Autodissemination of pathogens: A novel approach to controlling the Japanese beetle

Elizabeth E. Morris, and Parwinder S. Grewal

Department of Entomology, The Ohio State University, OARDC, Wooster, OH 44691 USA

Abstract

Since its discovery in the United States in 1916, Japanese beetle (*Popillia japonica*) has become the nemesis of agriculturists and home gardeners. Larvae in the soil, feed on grass roots, damaging turf, and adults feed on many species of plants, skeletonizing leaves. Together, they cost around 450 million dollars in control annually. Chemical and cultural methods are used to control the pest, with varying success. Pheromone traps containing feeding lures are effective at attracting the beetles from surrounding areas, but the beetles then have to be killed. We conducted a study to explore the use of the trap as an autodissemination device in which attracted beetles are infected with entomopathogenic nematodes (EPNs) prior to their release. A bioassay evaluated the effectiveness of twenty nematode EPNs to infect adult beetles and cause mortality. All twenty EPNs were able to infect and kill the beetles. Adult beetles were then infected with an EPN from the bioassay and placed in outdoor cages, to assess the vertical transfer of the EPNs from adults to offspring. As beetle larvae were unaffected by the treatment and no EPNs were recovered from the plots, the ability of four different EPNs to develop and reproduce in adult beetles was measured. Among the four tested, only the two *Steinernema* EPNs were able to develop into adults and reproduce in the beetle. Further studies will concentrate on the two most virulent *Steinernema* EPNs for developing control strategies for the adult Japanese beetles in the field.

Introduction

❖ Entomopathogenic nematodes (EPNs) *Heterorhabditis* and *Steinernema* are commercially available for the control of many insect pests.

❖ Several studies have evaluated the susceptibility of JB grubs to EPNs, but few studies are available that show the susceptibility of adults to the EPNs.

❖Due to different host search strategies, *Heterorhabditis* and *Steinernema* may differ in their ability to infect adult Japanese beetle (JB). Therefore, we compared the effectiveness of several different strains of *Heterorhabditis* and *Steinernema* spp to infect and kill, be vertically transferred, and reproduce in the adult beetles.

Objectives

❖ Identify EPN (s) most effective against adult JB

❖Determine ability of *Heterorhabditis bacteriophora* GPS11 to be vertically transferred from infected adults to larvae at oviposition sites

❖ Determine potential for EPN establishment in adult JB

❖ Determine reproduction potential of selected EPNs *(H. bacteriophora* GPS-11 strain, D98, R54, and R45) in adult JB

Methods*

*All data were subjected to the Analysis of Variance (ANOVA) procedure and significant differences were determined using a Tukey's t test.

PART I. Identify EPN(s) most effective against adult JB

Twenty different EPNs were tested against adult JB in a bioassay. For each of the four replicates, ten beetles were placed in a plastic cup with 10g of 9% moisture sand, containing 10,000 EPNs. Mortality was assessed daily for five days. Cadavers were removed at time of discovery and stored at 5˚C until a portion could be dissected to confirm the presence of live EPNs.

PART II. Determine ability of *H. bacteriophora* GPS-11 strain to be transferred vertically from infected adult JB to larvae at oviposition sites

For each treatment, four replicates were used in which twenty-five adult JB were placed in PVC tubes containing moist sand with either 25,000 EPNs or no EPNs as a control. Beetles were exposed for twelve hours, at which point they were moved to outdoor cages and allowed to lay eggs. Grubs were dug up in the fall and counted.

PART III. Determine potential for EPN establishment in adult JB

Four EPNs were tested, *H. bacteriophora* GPS11 and D98, and *Steinernema* R54 and R45. For each treatment, four replicates were used in which ten beetles were placed in a cup containing 10,000 EPNs in 10g of a 9% moisture sand for five days. Each day, dead beetles were removed and placed at 10˚C for one week, to allow EPNs to develop. After one week, cadavers were moved to 0˚C to halt EPN reproduction, until three beetles randomly picked from each replicate were dissected. The number of EPNs per beetle was recorded, and approximate life stage was observed.

PART IV. Determine reproduction potential of four EPNs in adult JB

Per treatment, four replicates were used in which ten beetles were placed in a cup containing 10,000 of the same EPNs from the prior experiment in 10g of a 9% moisture sand for five days. After five days, all dead beetles from each replicate were removed, counted, and placed on a White trap for 17 days at 25˚C, in order to collect and count the emerging EPNs.

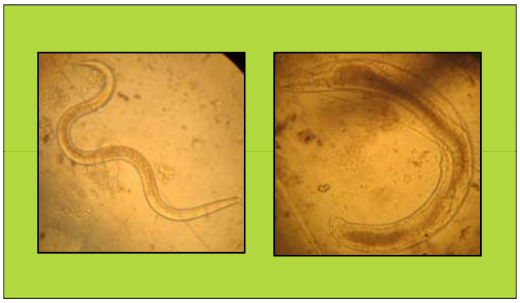

Figure 1. Infective juvenile of EPN D86 (left) and an adult of D86 (right), dissected from the beetle cadavers .

Results and Discussion

PART I. Identify EPN (s) most effective against adult JB

❖ EPNs D98, R54, R45, S.c., D59, F30, D60, and D61 caused significantly higher mortality of adult JB than other strains (Fig. 2). These results indicated no significant difference between *Steinernema* and *Heterorhabditis* EPNs even though the two genera are biologically different.

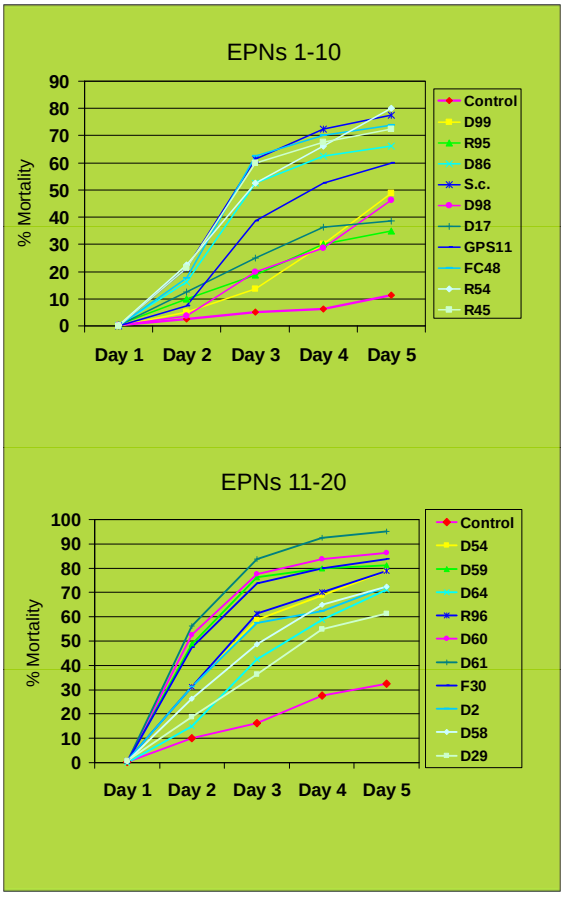

Figure 2. Japanese beetle mortality across five days, following treatment with twenty EPNs.

PART II. Determine ability of *H. bacteriophora* GPS11 strain to be transferred vertically from infected beetles to larvae at oviposition sites

❖ No evidence for vertical transfer of EPNs was found as there was no significant difference between larvae found in treated and untreated plots. Soil baiting revealed that the EPNs did not survive in the soil.

PART III. Determine potential for EPN establishment in adult JB

❖ There were significant differences among treatments in the number of EPNs per beetle. *Steinernema* EPNs were able to develop into adults, but *Heterorhabditis* EPNs could not (Fig. 3).

PART IV. Determine reproduction potential of four EPNs in adult JB

❖ *Steinernema* EPNs could reproduce in adult beetles and produced about 2,400 EPNs per beetle(Fig. 4). No reproduction of *Heterorhabitis* EPNs was observed in the beetles. Differences between *Steinernema* and *Heterorhabitis* may be due to differences in colonization ability of the symbiotic bacteria associated with the two EPNs.

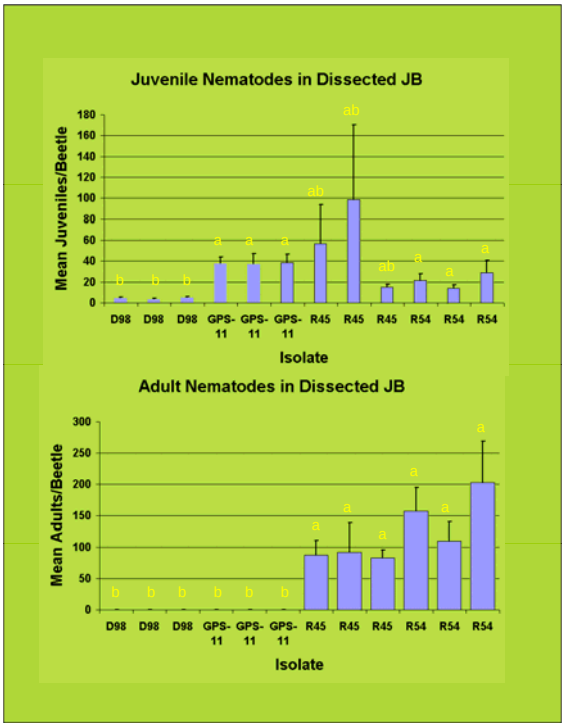

Figure 3. Amount and life stage of EPNs found in dissected.

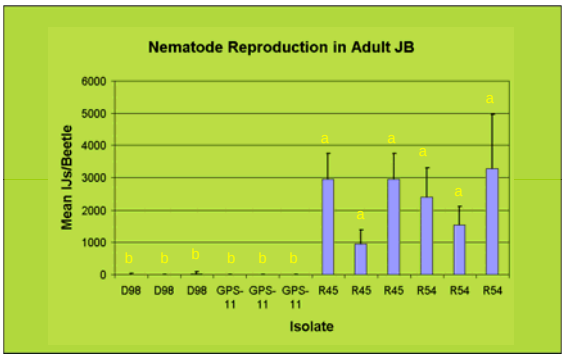

Figure 4. Reproduction ability of *Heterorhabditis* and *Steinernema* in Japanese beetle adults.

Conclusions

❖ Both *Steinernema* and *Heterorhabditis* EPNs were able to kill adult JB and thus have potential for its biocontrol.

❖ *Steinernema* EPNs could develop into adults and reproduce in adult JB, but *Heterorhabditis* EPNs could not. Therefore, only *Steinernema* EPNs have potential to be disseminated by adult beetles to oviposition sites. This could explain why no EPNs were found in the plots from the vertical transfer experiment, as a *Heterorhabditis* EPN was used.

❖ While *Heterorhabditis* EPNs are preferred for treatment of JB grubs, *Steinernema* EPNs seem to be more effective against the adults.

Major References

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