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Susceptibility of lady beetles (Coleoptera: Coccinellidae) to entomopathogenic nematodes

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Abstract

We investigated differential susceptibility of lady beetles to entomopathogenic nematodes, for two reasons: (1) to estimate potential nontarget effects on natural lady beetle populations, (2) to compare the susceptibility of exotic versus native lady beetle species. We hypothesize that successful establishment of some exotically introduced arthropods may be due, in part, to a lower susceptibility relative to competing native species. In laboratory studies, we compared the pathogenicity, virulence, and reproductive capacity of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* among two native (*Coleomegilla maculata* and *Olla v-nigrum*) and two successfully established exotic (*Harmonia axyridis* and *Coccinella septempunctata*) lady beetles, and a known susceptible lepidopteran host, *Agrotis ipsilon*. After 1 and 2 days of exposure to either nematode species, mortality of *A. ipsilon* was higher than in all lady beetles. Thus, we predict that nematode field applications would have significantly less impact on lady beetle populations than on a susceptible target pest. Additionally, the impact of soil-applied nematodes may be lower on lady beetles than on soil-dwelling hosts because the former spends relatively less time on the soil. Exotic lady beetles were less susceptible to nematode infection than native species. Reproductive capacity data also indicated lower host suitability in *H. axyridis*, but not in *C. septempunctata*. Overall, the hypothesis that low susceptibility to pathogens in certain exotic lady beetles may have contributed to competitive establishment was supported (especially for *H. axyridis*). Additional studies incorporating different hosts and pathogens from various geographic locations will be required to further address the hypothesis.
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Keywords: *Agrotis ipsilon*; Lady beetle; *Harmonia axyridis*; *Olla v-nigrum*; *Coleomegilla maculata*; *Coccinella septempunctata*; Entomopathogenic nematode; *Heterorhabditis bacteriophora*; *Steinernema carpocapsae*; Nontarget

1. Introduction

Evaluation of entomopathogen effects on nontarget organisms is an important yet relatively neglected area of study (Hajek and Goettel, 2000). Elucidation of entomopathogen effects on nontarget organisms such as beneficial insects will increase our understanding of a biocontrol agent's ecology in both natural and agricultural ecosystems, and facilitate effective pest management strategies. Pathogen groups with relatively specific host ranges, such as the nucleopolyhedroviruses (Tanada and Kaya, 1993), are considerably less likely to impact nontarget arthropods compared with those groups that contain wide host ranges. Entomopathogenic nematodes (families Steinernematidae and Heterorhabditidae) include many species with relatively wide host ranges that may suppress a variety of insect pest populations in various orders and families (Grewal et al., 2005; Klein, 1990).

Entomopathogenic nematodes are biological control agents that kill their arthropod hosts through a mutualistic relationship with a bacterium (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respectively) (Poinar, 1990). Infective juveniles

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(IJs), the only free-living stage, enter hosts through natural openings (mouth, anus, and spiracles), or in some cases, through the cuticle. After entering the host's hemocoel, nematodes release their symbiotic bacteria, which are primarily responsible for killing the host, defending against secondary invaders, and providing the nematodes with nutrition (Dowds and Peters, 2002). The nematodes molt and complete up to three generations within the host after which IJs exit the cadaver to search out new hosts (Kaya and Gaugler, 1993).

For the most part, entomopathogenic nematodes have been reported to be pathogenic to a number of beneficial insects (predators and parasitoids) under laboratory conditions (Akhurst and Smith, 2002; Georgis et al., 1991; Mráček and Ružička, 1990), but field studies to-date report little or no effect on natural nontarget arthropod populations (Akhurst and Smith, 2002; Bathon, 1996; Georgis et al., 1991). The data, however, are insufficient to make blanket conclusions. Additional research is needed to characterize the impact or potential impact of entomopathogenic nematodes on other biocontrol agents that are important to pest regulation.

Our primary objective was to estimate the potential impact of entomopathogenic nematodes on lady beetles. We determined the innate pathogenicity (ability to cause disease) and virulence (degree of disease-causing power) of entomopathogenic nematodes to four important lady beetle species (Coleoptera: Coccinellidae): the seven spotted lady beetle, *Coccinella septempunctata* L.; *Coleomegilla maculata* (De Geer); the multicolored Asian lady beetle, *Harmonia axyridis* Pallas; and *Olla v-nigrum* Mulsant. There is a dearth of information on entomopathogenic nematode pathogenicity in lady beetles (Laumond et al., 1979; Mráček and Ružička, 1990), and differential susceptibility among lady beetles has not been previously examined. We measured relative susceptibility of lady beetles to two nematodes, *Steinernema carpocapsae* (Weiser) and *Heterorhabditis bacteriophora* Poinar. Both nematodes are ubiquitous, widely studied, and sold commercially on a large scale (Gaugler, 2002; Grewal and Georgis, 1999). To estimate the potential impact of entomopathogenic nematode applications, we compared susceptibility among lady beetle species relative to each other as well as to a known susceptible host, the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) (Capinera et al., 1988; Levine and Oloumi-Sadeghi, 1992).

The study was conducted under laboratory conditions. Certainly, detection of pathogenicity to a particular host in the laboratory does not necessarily mean that the pathogen impacts or can impact disease prevalence in the host's natural population; physiological host range does not necessarily equal ecological host range (Federici and Maddox, 1996; Hajek and Goettel, 2000). Nonetheless, laboratory evaluations that include negative or positive controls (a susceptible host) can serve as a valuable starting point for estimating potential impact in natural populations (Hajek and Goettel, 2000).

The susceptibility of *H. axyridis* to entomopathogenic nematodes compared with the susceptibility of *O. v-nigrum* is of particular interest because differential susceptibility between the two species to the fungal pathogen *Beauveria bassiana* (Balsamo) Vuillemin was reported previously (Cottrell and Shapiro-Ilan, 2003). Endemic *B. bassiana* isolates were substantially more virulent to *O. v-nigrum* than to *H. axyridis* (Cottrell and Shapiro-Ilan, 2003). We find the comparison especially interesting because *O. v-nigrum* is native to North America, and *H. axyridis* is an introduced species from Asia. *H. axyridis* was first documented as being released in the US during the early 1900s with continued releases occurring periodically through the 1980s (Gordon, 1985; Tedders and Schaefer, 1994). *H. axyridis* has since successfully established and become dominant in various row-crop and orchard habitats occupied by native lady beetles (Brown and Miller, 1998; Cottrell and Yeargan, 1998; Michaud, 2002). The success of *H. axyridis* relative to native species has been attributed to high fecundity (Michaud, 2002), aggressiveness, and size (Cottrell and Yeargan, 1998; Michaud, 2002). We hypothesize that the success *H. axyridis* in establishing itself is also due to low susceptibility to entomopathogens relative to native lady beetle species. The results of Cottrell and Shapiro-Ilan (2003) support this hypothesis. Our second objective in the present study was to determine if the same trend (i.e., greater susceptibility in native versus exotic lady beetles) exists when examining entomopathogenic nematodes. Additionally, we have expanded the comparison to include the native *C. maculata* and exotic *C. septempunctata*. To further assess host suitability, we also compared reproductive potential of the entomopathogenic nematodes in each species of lady beetle and in *A. ipsilon*.

2. Materials and methods

2.1. Nematodes and insects

The nematodes used in this study, *S. carpocapsae* (Cxrd strain) and *H. bacteriophora* (VS strain), were isolated near West Helena, Arkansas, USA and Barnesville, Georgia, USA, respectively (Shapiro-Ilan et al., 2003). We purposefully chose nematodes isolated from the southeastern USA to represent populations that occur within the overlapping geographical ranges of the lady beetle species being studied. The nematodes were cultured in parallel in last instar *Galleria mellonella* (L.) (obtained from Webster's Waxie Ranch, Webster, WI) according to Kaya and Stock (1997). Nematodes were stored at 13 °C for less than 3 weeks prior to use in experiments. Prior to this study, the number of passages

that the nematodes had been cultured through *G. mellonella* since their original isolation did not exceed five.

Third instar *A. ipsilon*, reared on artificial diet, were obtained from the USDA-ARS Corn Insects Research unit (Ames, IA). Laboratory colonies of *O. v-nigrum* and *H. axyridis* originated from adult beetles collected from pecan orchards at the USDA, Agricultural Research Service, Southeastern Fruit and Tree Nut Research Laboratory at Byron, GA. The *C. maculata* colony originated from overwintering adult beetles collected from near Lexington, KY, USA. Each colony was supplemented with intermittent addition of field-collected adults from the USDA laboratory at Byron, GA, USA. Each species was housed in 9-cm-diameter petri dishes in an environmental chamber at 25 ± 1 °C and a photoperiod of 14:10 (L:D) h. *O. v-nigrum* and *H. axyridis* were fed live pecan aphids (blackmargined aphids [*Monellia caryella*] and yellow pecan aphids [*Monelliopsis pecanis*] [Homoptera: Aphididae]), frozen *Ephestia kuhniella* eggs [Lepidoptera: Pyralidae], supplemented with a ground beef-beef liver diet (Cohen, 1985) and water provided with a moistened cotton dental wick. The polyphagous *C. maculata* was fed the beef diet and supplemented with *E. kuhniella* eggs. Aphids were reared on foliage of greenhouse-grown seedling pecans (Cottrell et al., 2002). Adult beetles of all three species were from 10 to 20 days old when used in assays. Due to difficulty in establishing laboratory cultures, adult *C. septempunctata* were field collected during the spring of 2004 from Georgia and Minnesota, fed *E. kuhniella* eggs, and used directly in experiments.

2.2. Evaluation of nematode virulence and reproductive capacity

Virulence assays were conducted in plastic Petri dishes (60 mm) based on procedures described by Kaya and Stock (1997). One leaf of filter paper (Whatman No. 1) was placed on the top and on the bottom of each dish and folded up around the sides so that all of the surface area inside the dish was covered. The continuous lining of filter paper allowed for exposure to nematodes throughout the dish regardless of where the insects crawled. Approximately 125 IJs were applied in 0.35 ml water to top and bottom filter paper in each dish (250 IJs per dish total). The rate of application was chosen based on preliminary trials conducted to estimate a distinguishing dose (Shapiro-Ilan and Cottrell, unpublished data). One insect was then added to each dish, and the dishes were incubated at 25 °C. Treatments included the two nematode species and a water-only control applied to the four lady beetle species and *A. ipsilon* (15 total treatments). Insect mortality was evaluated 24 and 48 h after application. The experiment was repeated once in entirety (i.e., two trials were conducted).

Nematode reproductive capacity was evaluated by placing insect cadavers from both virulence trials on White traps (Kaya and Stock, 1997). Emerging IJs were collected from White traps (incubated at 25 °C) for up to 21 days. Nematode reproductive capacity was compared among insect hosts based on total number of IJs emerged (estimated through dilution counts on a dissecting microscope), and number of IJs produced per mg of host.

2.3. Data analysis

Treatment effects in virulence assays were analyzed through ANOVA in a factorial analysis with nematode and insect as the main factors (Steel and Torrie, 1980). If the ANOVA detected a significant difference ($P \leqslant 0.05$) then treatment differences were elucidated through Student–Newman–Keuls' test (SAS, 2001). Reproductive capacity was also analyzed through ANOVA and Student–Newman–Keuls' test (SAS, 2001). Reproductive capacity of *H. bacteriophora* in *H. axyridis* was not included in the analysis because only one insect died in that treatment; all other treatments were included in the analysis and contained a minimum of five replicates (insect cadavers). Prior to analysis percentage mortality was arcsine transformed (arcsine of square root), and number of IJs produced per insect or mg of insect was square root transformed (Southwood, 1978) (nontransformed means are presented in figures).

3. Results

In the virulence assays, no significant difference was detected between trials ($F = 0.06$; df = 1,105; $P = 0.80$), and no significant interactions were detected between trial effect and the main effects ($F = 0.54$; df = 2,105; $P = 0.58$ for the nematode * trial interaction, and $F = 0.19$; df = 4,105; $P = 0.94$ for the trial * insect interaction). Therefore, data from trials were combined in all subsequent analyses. A significant interaction was detected between the main effects (nematode and insect) ($F = 20.32$; df = 2,104; $P < 0.0001$). Therefore, main effects were considered separately (Steel and Torrie, 1980). Differential insect susceptibility (and nematode reproduction within each insect) was addressed separately for each nematode. Additionally, differential nematode virulence was also addressed separately (virulence of *S. carpocapsae* versus *H. bacteriophora* addressed in each insect separately).

In *H. bacteriophora* assays, lady beetle susceptibility was only detected in *O. v-nigrum*. At 1 day after treatment, *H. bacteriophora* pathogenicity (as indicated by a significant difference between corresponding treated and control insects) was only detected in *A. ipsilon* ($F = 6.0$; df = 9,70; $P < 0.0001$); lady beetle mortality did not differ

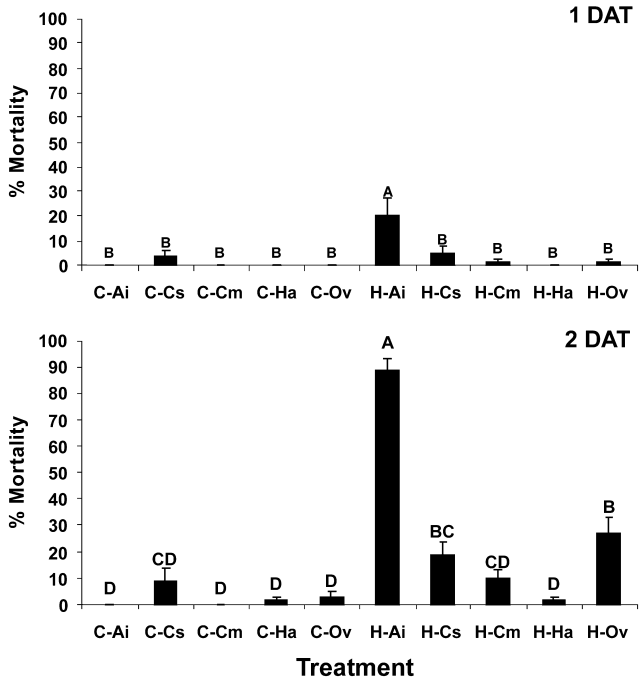

Fig. 1. Mean (±SEM) percentage insect mortality 1 or 2 days after treatment (DAT) with *H. bacteriophora* (H-) or a water-only control (C-). Insects in treatments were *A. ipsilon* (Ai), *C. septempunctata* (Cs), *C. maculata* (Cm), *H. axyridis* (Ha), and *O. v-nigrum* (Ov). Different letters above bars indicate statistical differences ($P \leqslant 0.05$).

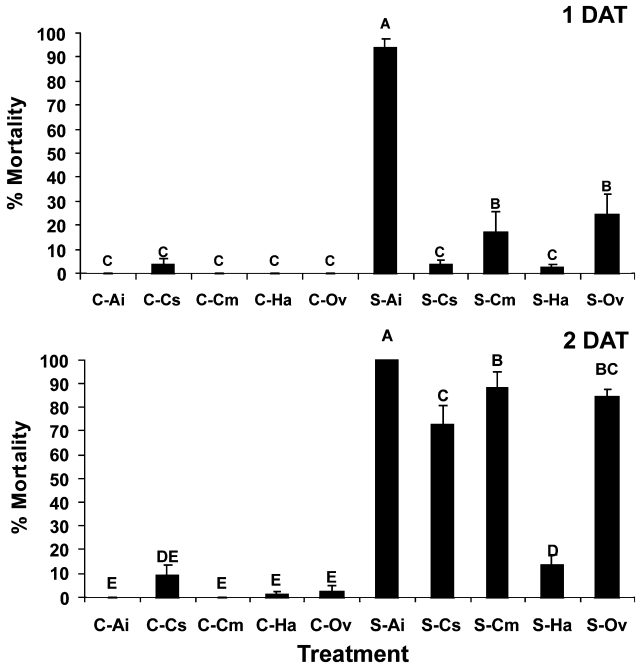

Fig. 2. Mean (±SEM) percentage insect mortality 1 or 2 days after treatment (DAT) with *S. carpocapsae* (S-) or a water-only control (C-). Insects in treatments were *A. ipsilon* (Ai), *C. septempunctata* (Cs), *C. maculata* (Cm), *H. axyridis* (Ha), and *O. v-nigrum* (Ov). Different letters above bars indicate statistical differences ($P \leqslant 0.05$).

between corresponding treated and control insects (Fig. 1). Two days after treatment, *H. bacteriophora* pathogenicity was detected in *A. ipsilon* and in *O. v-nigrum* ($F = 38.56$; df = 9,70; $P < 0.0001$), but not in other lady beetles (Fig. 1). *H. bacteriophora* virulence (as indicated by relative mortality between treated insects) in *A. ipsilon* was greater than in *O. v-nigrum* (Fig. 1).

In *S. carpocapsae* assays, susceptibility of lady beetles was higher in the native species, *C. maculata* and *O. v-nigrum*, compared with the exotic lady beetles, *C. septempunctata* and *H. axyridis*. One day after treatment, *S. carpocapsae* pathogenicity was detected in *A. ipsilon*, *C. maculata*, and *O. v-nigrum* ($F = 45.56$; df = 9,69; $P < 0.0001$), but not in *C. septempunctata* or *H. axyridis* (Fig. 2). *S. carpocapsae* virulence 1 day after treatment was greater in *A. ipsilon* than in *O. v-nigrum* or *C. maculata* (which were not different from each other) (Fig. 2). Two days after treatment, *S. carpocapsae* pathogenicity was detected in *A. ipsilon* and all four lady beetle species ($F = 81.04$; df = 9,69; $P < 0.0001$) (Fig. 2). *S. carpocapsae* virulence 2 days after treatment was greatest in *A. ipsilon* and least in *H. axyridis*; virulence in *C. maculata* was greater than in *C. septempunctata*, and virulence in *O. v-nigrum* was intermediate between *C. maculata* and *C. septempunctata* (Fig. 2).

Steinernema carpocapsae was more virulent than *H. bacteriophora* in all insects tested. Differences in nematode effects were evaluated in separate analyses within each insect. One day after treatment, *S. carpocapsae* caused greater mortality than *H. bacteriophora* in three of the insects: *A. ipsilon*, *C. maculata*, and *O. v-nigrum* ($P < 0.0001$ for *A. ipsilon* and *O. v-nigrum*, and $P = 0.009$ for *C. maculata*); no differences were detected in the other two insects ($P > 0.05$) (see Figs. 1 and 2 to compare means). Two days after treatment, *S. carpocapsae* caused greater mortality in all insects ($P < 0.0001$ for all insects except *H. axyridis* for which $P = 0.009$) (see Figs. 1 and 2 to compare means).

Nematode reproductive capacity varied among insects. Reproductive capacity of *H. bacteriophora* per insect and per mg insect was higher in *A. ipsilon* than in the lady beetles ($F = 42.08$; df = 3,59; $P < 0.0001$ for IJs per insect, and $F = 21.93$; df = 3,59; $P < 0.0001$ for IJs per mg insect); no nematodes were produced in *C. maculata* (Fig. 3). Also, no progeny was produced in the single *H. axyridis* beetle that died following exposure to *H. bacteriophora* (data not included in Fig. 3 or in the analysis).

Reproductive capacity of *S. carpocapsae* per insect was higher in *A. ipsilon* than in the lady beetles ($F = 6.19$; df = 4,107; $P = 0.0002$) (Fig. 3). Reproductive capacity of *S. carpocapsae* per mg insect was higher in *A. ipsilon* than in all lady beetles except *C. septempunctata* ($F = 5.07$; df = 4,107; $P = 0.0009$) (Fig. 3). Reproductive capacity of *S. carpocapsae* per mg insect was significantly higher in *C. septempunctata* than in *H. axyridis* (in which no reproduction occurred), but not different from *C. maculata* or *O. v-nigrum* (Fig. 3).

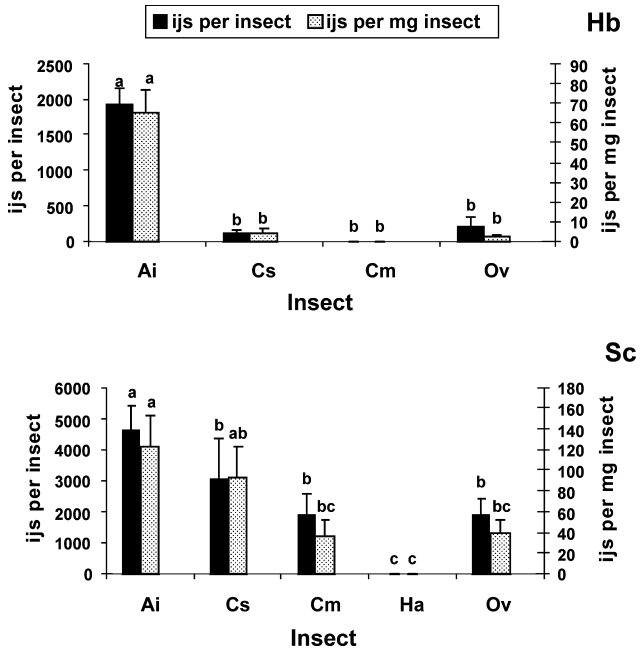

Fig. 3. Mean (±SEM) infective juvenile (ijs) *H. bacteriophora* (Hb) or *S. carpocapsae* (Sc) produced per insect, or per mg insect. Insects were *A. ipsilon* (Ai), *C. septempunctata* (Cs), *C. maculata* (Cm), *H. axyridis* (Ha), and *O. v-nigrum* (Ov). The number of replicates (infected insects) evaluated for Ai, Cs, Cm, Ha, Ov was 27, 15, 5, 0, 16, and 27, 27, 27, 7, 24 for Hb and Sc, respectively. Different letters above bars indicate statistical differences within each series (per insect, or per mg insect) ($P \leqslant 0.05$).

4. Discussion

We predict that nematode field applications would have significantly less impact on the lady beetle species than on a susceptible target pest such as *A. ipsilon*. Although nematode pathogenicity was detected in the lady beetles, virulence was, overall, substantially lower than in the positive control, *A. ipsilon*. Unlike with *H. bacteriophora*, mortality of some of the lady beetle species reached relatively high levels of mortality on the second day after exposure to *S. carpocapsae*, e.g., greater than 80% mortality in the native species *C. maculata* and *O. v-nigrum*. Thus, under optimum conditions and exposure, it is conceivable that some lady beetle populations could be negatively affected by nematode applications (albeit predictably less so than a susceptible target pest such as *A. ipsilon*). However, due to several biological and ecological factors the potential for notable impact on lady beetle populations may be reduced. First, we can expect that infectivity will be reduced under field conditions compared to a protected environment (such as the laboratory or greenhouse) (Hajek and Goettel, 2000; Shapiro and McCoy, 2000). The likelihood of impact on natural lady beetle populations is further minimized because lady beetles spend considerable time searching for prey on plants (Ewert and Chiang, 1966) above the nematode-treated soil, and thus contact between the pathogen and coccinellid would be relatively limited (compared with soil-dwelling host organisms). Finally, potential for contact is also reduced by the fact that nematode applications generally do not persist, usually falling to pre-treatment levels within 2–6 weeks following application (Shapiro-Ilan et al., 2002). Field testing is required to verify our predictions and determine the extent of infection that natural lady beetle populations experience from nematode applications.

Many classical biocontrol programs have resulted in successfully established natural enemy populations, but the majority of introductions have failed (Van den Bosch, 1968). The discipline of biological control will be enhanced by continued elucidation of factors that contribute to success or failure. The differences we observed in nematode pathogenicity and virulence to lady beetles support our hypothesis that success may be due, in part, to an introduced species' relatively low susceptibility to pathogens. Both introduced lady beetle species, *C. septempunctata* and *H. axyridis*, have been quite successful in competing with native species (Brown and Miller, 1998; Cottrell and Yeargan, 1998; Elliott et al., 1996), and in this study we observed that both species were relatively less susceptible to entomopathogenic nematode infection compared with the native species. The evidence provided in this study is consistent with Cottrell and Shapiro-Ilan's (2003) data that indicated lower susceptibility of *H. axyridis* to *B. bassiana* compared with susceptibility of the native lady beetle, *O. v-nigrum*.

Nematode reproductive capacity data also provided evidence of poor host suitability in *H. axyridis* but not in *C. septempunctata*. No reproduction was observed in *H. axyridis*. Yet for *C. septempunctata* it appears that once nematodes establish a successful infection, efficiency of reproduction will be similar to reproduction in the native lady beetles tested. Furthermore, on a per weight basis, efficiency of reproduction was similar in *C. septempunctata* and *A. ipsilon*. The low number of replicates in estimating some of the reproduction in lady beetles may have led to variation and masking of some treatment differences. Nematode reproductive capacity is known to vary substantially among insects of the same species (Shapiro-Ilan, 2001; Shapiro-Ilan and Gaugler, 2002). Nonetheless, we feel the level of replication (a minimum of five insects) was sufficient to make statistical comparisons among the treatments for our purposes.

Observations on differential susceptibility in exotic and native insects could stem from the arbitrary choice pathogen species and strains. For example, one might wonder if we would have observed a different trend had we used other nematode species or strains, e.g., strains not endemic to geographic regions in which the lady beetles occur within the USA. Berry et al. (1997) compared the virulence of two strains of an endemic *Heterorhabditis* sp. with three exotic species of heterorhabditids and two

exotic steinernematids in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), and concluded that the exotic heterorhabditid species were more virulent than the endemic species. In the case of Berry et al. (1997), however, it is not clear whether the differential virulence was due to the nature of being endemic or exotic, or simply due to innate differences in virulence among nematode species. When exploring endemic versus exotic effects among pathogens it is more instructive to eliminate species effects by investigating strain differences within species. Cottrell and Shapiro-Ilan (2003) reported differences in virulence to lady beetle species between strains of *B. bassiana*.

Observations on differential susceptibility in exotic and endemic insects might also stem from the arbitrary choice of host species. Grewal et al. (2002) compared the susceptibility of an exotic and native white grub species (Coleoptera: Scarabaeidae), the Japanese beetle, *Popillia japonica* Newman and the northern masked chafer, *Cyclocephala borealis* Arrow, to 10 strains of *H. bacteriophora* within the native insect's range, and four strains of *H. bacteriophora* and four other *Heterorhabditis* spp. outside the range; the authors concluded that, as a group, the endemic and exotic nematodes and insects did not differ in virulence or susceptibility. However, when Grewal et al. (2002) added two additional exotic hosts, the European chafer, *Rhizotrogus majalis* (Razoumowsky) (Coleoptera: Scarabaeidae) and oriental beetle, *Anomala orientalis* Waterhouse (Coleoptera: Scarabaeidae), one of the exotic species (*R. majalis*) was less susceptible than the native white grub, but the other exotic hosts were not. The results of Grewal et al. (2002) illustrate that conclusions on differential susceptibility can vary depending on which hosts are selected.

In the case of differential susceptibility among native and exotic lady beetles to pathogens, the trend we observed may also have been due to the particular selection of hosts and pathogens. However, the trend of lower susceptibility in the exotic lady beetle, *H. axyridis*, relative to the native *O. v-nigrum* has now been observed with two pathogen groups (nematodes and fungi), and a similar trend was observed with nematodes when two additional lady beetles (one native and one exotic) were tested. Thus, the strength of evidence has increased supporting the concept that successful establishment of certain exotic lady beetles (especially, *H. axyridis*) is due in part to low susceptibility to pathogens. Nonetheless, additional laboratory and field studies incorporating different hosts and pathogens from various geographic locations will be required to further address the hypothesis.

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