***Title:*** Managing Grubs in Turf with Persistent Entomopathogenic Nematodes - 2014

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***Abstract:***

White grubs are the most prevalent insect pests of both high and low maintenance turfgrass in New York State. Grub control in turf with insecticides is complicated because the lower toxicity insecticides need to be applied before the need for their application can be assessed and the two labeled curative insecticides are highly toxic and have variable efficacy against white grubs. Demand is increasing among homeowners, grounds managers and golf course superintendents for low-impact grub control practices and products, such as biological control. Entomopathogenic nematodes (EPNs) are the only effective biological control for grubs.

This project builds on work begun in 2013, funded by a Community IPM grant. It tests the feasibility of classical biological control by reintroducing native NY strains of entomopathogenic nematodes into the turf system to augment existing nematode populations to suppress grub populations over multiple years. A second evaluation assay sampling for grubs and treatment impact prior to insect pupation in the treated fairways was done in spring 2014. Results showed continued persistence for EPNs introduced as an augmentation to current populations in the turf although *S. carpocapsae* presence had declined since the fall 2013 assay while *H. bacteriophora* had not increased. Comparison of white grub totals from both the fall 2013 and spring 2014 were inconclusive. In other systems, impact of native nematodes tends to phase in over 2-3 years before their full impact is observed.

The poor persistence of *S. carpocapsae* and *H. bacteriophora* raised questions about the loss of the persistence genes from the laboratory cultures. Since the cultures were reinitiated from new isolations in the late summer and fall of 2013, a decision was made to reinoculate those nematodes on the appropriate fairways. The lack of efficacy from *H. bacteriophora* , a species sold commercially for grub control also influenced the decision to increase the rate of application to the commercial levels (1 billion per acre) and follow the application with irrigation.

A third evaluation sampling for grubs in the fall 2014 indicated a reduction in grub numbers in some of the treated sides of the fairways. In early summer 2014, biocontrol nematodes were applied to 9 greens and 9 greens were left untreated as a treatment plan for cutworms. The greens were treated with a combination of *S. carpocapsae* and *S. feltiae.*

***Background and Justification:***

White grubs are the most prevalent insect pests of both high and low maintenance turfgrass in New York State. Their damage ranges from loss of a small percentage of roots that

may weaken a turf stand and allow encroachment of weeds and bare spots, to total loss of turf cover resulting from massive root destruction. Furthermore, substantial damage from predators such as raccoons and skunks destroying turf in search for grubs occurs either high or low populations of grubs present.

Good IPM for grubs in turf with insecticides is complicated by two factors: **1)** To be efficacious, the products of lower toxicity (e.g. chlorantraniliprole, imidacloprid) need to be applied preventatively, before the need for their application can be assessed through sampling and comparison with treatment thresholds and **2)** The two labeled curative products are both of high toxicity. Trichlorfon is experiencing an increased failure rate and carbaryl has always demonstrated low efficacy against white grubs.

The demand is high for low-impact grub control practices and products, such as biological control. Schools in NY are no longer allowed to use synthetic pesticides without an emergency exemption, while being responsible for valuable athletic field turf and the safety of the student athletes playing on it. Homeowners, grounds managers and golf course superintendents are also interested in low impact alternatives.

Entomopathogenic nematodes (EPNs) are the only effective biological control for grubs and commercially purchased nematodes are not commonly used because of their high cost and variable efficacy. Applications with commercial EPNs are often timed after a problem is detected and commercial nematodes only remain active for a relatively short time (2-4 wks) due to intensive selection for a fast acting nematode. Application of commercial nematodes can be best viewed as a short persistence bio-pesticide. The concept of utilizing native NY EPNs with the ability to persist in the NY soil environment has been developed in Northern NY as a successful biological control strategy for alfalfa snout beetle. These native NY strains of entomopathogenic nematodes are adapted to NY conditions since they occur naturally in NY agricultural systems. In 25 years of field research utilizing these nematode strains under NY conditions, a single application will inoculate a field for many years providing there is a constant supply of susceptible soil insects to attack and reproduction. This concept can be best viewed as a classical approach to biological control and a direct opposite approach from the application of commercial EPNs as a biopesticide. In addition, the turf system provides a wide array of susceptible hosts for entomopathogenic nematodes to attack and for reproduction. With the genetics intact in the native nematode population to persist across adverse conditions, these native NY nematodes should persist in most turf systems for many years. The Shields' lab has native NY strains of *Steinernema carpocapsae, S. feltiae and Heterorhabditis bacteriophora* in culture and these species are the same species which are commonly purchased from commercial suppliers. Great care is taken to preserve the ability of these NY strains to persist under NY conditions. In addition, the Shields' lab has developed a nematode mass rearing method that dramatically reduces the cost of the nematodes and application techniques which are compatible with commercial pesticide sprayers.

In August 2013 (previous grant), fifteen fairways were divided down the middle and EPN

combinations (*S. carpocapsae x H. bacteriophora*, *S. feltiae x H. bacteriophora*, and *S. carpocapsae x S. feltiae*) were applied to one-half of the length of each fairway (5 fairways/combination). Fall sampling results (October 2013) indicated successful EPN establishment but no differences the grub populations were observed between the treated and untreated sides of all fairways regardless of EPN combination. As grubs feed, they create an earthen cell which may protect them from EPN attack. The larvae leave these cells to move down in the soil profile for overwintering and when they return to the upper soil layers in the spring. The spring grub sampling will be used to evaluate the effectiveness of these nematodes during these two periods of enhanced exposure. A spring sampling for grubs and EPN persistence was planned for late May 2014.

***Objectives:***

1) **Evaluate the efficacy of 3 different nematode species combinations against white grubs in the commercial turf environment.**
2) **Evaluate the efficacy of NY persistent strain of *Steinernema feltiae* against black cutworm on golf putting greens.**
3) **Document the persistence of these native NY nematode strains in the commercial turf environment.**
4) **Evaluate the project's objectives and publicize the results.**

***Procedures:***

Research for this project was conducted at the Battle Island State Park Golf Course (Figure 1), an 18-hole public course in Fulton NY. The facility has a long history of grub infestation, mainly Japanese beetles, and the superintendent is an excellent cooperator with a keen interest in the project.

Fig.1 Battle Island State Park Golf Course - Fulton, NY

Spring 2014

In late May, all EPN-treated fairways were sampled for both EPNs and grubs. Both the untreated and treated sides of each fairway were sampled for both grubs and EPNs. Since all fall sample sites were recorded by GPS, those fall sites positive for grubs were more intensively sampled in the spring by taking a total of 5 cup cutter cores at each fall positive site. At the fall-negative sites, the more intensive sampling protocol was used if the single initial cup cutter core yielded a grub. In addition, 4 EPN soil samples were collected at each GPS site and returned to the laboratory for a *Galleria* based bioassay.

Summer 2014

Black cutworms are another perennial pest in turf, resulting in frequent insecticide applications to golf course putting greens. In June 2014, we applied persistent EPN applications (*S. carpocapsae* x *S. feltiae*) to a nine putting greens to test efficacy against black cutworm populations and preventing damage. Although planned, soil cores were never collected in the fall from the treated greens to verify the successful establishment of EPNs on the treated greens.

The poor persistence of *S. carpocapsae* and *H. bacteriophora* recorded from the spring 2014 sampling raised questions about the loss of the persistence genes from the laboratory cultures. Since the cultures were reinitiated from new isolations in the late summer and fall of 2013, a decision was made to reinoculate those nematodes on the appropriate fairways in late July and early August 2014. The lack of efficacy from *H. bacteriophora* , a species sold commercially for grub control also influenced the decision to increase the rate of application to the commercial levels (1 billion per acre) and follow the application with irrigation.

Fall 2014

In October 2014, all fairways were sampled for grubs, separated into the EPN treated side and the untreated side. An equal number of cup cutter cores were collected on both the treated and untreated sides and location of each site was recorded with GPS. At each site, the cup cutter soil core was inspected for grubs and any grubs found were returned to the lab for species identification. In addition, four soil cores for EPN persistence were collected at each GPS sampling location on the treated side. EPN soil cores were returned to the laboratory to be bioassayed for the presence of EPNs.

**Results:**

Spring 2014

<u>Grub counts:</u>

The grub counts for each fairway for the fall 2013 and the spring of 2014 are listed in Tables 1, 3, 5. Fairways treated with each of three EPN combinations are grouped together. As

reported earlier, no significant differences were recorded in the fall 2013 grub survey between the treated and untreated sides of the fairways across all three of the nematode combinations. During the spring 2014 grub survey, an increased number of cup cutter cores were taken at GPS locations where grubs were either collected in the fall or during the spring sampling. Data from the spring 2014 sampling needed to be standardized due to the variable number of samples taken per fairway. The final column in Table 1 are the standardized values which can be directly compared. A careful examination of the standardized data indicates no significant difference between the treated and untreated sides of all treatments. EPNs appear to have no significant impact on the grub population during the first grub cycle (Fall 2013-Spring 2014). It is also interesting to note that overwintering mortality reduces the grub population approximately equally in both the treated and untreated sides of the fairways.

Nematode persistence:

*S. carpocapsae x S. feltiae:* In the first sampling after inoculation in the fall 2013, establishment rates *S. carpocapse* were lower than expected across all fairways inoculated with this combination and they remained low at the spring 2014 sampling. Based on these results, *S. carpocapsae* was reapplied during the summer 2014 because we reinitiated the cultures from wild populations during the fall 2013. Establishment levels of *S. feltiae* was as expected, based on our research in other cropping systems. The increase of *S. feltiae* populations from fall to spring is expected, indicating the EPNs were recycling in soil insects after inoculation (Table 2).

*S. carpocapsae x H. bacteriophora:* In the first sampling after inoculation in the fall 2013, the establishment rates of both EPN species was lower than expected across all fairways and remained low at the spring 2014 sampling. Based on these low spring nematode numbers, both species were reapplied in the summer ofl 2014. Since there was no impact of the EPNs on grub numbers, the rate of *H. bacteriophora* was increased to the commercial rate of 1 billion IJ/acre and the fairway was irrigated after application to assist the nematodes entering the soil (Table 4).

*S. feltiae x H. bacteriophora:* In the first sampling after inoculation in the fall 2013, the establishment rates of *S. feltiae* was lower than expected. However, when matched with *H. bacteriophora, S. feltiae* is forced into the upper 2-3 inches of the soil profile where there is often less hosts. In contrast, the establishment rates of *H. bacteriophora* was as expected and increased over the winter, indicating a level of recycling in the soil. Levels of *S. feltiae* also increased in three of the fairways. Since there was no impact of the EPNs on grub numbers, the rate of *H. bacteriophora* was increased to the commercial rate of 1 billion IJ/acre and the fairway was irrigated after application to assist the nematodes entering the soil (Table 6).

| ***S. carpocapsae* X *S. feltiae* combination** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fairway # | Fall 2013 | | | # JB Grubs Fall 2013 | | Fall 2013 Std. Values | | | Spring 2014 | | | # JB Grubs Spring 2014 | | Spring 2014 Std. Values | |
| | # Loc. | # Unt Samp. | # Trt Samp. | Unt | Trt | Unt | Trt | | # Loc. | # Unt Samp. | # Trt Samp. | Unt | Trt | Unt | Trt |
| 1 | 20 | 20 | 20 | 3 | 1 | 0.15 | 0.05 | | 20 | 43 | 56 | 10 | 15 | 0.23 | 0.27 |
| 3 | 30 | 30 | 30 | 15 | 13 | 0.50 | 0.43 | | 30 | 106 | 98 | 24 | 24 | 0.23 | 0.24 |
| 4 | 15 | 15 | 15 | 6 | 9 | 0.40 | 0.60 | | 15 | 51 | 35 | 17 | 14 | 0.33 | 0.40 |
| 5 | 15 | 15 | 15 | 8 | 4 | 0.53 | 0.27 | | 15 | 43 | 51 | 0 | 7 | 0.00 | 0.14 |
| 12 | 30 | 30 | 30 | 13 | 17 | 0.43 | 0.57 | | 30 | 90 | 102 | 31 | 22 | 0.34 | 0.22 |
| | | | | | | | | | | | | | | | |
| Totals | 110 | 110 | 110 | 45 | 44 | 0.41 | 0.40 | | 110 | 333 | 342 | 82 | 82 | 0.25 | 0.24 |
| | | | | | | | | | | | | | | | |

Table 1.Battle Island Grub Assay - Sc/Sf Combination Fairways

| ***S. carpocapsae* X *S. feltiae* combination** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Fairway # | Fall 2013 | | % Pos. Fall 2013 Cores-Species | | | Spring 2014 | | % Pos. Spring 2014 Cores-Species | |
| | # Locations | # Cores | *S. carpocapsae* | *S. feltiae* | | # Locations | # Cores | *S. carpocapsae* | *S. feltiae* |
| 1 | 20 | 80 | 5 | 23 | | 20 | 80 | 2 | 49 |
| 3 | 30 | 120 | 3 | 20 | | 30 | 120 | 3 | 30 |
| 4 | 15 | 60 | 2 | 35 | | 15 | 60 | 5 | 27 |
| 5 | 15 | 60 | 7 | 8 | | 15 | 60 | 3 | 22 |
| 12 | 30 | 120 | 7 | 12 | | 30 | 120 | 1 | 34 |
| | | | | | | | | | |
| Totals | 110 | 440 | 5 | 19 | | 110 | 440 | 11 | 33 |

Table 2.Battle Island EPN Establishment and Persistence Assay- Sc/Sf Combination Fairways

| ***S. carpocapsae* X *H. bacteriophora* combination** | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fairway # | Fall 2013 | | | # JB Grubs Fall 2013 | | Fall 2013 Std. Values | | Spring 2014 | | | # JB Grubs Spring 2014 | | Spring 2014 Std. Values | |
| | # Loc. | # Unt Samp. | # Trt Samp. | Unt | Trt | Unt | Trt | # Loc. | # Unt Samp. | # Trt Samp. | Unt | Trt | Unt | Trt |
| 8 | 25 | 25 | 25 | 9 | 15 | 0.36 | 0.60 | 25 | 89 | 81 | 23 | 40 | 0.26 | 0.49 |
| 10 | 20 | 20 | 20 | 5 | 6 | 0.25 | 0.30 | 20 | 64 | 52 | 17 | 23 | 0.27 | 0.44 |
| 13 | 15 | 15 | 15 | 5 | 10 | 0.33 | 0.67 | 15 | 43 | 51 | 8 | 12 | 0.19 | 0.24 |
| 14 | 25 | 25 | 25 | 1 | 7 | 0.04 | 0.28 | 25 | 61 | 70 | 9 | 10 | 0.15 | 0.14 |
| 16 | 25 | 25 | 25 | 7 | 14 | 0.28 | 0.56 | 25 | 73 | 89 | 14 | 29 | 0.19 | 0.33 |
| | | | | | | | | | | | | | | |
| Totals | 110 | 110 | 110 | 27 | 52 | .025 | 0.47 | 110 | 330 | 343 | 71 | 114 | 0.22 | 0.33 |

Table 3.Battle Island Grub Assay - Sc/Hb Combination Fairways

| ***S. carpocapsae* X *H. bacteriophora* combination** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Fairway # | Fall 2013 | | % Pos. Fall 2013 Cores-Species | | Spring 2014 | | % Pos. Spring 2014 Cores-Species | |
| | # Locations | # Cores | *S. carpocapsae* | *H. bacteriophora* | # Locations | # Cores | *S. carpocapsae* | *H. bacteriophora* |
| 8 | 25 | 100 | 8 | 5 | 25 | 100 | 6 | 11 |
| 10 | 20 | 80 | 16 | 5 | 20 | 80 | 8 | 10 |
| 13 | 15 | 60 | 13 | 12 | 15 | 60 | 7 | 5 |
| 14 | 25 | 100 | 4 | 19 | 25 | 100 | 5 | 9 |
| 16 | 25 | 100 | 13 | 14 | 25 | 100 | 6 | 6 |
| | | | | | | | | |
| Totals | 110 | 440 | 10 | 11 | 110 | 440 | 6 | 8 |

Table 4.Battle Island EPN Establishment and Persistence Assay- Sc/Hb Combination Fairways

| ***S. feltiae* X *H. bacteriophora* combination** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fairway # | Fall 2013 | | | # JB Grubs Fall 2013 | | Fall 2013 Std. Values | | Spring 2014 | | | # JB Grubs Spring 2014 | | Spring 2014 Std. Values | |
| | # Loc. | # Unt Samp. | # Trt Samp. | Unt | Trt | Unt | Trt | # Loc. | # Unt Samp. | # Trt Samp. | Unt | Trt | Unt | Trt |
| 2 | 25 | 25 | 25 | 3 | 8 | 0.12 | 0.32 | 25 | 69 | 76 | 12 | 16 | 0.17 | 0.21 |
| 7 | 20 | 20 | 20 | 4 | 5 | 0.20 | 0.25 | 20 | 60 | 56 | 9 | 11 | 0.15 | 0.20 |
| 11 | 15 | 15 | 15 | 5 | 5 | 0.33 | 0.33 | 15 | 51 | 47 | 8 | 25 | 0.16 | 0.53 |
| 15 | 15 | 15 | 15 | 5 | 7 | 0.33 | 0.47 | 15 | 47 | 55 | 11 | 9 | 0.23 | 0.16 |
| 18 | 30 | 30 | 30 | 12 | 21 | 0.40 | 0.70 | 30 | 106 | 118 | 34 | 20 | 0.32 | 0.17 |
| | | | | | | | | | | | | | | |
| Totals | 105 | 105 | 105 | 29 | 46 | 0.28 | 0.44 | 105 | 333 | 352 | 74 | 81 | 0.22 | 0.23 |

Table 5.Battle Island Grub Assay - Sc/Sf Combination Fairways

| ***S. feltiae* X *H. bacteriophora* combination** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Fairway # | Fall 2013 | | % Pos. Fall 2013 Cores-Species | | Spring 2014 | | % Pos. Spring 2014 Cores-Species | |
| | # Locations | # Cores | *S. feltiae* | *H. bacteriophora* | # Locations | # Cores | *S. feltiae* | *H. bacteriophora* |
| 2 | 25 | 100 | 6 | 28 | 25 | 100 | 1 | 40 |
| 7 | 20 | 80 | 5 | 25 | 20 | 80 | 8 | 24 |
| 11 | 15 | 60 | 2 | 15 | 15 | 60 | 12 | 23 |
| 15 | 15 | 60 | 0 | 27 | 15 | 60 | 10 | 37 |
| 18 | 30 | 120 | 13 | 10 | 30 | 120 | 10 | 19 |
| | | | | | | | | |
| Total | 105 | 420 | 6 | 20 | 105 | 420 | 8 | 28 |

Table 6.Battle Island EPN Establishment and Persistence Assay- Sf/Hb Combination Fairways

Fall 2014:

Grub counts:

Since a new population is initiated with the oviposition of eggs in mid-summer, the year-2 sampling is independent of the previous year's results. For that reason, only a single cup cutter core was collected at each GPS location since the distribution of the grub larval population was unknown. Analysis of the grub counts across all three nematode combination show no significant difference between the nematode treated side of the fairway and the untreated side of the fairway (Tables 7, 9, 11). There seems to be no impact on the grub population from the nematodes. In the laboratory, all three species have shown the ability to kill grubs so these data indicate other factors are in play.

Nematode persistence:

*S. carpocapsae x S. feltiae:* In spite of re-inoculating *S. carpocapsae* into these fairways, the level of this nematode remains low, suggesting that the environment/soil type is not favorable for high level persistence for *S. carpocapsae*. As in year 1, the persistence levels for *S. feltiae* is as expected, indicating they are recycling on some soil insects although the lack of difference between the treated and untreated side would suggest that it is not grubs (Table 8).

*S. carpocapsae x H. bacteriophora:* As in the previous nematode combination, *S. carpocapsae* levels remain low in spite of the fairways being reinoculated in 2014. *H. bacteriophora* levels are lower than expected with the re-inoculation of 1 billion IJ/acre followed by 0.25" irrigation. However, the population numbers are sufficient high to impact the grub population if the grubs were available to the nematodes. These data would suggest that the high clay soil content with the very small pore size inhibit the effectiveness of the nematodes to find the grubs (Table 10).

*S. feltiae x H. bacteriophora:* The levels of both of these nematodes is sufficiently high to impact the grub population if the grubs were exposed. I am surprised at the lack of impact for this combination. These data would suggest that the high clay soil content with the very small pore size inhibit the effectiveness of the nematodes to find the grubs (Table 12).

| ***S. carpocapsae* X *S. feltiae* combination** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Fairway # | Fall 2014 | | | # JB Grubs Fall 2014 | | Fall 2014 Std. Values | |
| | # Locations | # Unt Samples | # Trt Samples | Unt | Trt | Unt | Trt |
| 1 | 20 | 20 | 20 | 10 | 10 | 0.50 | 0.50 |
| 3 | 30 | 30 | 30 | 11 | 13 | 0.37 | 0.43 |
| 4 | 15 | 15 | 15 | 10 | 1 | 0.67 | 0.07 |
| 5 | 15 | 15 | 15 | 3 | 3 | 0.20 | 0.20 |
| 12 | 30 | 30 | 30 | 1 | 9 | 0.03 | 0.30 |
| | | | | | | | |
| Totals | 110 | 110 | 110 | 35 | 35 | 0.32 | 0.32 |

Table 7. Battle Island Fall 2014 Grub Assay - Sc/Sf Combination Fairways

| ***S. carpocapsae* X *S. feltiae* combination** | | | | |
|---|---|---|---|---|
| Fairway # | Fall 2014 | | % Pos. Fall 2014 Cores-Species | |
| | # Locations | # Cores | *S. carpocapsae* | *S. feltiae* |
| 1 | 20 | 80 | 3 | 28 |
| 3 | 30 | 120 | 3 | 21 |
| 4 | 15 | 60 | 10 | 20 |
| 5 | 15 | 60 | 3 | 27 |
| 12 | 30 | 120 | 6 | 11 |
| | | | | |
| Totals | 110 | 440 | 5 | 20 |

Table 8. Battle Island Fall 2014 EPN Establishment Assay- Sc/Sf Combination Fairways

| ***S. carpocapsae* X *H. bacteriophora* combination** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Fairway # | Fall 2014 | | | # JB Grubs Fall 2014 | | Fall 2014 Std. Values | |
| | # Locations | # Unt Samples | # Trt Samples | Unt | Trt | Unt | Trt |
| 8 | 25 | 25 | 25 | 17 | 11 | 0.68 | 0.44 |
| 10 | 20 | 20 | 20 | 13 | 11 | 0.65 | 0.55 |
| 13 | 15 | 15 | 15 | 3 | 9 | 0.20 | 0.60 |
| 14 | 25 | 25 | 25 | 6 | 5 | 0.24 | 0.20 |
| 16 | 25 | 25 | 25 | 8 | 9 | 0.32 | 0.36 |
| | | | | | | | |
| Totals | 110 | 110 | 110 | 47 | 45 | 0.43 | 0.41 |

Table 9. Battle Island Fall 2014 Grub Assay - Sc/Hb Combination Fairways

| ***S. carpocapsae* X *H. bacteriophora* combination** | | | | |
|---|---|---|---|---|
| Fairway # | Fall 2014 | | % Pos. Fall 2014 Cores-Species | |
| | # Locations | # Cores | *S. carpocapsae* | *H. bacteriophora* |
| 8 | 25 | 100 | 5 | 11 |
| 10 | 20 | 80 | 20 | 21 |
| 13 | 15 | 60 | 8 | 18 |
| 14 | 25 | 100 | 9 | 25 |
| 16 | 25 | 100 | 13 | 2 |
| | | | | |
| Totals | 110 | 440 | 11 | 15 |

Table 10. Battle Island Fall 2014 EPN Establishment Assay- Sc/Hb Combination Fairways

| ***S. feltiae* X *H. bacteriophora* combination** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Fairway # | Fall 2014 | | | # JB Grubs Fall 2014 | | Fall 2014 Std. Values | |
| | # Locations | # Unt Samples | # Trt Samples | Unt | Trt | Unt | Trt |
| 2 | 25 | 25 | 25 | 11 | 7 | 0.44 | 0.28 |
| 7 | 20 | 20 | 20 | 10 | 7 | 0.50 | 0.35 |
| 11 | 15 | 15 | 15 | 7 | 4 | 0.47 | 0.27 |
| 15 | 15 | 15 | 15 | 5 | 10 | 0.33 | 0.67 |
| 18 | 30 | 30 | 30 | 2 | 9 | 0.07 | 0.30 |
| | | | | | | | |
| Totals | 105 | 105 | 105 | 35 | 37 | 0.33 | 0.35 |

Table 11. Battle Island Fall 2014 Grub Assay - Sc/Sf Combination Fairways

| ***S. feltiae* X *H. bacteriophora* combination** | | | | |
|---|---|---|---|---|
| Fairway # | Fall 2014 | | % Pos. Fall 2014 Cores-Species | |
| | # Locations | # Cores | *S. feltiae* | *H. bacteriophora* |
| 2 | 25 | 100 | 17 | 17 |
| 7 | 20 | 80 | 4 | 28 |
| 11 | 15 | 60 | 25 | 8 |
| 15 | 15 | 60 | 10 | 18 |
| 18 | 30 | 120 | 22 | 8 |
| | | | | |
| Totals | 105 | 420 | 16 | 15 |

Table 12. Battle Island Fall 2014 EPN Establishment Assay- Sf/Hb Combination Fairways

**Summary:**

After two seasons of work on the Battle Island course with no success in reducing the grub population, we conclude that the soil type on the course (high clay) has a major impact of the ability of the nematodes to locate and attack the grubs. However, the nematodes have no problem persisting in this environment and must be recycling on some prey. Perhaps on a different soil type, the concept of native EPNs having a long-term impact on grub populations would be effective.

## Appendix:

**Fig.2 Wax worm cups infected with IJs prior to wash**

**Fig. 3 50 gallon water tanks used in EPN applications**

**Fig.4 Filling water tanks with EPNs using a pump**

**Fig.5 Truck with spray boom attached applying EPNs to fairways**


**Fig.6 Removing soil core for grub evaluation**

**Fig.7 Collecting grubs for identification**

Fig.8 Soil sampling for EPNs within treated fairways

Fig.9 Battle Island Golf Course patrons observing sampling


Fig.10 Battle Island State Golf Course fairways treated with EPNs