

Virulence of Four New Strains of Entomopathogenic Nematodes from Thailand Against Second Instar Larva of the Japanese Beetle, *Popillia japonica* (Coleoptera: Scarabaeidae)

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Abstract

Four new entomopathogenic nematode strains from Thailand were evaluated for virulence against the second instar larva of the Japanese beetle, *Popillia japonica* in laboratory bioassays. *Heterorhabditis indica* strains MP17 and MP111 and *Heterorhabditis* sp. strain MP68 showed higher virulence toward *P. japonica* than *Steinernema minuta* strain MP10. Based on the LC₅₀ (lethal nematode concentration for 50% larval mortality) value MP111 strain was the most virulent with only 136 nematode infective juveniles (IJs) required for 50% larval mortality at 5 days after treatment whereas 199, 254 and 501 IJs were required for MP68, MP17 and MP10 strain, respectively. Also, LT₅₀ (lethal time in days for 50% larval mortality) values at 100 IJs/larva revealed that MP111 killed grubs faster (7.4 days) than MP68 (9.4 days) followed by MP17 (10.5 days) and MP10 (15.7 days), respectively. At a concentration of 1,000 IJs/larva the MP111 strain caused the highest total larval mortality (84.81%) which was higher than MP17 (72.15%), MP68 (72.15%), and MP10 (36.71%), respectively at 5 days after treatment. We conclude that the MP111 strain of *H. indica* was the most virulent against the Japanese beetle second instar larva.

Keywords: entomopathogenic nematodes; *Steinernema*; *Heterorhabditis*; Japanese beetle; *Popillia japonica*; second instar larva

Introduction

The Japanese beetle white grub, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), is native to Japan. The larvae entered the USA in a shipment of iris bulbs prior to 1912 when inspections of commodities entering the country was started. This insect was first found in the USA in 1916 in a nursery near Riverton, New Jersey (Gambrell et al., 1942). It is considered as the most serious insect pests of turfgrass while the adults damage foliage and flowers of over 400 plant species. Japanese beetle is now established throughout the eastern United States and spreading southwards and westwards. The adult beetles emerge in early July. After mating, the female

beetles lay eggs in turfgrass under the host plants. The eggs hatch within 2-3 weeks. The first and second instars occur in August, followed by the final instars which feed during September and then dig deep into the soil for overwintering (Potter, 1998; Vittum et al., 1999). Although all instars of the white grubs feed on plant roots, the third instars are the most damaging (Potter, 1998).

Since the Japanese beetle entered the USA without its natural enemies, several control strategies were used to suppress damaging populations of adults and grubs. The chemical products most commonly used to control the beetles are trichlorofon, chlorpyrifos, carbaryl, diazinon, imidacloprid and halofenozide (George et al., 2007; Oliver et al., 2009). *Tiphia vernalis* Rohwer, a

parasite of the Japanese beetle grub and *Istocheta aldrichi* Mesnil, a parasite of the adult, have been brought from Asia, but are not commercially available (Oliver et al., 2005). The bacterium that is the causative agent of milky disease, *Paenibacillus popilliae* Dutky, can be used against the grub as a long term control but it needs two to three years to build up in the soil to be effective (Koppenhöfer et al., 2000).

Steinernematidae and Heterorhabditidae are two families of the entomopathogenic nematodes with tremendous potential as biocontrol agents (Grewal et al., 2005). They have been isolated from every continent but Antarctica (Poinar, 1990). We discovered four new nematode strains including *Heterorhabditis indica* strains MP17 and MP111, *Heterorhabditis* sp. strain MP68 and *Steinernema minuta* strain MP10 (Maneesakorn, 2010). The infective juveniles (IJs) of the entomopathogenic nematodes seek the insect host, enter through natural body openings and release their symbiotic bacteria into the insect hemolymph (Ciche and Ensign, 2003; Martens and Goodrich-Blair, 2005). *Heterorhabditis* nematodes are associated with *Photorhabdus* bacteria and *Steinernema* nematodes are associated with *Xenorhabdus* bacteria (Boemare, 2002). The bacteria multiply and kill the insect within 24-48 h. The bacteria convert the host cadaver into a suitable food source for nematode growth, development and reproduction. Finally, the nematodes emerge as IJs when food resources are depleted and move into the soil searching for a new host (Poinar, 1990).

The entomopathogenic nematodes *Steinernema* and *Heterorhabditis* have been successfully used to control white grubs, however, virulence of different nematode species and strains against the Japanese beetle grubs differs substantially (Grewal et al., 2002; 2004; Koppenhöfer et al., 2006). The first and second instar larva of the Japanese beetle have been found to be more susceptible to the nematodes than the third instar, both in laboratory and field conditions (Klein, 1990; Koppenhöfer and Fuzy, 2004; Power et al., 2009). Power et al. (2009) recommended that the best time to apply EPNs is when the second instar larva are present in the soil. Here, we investigated the virulence of four new nematode strains isolated from Thailand against the second instar larva of *P. japonica*, in order to

identify the most virulent strains for use against the Japanese beetle larva. The information gained from this study would be served as the guide line for controlling several scarabaeid beetles such as *Meladera* sp. and *Leucopholis* sp. which are the pests of peanut (*Arachis hypogaea* L.) (DOAE, 2010a) and sugarcane (*Saccharum officinarum* L.) in Thailand (DOAE, 2010b).

Materials and Methods

Grubs, Nematodes and Soil

The *P. japonica* larvae were collected twice for two experiments (late August and late September 2008) in turf areas at the Wooster campus of the Ohio State University. Only second instar larvae were carefully taken from each collected batch to run the experiments. Larvae were kept in the soil mixed with grass seeds at room temperature ($22\pm 1^\circ\text{C}$) for three days before use in the experiments. Only actively moving grubs were used in all bioassays.

All four nematode strains used in this study were recently isolated from Thailand. There were two strains of *H. indica* (Maneesakorn, 2010), one of an undescribed *Heterorhabditis* species (Maneesakorn, 2010) and one of a new species of *Steinernema*, *S. minuta* (Maneesakorn, 2010; Maneesakorn et al., 2010). All nematodes were reared in the last instar of *Galleria mellonella* L. at 25°C . Nematodes were harvested from the White traps (White, 1927) and stored in tap water at 10°C for 3 to 30 days prior to use in the experiments.

The soil used in this experiment was identified as silty clay (61.2% silt, 26.2% clay, 12.6% sand) with 3.6% organic matter and 7.1 pH. The soil was pasteurized for 10 h at 121°C and air-dried before using. The soil moisture was adjusted to 24% (w/w) by adding sterilized tap water immediately before setting up the experiments.

Virulence Bioassays

Virulence bioassays were conducted at room temperature ($22 \pm 1^\circ\text{C}$). Individual larvae were placed at the bottom of a 30-ml plastic cup which was filled with 20 g of 24% moisture soil mixed with perennial ryegrass seed (0.2 g per 20 g soil) as a food source. The nematode concentrations were 0 (control), 10, 33, 100, 330, and 1,000 IJs/larva.

Treatments were replicated three times with 10 cups per replication. The entire experiment was also repeated once using a different batch of grubs but the same batch of nematodes. Larval mortality was assessed at 5, 10, and 15 days after treatment (DAT).

Statistical Analyses

The larval mortalities at different nematode concentrations and DAT were analyzed using Probit analysis. Lethal concentration for 50% mortality (LC_{50}) at 5 DAT and lethal time in days to 50% mortality (LT_{50}) at 100 IJs/larva for each nematode strain were calculated. The differences in the LC_{50} s and LT_{50} s were considered significant if there was no overlap between the 95% fiducial limits. Percentage larva mortalities were corrected for control mortality using Henderson-Tilton's formula (Henderson and Tilton, 1955) and arcsine transformed before subjecting to analysis of variance (ANOVA). Minitab statistical software, version 15.1.20.0, 2007 (Minitab Inc., State College, PA, USA) was used for data analyses.

Results and Discussion

The three *Heterorhabditis* strains, *H. indica* strains MP17 and MP111, and *Heterorhabditis* sp. strain MP68 were more virulent than *S. minuta* strain MP10 against the Japanese beetle second instar larvae in both experiments. Since there was no difference in the two trials, the data were pooled and reanalyzed. Among the *Heterorhabditis* strains, MP111 was the most virulent with LC_{50} of 136 IJs/larva (95% Confident Interval (CI): 77 - 199) which was lower than that of the MP68 (199, 95% CI: 123 - 287) and MP17 (254, 95% CI: 176 - 353). *S. minuta* MP10 was the least virulent with the mean LC_{50} of 501 IJs/larva (95% CI: 332 - 917). The MP111 strain was significantly more virulent than MP10 but not from MP17 and MP68 at 5 days after treatment (Figure 1). These results corroborate the finding of Grewal et al. (2002) who reported that the pathogenicity of different nematode species and strains can vary substantially toward the white grub species. Power et al. (2009) reported that 127 IJs of *H. bacteriophora* strain GPS11 caused 50% mortality of the second instar larva *P. japonica* at 5 DAT in laboratory tests.

Thus, the MP111 strain is similar in virulence to the commercially available GPS11 strain of *H. bacteriophora*.

The nematode strains also differed significantly in the speed of kill (Figure 2). The LT_{50} values at 100 IJs/larva against the second instar larva of *P. japonica* were lowest (7.4 days) for *H. indica* (MP111) followed by *H. sp.* (MP68), *H. indica* (MP17), and *S. minuta* (MP10), respectively. The MP111 strain killed the larvae significantly faster than MP10 but not from MP17 and MP68.

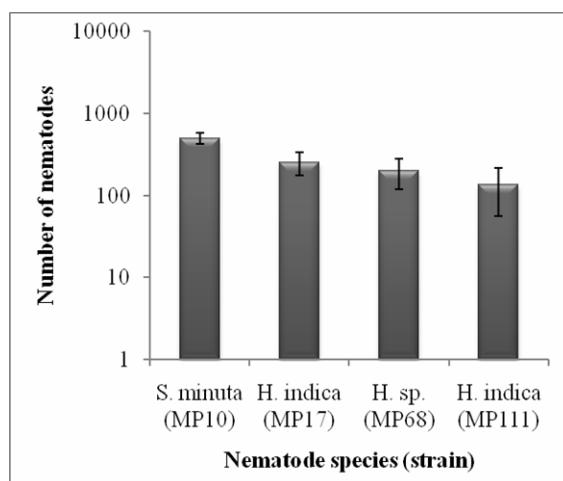


Figure 1 LC_{50} value of four Thai entomopathogenic nematode strains against the second instar larva of *Popillia japonica* at 5 DAT at $22\pm 1^\circ\text{C}$.

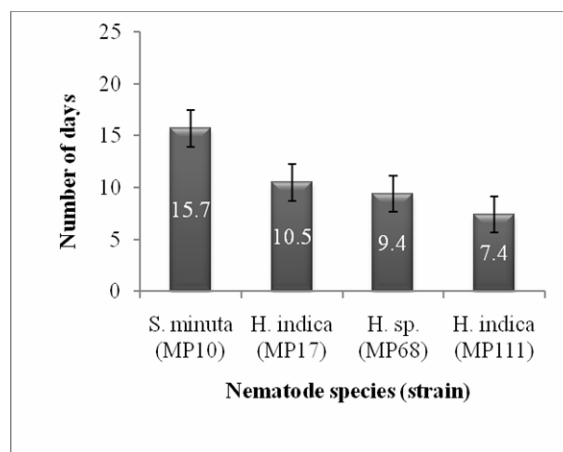


Figure 2 LT_{50} value of four Thai entomopathogenic nematode strains against the second instar larva of *Popillia japonica* at 100 IJs/larva at $22\pm 1^\circ\text{C}$.

Significant differences in total mortality were recorded among the four nematode strains at 5 DAT ($P < 0.05$, $df = 3$, $F = 3.10$). At the concentration of 1,000 IJs/larva, *H. indica* (MP111) was the most virulent toward *P. japonica* resulting in 84.8% mortality and it differed significantly from all other strains. There was no significant difference between MP68 (72.2%) and MP17 (72.2%), but the two strains differed significantly from MP10 (36.7%). Furthermore, *H. indica* (MP111) caused the highest mortality of the Japanese beetle larva at each concentration (Figure 3). There was a significant difference in grub mortality at 1,000 IJs/larva compared with 100 IJs/larva at 5 DAT ($P < 0.05$, $df = 4$, $F = 2.76$). At 100 IJs/larva the MP111, MP68 and MP17 strains caused 34.2, 21.5, and 19% mortality of the second instar larva of *P. japonica* at 5 DAT. According to Koppenhöfer and Fuzy (2004), *H. bacteriophora* TF strain caused about 30% mortality of the second instar larva of *P. japonica* at 100 IJs/larva at 7 DAT. *S. minuta* strain MP10 at 100 IJs/larva caused only 16.5 and 43.9% mortality of the second instar larva of *P. japonica* at 5 and 15 DAT whereas Koppenhöfer and Fuzy (2004) reported that *S. scarabaei* at 20 IJs/larva caused about 43 and 65% mortality of the second instar larva *P.*

japonica at 7 and 14 DAT, respectively. Therefore, *S. minuta* is significantly less virulent to *P. japonica* compared with *S. scarabaei*. In addition, there was no interaction between nematodes strains and concentrations ($P > 0.05$, $df = 15$, $F = 1.71$) (Figure 4).

In summary, our results show that all new Thai nematode strains are pathogenic to the second instar larvae of the Japanese beetle but *H. indica* strain MP111 is the most virulent. Future studies should focus on field efficacy of this nematode strain against other insect pests, especially scarabaeid beetles which are pests on cash crops in Thailand.

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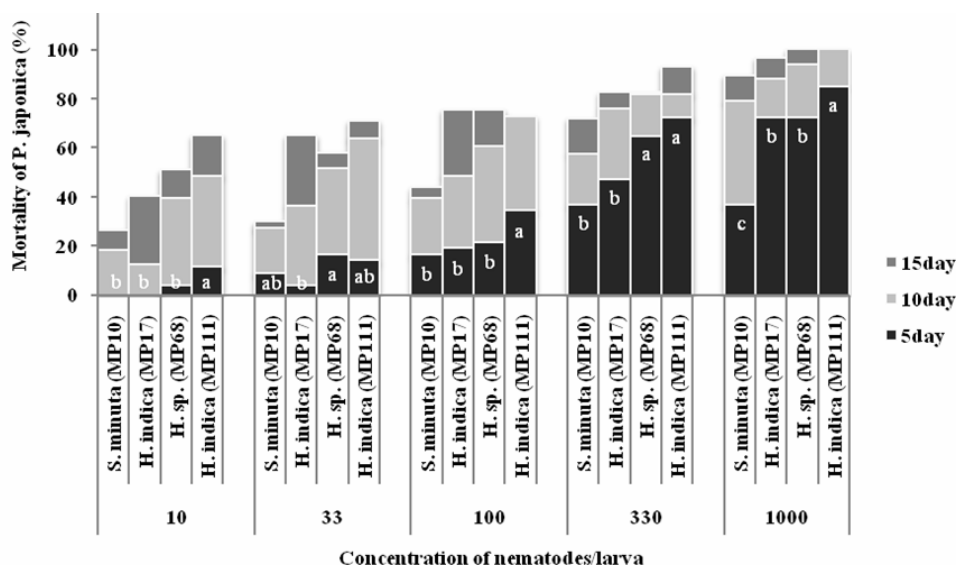


Figure 3 Percent mortality of the second instar larva of *Popillia japonica* caused by four Thai entomopathogenic nematode strains at different concentrations at 5, 10, and 15 days after treatment at $22 \pm 1^\circ\text{C}$. Bars with different letters show significant differences among nematode strains at each concentration at 5 DAT. Data shown are corrected for control mortality.

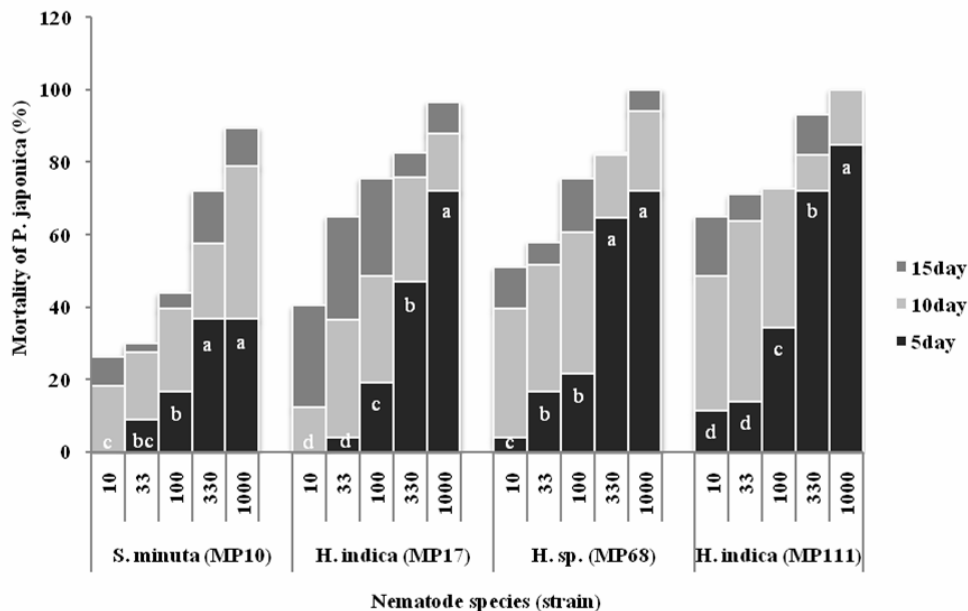


Figure 4 Percent mortality of the second instar larva of *Popillia japonica* at different concentrations of four Thai entomopathogenic nematode strains at 5, 10, and 15 days after treatment at $22\pm 1^\circ\text{C}$. Bars with different letters show significant differences among nematode concentrations for each nematode strain at 5 DAT. Data shown are corrected for control mortality.

References

- Boemare, N. 2002. Biology, taxonomy and systematics of *Photorhabdus* and *Xenorhabdus*, pp. 35-36. In R. Gaugler, ed., *Entomopathogenic Nematology*. CABI Publishing, Wallingford, UK.
- Ciche, T.A. and J.C. Ensign. 2003. For the insect pathogen *Photorhabdus luminescens*, which end of a nematode is out? *Appl. Environ. Microbiol.* 69: 1890-1897.
- DOAE. 2010a. White grub. Available source: <http://agriqua.doae.go.th/plantclinic/clinic/plant/peanut/grub.htm>
- DOAE. 2010a. White grub. Available source: http://agriqua.doae.go.th/plantclinic/plant/cane/wh_grub.htm
- Gambrell, F.L., S.C. Mendel and E.H. Smith. 1942. A destructive European insect new to United States. *J. Econ. Entomol.* 35: 289.
- George, J., C.T. Redmond, R.N. Royalty and D.A. Potter. 2007. Residual effects of imidacloprid on Japanese beetle (Coleoptera: Scarabaeidae) oviposition, egg hatch, and larval viability in turfgrass. *J. Econ. Entomol.* 2: 431-439.
- Grewal, P.S., R.U. Ehlers and D.I. Shapiro-Ilan. 2005. *Nematodes as Biological Control Agents*. CABI Publishing, CAB International, Wallingford, Oxfordshire, UK.
- Grewal, P.S., S.K. Grewal, V.S. Malik and M.G. Klein. 2002. Difference in susceptibility of introduced and native white grub species to entomopathogenic nematodes from various geographic localities. *Biol. Control.* 24: 230-237.
- Grewal, P.S., K.T. Power, S.K. Grewal, A. Suggars and S. Haupricht. 2004. Enhanced consistency in biological control of white grubs (Coleoptera: Scarabaeidae) with new strains of entomopathogenic nematodes. *Biol. Control.* 30: 73-82.
- Henderson, C.F. and E.W. Tilton. 1955. Tests with acaricides against the brow wheat mite. *J. Econ. Entomol.* 48: 157-161.
- Klein, M.G. 1990. Efficacy against soil-inhabiting insect pests, pp. 195-214. In R. Gaugler and H.K. Kaya, eds., *Entomopathogenic Nematodes in Biological Control*. CRC Press, Boca Raton, FL.
- Koppenhöfer, A.M. and E.M. Fuzy. 2004. Effect of white grub developmental stage on susceptibility to entomopathogenic nematodes. *J. Econ. Entomol.* 97: 1842-1849.
- Koppenhöfer, A.M., P.S. Grewal and E.M. Fuzy. 2006. Virulence of the entomopathogenic nematodes *Heterorhabditis bacteriophora*, *Heterorhabditis zealandica*, and *Steinernema scarabaei* against five white grub species of economic importance in turfgrass in North America. *Biol. Control.* 38: 397-404.
- Koppenhöfer, A.M., M. Wilson, I. Brown, H.K. Kaya and R. Gaugler. 2000. Biological control agents for white grubs (Coleoptera: Scarabaeidae) in anticipation of the establishment of the Japanese beetle in California. *J. Econ. Entomol.* 93: 71-80.

- Maneesakorn, P. 2010. Discovery, Identification and Molecular Phylogeny of Entomopathogenic Nematodes and Their Symbiotic Bacteria. Ph.D. Thesis. Kasetsart University.
- Maneesakorn, P., P.S. Grewal and A. Chandrapatya. 2010. *Steinernema minuta* sp. nov. (Rhabditida: Steinernematidae): a new entomopathogenic nematode from Thailand. *Int. J. Nematol.* (in press).
- Martens, E.C. and H. Goodrich-Blair. 2005. The *Steinernema carpocapsae* intestinal vesicle contains a subcellular structure with which *Xenorhabdus nematophila* associates during colonization initiation. *Cell. Microbiol.* 7: 1723-1735.
- Oliver, J.B., C.M. Mannion, M.G. Klein, J.J. Moysenko and B. Bishop. 2005. Effect of insecticides on *Tiphia vernalis* (Hymenoptera: Tiphidae) oviposition and survival of progeny to cocoon stage when parasitizing *Popillia japonica* (Coleoptera: Scarabaeidae) larvae. *J. Econ. Entomol.* 98: 694-703.
- Oliver, J.B., M.E. Reding, N.N. Youssef, M.G. Klein, B.L. Bishop and P.A. Lewis. 2009. Surface-applied insecticide treatments for quarantine control of Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), larvae in field-grown nursery plants. *Pest Manag. Sci.* 6: 381-390.
- Poinar, Jr., G.O. 1990. Taxonomy and biology of Steinernematidae and Heterorhabditidae, pp. 75-90. In R. Gaugler and H.K. Kaya, eds., *Entomopathogenic Nematodes in Biological Control*. CRC Press, Boca Raton, FL.
- Potter, D.A. 1998. *Destructive Turfgrass Insects: Biology, Diagnosis, and Control*. Ann. Arbor Press, Chelsea, Michigan.
- Power, K.T., R. An and P.S. Grewal. 2009. Effectiveness of *Heterorhabditis bacteriophora* strain GPS11 applications targeted against different instars of the Japanese beetle *Popillia japonica*. *Biol. Control.* 48: 232-236.
- Vittum, P.J., M.G. Villani and H. Tashiro. 1999. *Turfgrass Insects of the United States and Canada*. Cornell University Press, Ithaca, NY.
- White, G.F. 1927. A method for obtaining infective nematode larvae from cultures. *Science* 66: 302-303.

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