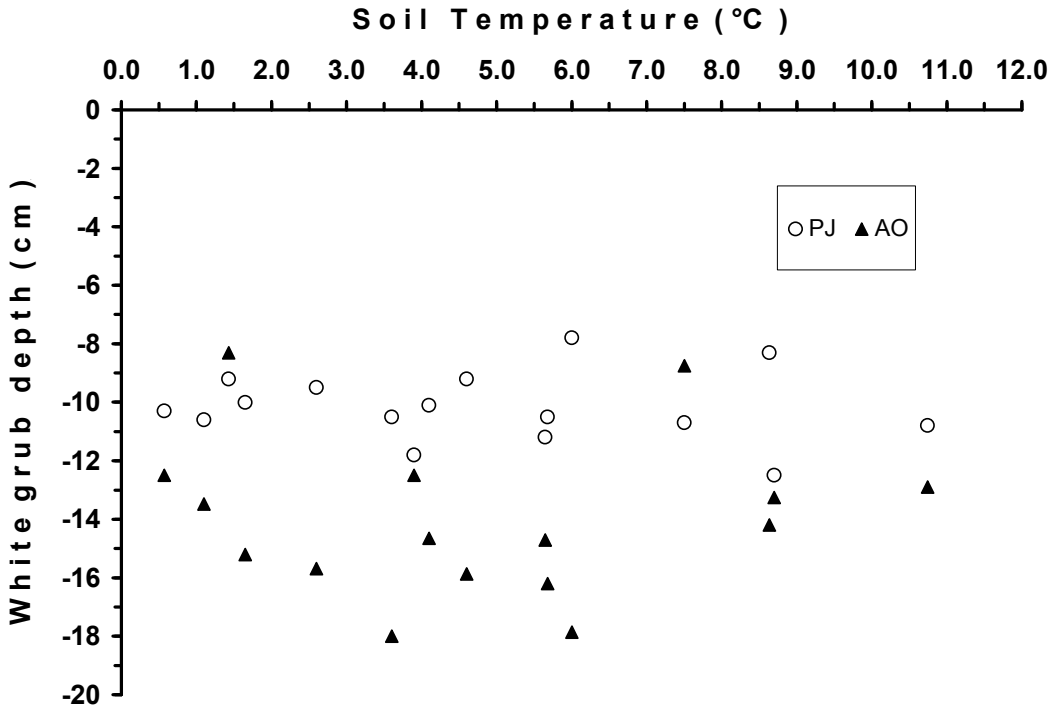

Soil Temperature (°C)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
White grub depth (cm)
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
PJ
AO
Linear (PJ)
Linear (AO)

Figure 12. Relationship between white grub depth and soil temperature from November to March of the 2006/2007 and 2007/2008 seasons combined. Linear relationships were not detected for *Popillia japonica* ($P = 0.27$; $r^2 = 0.0006$) or *Anomala orientalis* ($P = 0.30$; $r^2 = 0.0004$). White grub and soil temperature data from both seasons were combined since we failed to reject the null hypothesis of equal variance using Levene's test for equality of variance ($P = 0.05$). Temperature is averaged across three depths (5 cm, 15 cm, 25 cm) and the 5 days prior to each sample date.


Soil Temperature (°C)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
White grub depth (cm)
PJ
AO

CURRICULUM VITA

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EDUCATION

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PUBLICATIONS:

Tanny, J., S. Cohen, D.E. Elmowitz. 2006. Measuring and Predicting Evapotranspiration in banana screenhouse. *Acta Hort.* 718: 539- 543.