# Occurrence of Entomopathogenic Nematodes in Turfgrass: A Principal Components Analysis



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A Heterorhabditis - infected white grub



We conducted a study to determine the relationship between turfgrass management intensity and natural occurrence of entomopathogenic nematodes (EPNs) on golf courses in Ohio. 159 soil samples were collected from putting greens, fairways and rough areas – three distinct surfaces that are managed differently. EPNs were recovered from soil samples using the insect baiting technique. Soil samples were also analyzed for texture, organic matter, pH, phosphorus, calcium, magnesium and potassium. Principal components and correlation estimates were used to determine possible predictors of nematode occurrence in turfgrass. We found that surface type and soil texture were important factors in predicting nematode occurrence. Putting greens differed significantly from fairways and rough areas in the number of EPN-positive sites. EPNs were recovered from 42.9% of the fairways and 57.1% of the rough areas, but not from putting greens. Putting greens also differed significantly from fairways and rough areas, but not from putting greens. The fairways and rough areas did not however differ in EPN-positive sites and soil parameters. Presence of EPNs significantly correlated with sand, silt, phosphorus, organic matter and magnesium, but not with clay, pH, calcium or potassium. The nematode species were identified as *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, and *S. glaseri*. These results suggests that EPNs are more likely to occur in less intensively managed sites that receive fewer inputs, and have relatively high sand, low pH, and moderate phosphorus and magnesium content.

# Introduction

Entomopathogenic nematodes (EPNs) are biological control agents of many soil-inhabiting insect pests (Grewal et al., 2005). However, at present they are almost exclusively used as biological insecticides, although they have the potential to provide long-term suppression of pests.

In order to build a conservation approach for the use of nematodes, factors affecting the natural populations must be understood. We hypothesized that the intensity of management would affect the natural occurrence of EPNs in turfgrass.

Thus, the objectives of this study were to:

Determine the influence of management intensity on the occurrence of EPNs on three distinct turfgrass surface types (Fig. 1).

Determine what other factors influence the natural occurrence of EPNs in turfgrass.



Figure 1. Surface types common to turfgrass. Putting greens are the most intensively managed whereas rough areas are the least intensively managed surfaces on golf courses.



#### Soil sampling, nematode extraction and identification

□ 159 soil samples were collected from 19 golf courses in Ohio (Fig. 3).

□ 3 putting greens, 3 contiguous fairways and 3 rough areas were selected as sampling sites per golf course.

• On each surface type, 20 soil cores (2 cm diameter x 15 cm deep) were taken along a 10 m transect and composited.

□ Nematodes were recovered from the samples using the insect baiting technique (Kaya and Stock, 1997).

□ Infected cadavers were transferred onto White traps (Fig. 2A) for nematodes to emerge.

□ Nematodes were identified by morphological analysis of infective juveniles (Fig. 2B) using light microscopy.

□ Soil samples were analyzed for physical and chemical parameters.



**Figure 2.** A White trap with *Heterorhabditis*-infected wax moth larvae (A), and a *H. bacteriophora* infective juvenile (B).

#### Data analysis

□ Presence/absence of EPNs on surface types was analyzed using ANOVA (SAS, 2001).

Comparisons in soil chemical parameters were made using least significance difference.

□ The influence of all soil parameters (fairways and rough areas only) was determined using principal components and correlation estimates (Statistica, 2002).

## **Results & Discussion**



Figure 3. Map of Ohio showing location of sampling sites by golf course. (●) EPN-positive sites; (▲) EPN-negative sites.

□ 9 of 19 (47.4%) golf courses were positive for EPNs (Fig. 3).

□ 6 fairways (42.9%), roughs areas (57.1%) and 0 greens (0%) were positive for EPNs (Fig. 4).

□ Nematode Species recovered: *H. bacteriophora*, *S.* carpocapsae, and S. glaseri.



Figure 4. Comparison of EPN-positive sites by surface type.

 $\Box$  Significant differences (P < 0.05) in the number of EPN-positive sites were observed between the putting greens and the fairways and rough areas, but no significant differences (P > 0.05) were detected between the fairways and the rough areas (Fig. 4).

□ Similarly, the putting greens differed significantly (P < 0.05) from the fairways and the rough areas in terms of the organic matter, pH, calcium and phosphorus contents (Table 1).

□ EPN presence on the surface types significantly correlated with sand, silt, organic matter, phosphorus and magnesium, but not with clay, pH, potassium or calcium.

| Table 1. Mean (±SE) soil c | chemical parameters | by surface type |
|----------------------------|---------------------|-----------------|
|----------------------------|---------------------|-----------------|

| Site/Surface<br>type | OM (% )     | pН          | Calcium <sup>1</sup><br>(%) | Magnesium <sup>1</sup><br>(%) | Phosphorus<br>(ug/g) | Potassium <sup>1</sup><br>(%) |
|----------------------|-------------|-------------|-----------------------------|-------------------------------|----------------------|-------------------------------|
| Greens               | 2.9 ± 0.22a | 7.1 ± 0.06a | 83.7 ± 1.15a                | 12.2 ± 0.86a                  | 54.0 ± 5.60a         | 2.9 ± 0.31a                   |
| Fairways             | 4.8 ± 0.19b | 6.0 ± 0.14b | 55.2 ± 2.96b                | 15.3 ± 0.90a                  | 47.1 ± 6.10b         | 3.2 ± 0.15a                   |
| Rough areas          | 4.8 ± 0.25b | 5.9 ± 0.14b | 52.9 ± 2.23b                | 13.8 ± 0.87a                  | 47.8 ± 6.96b         | 3.1 ± 0.15a                   |

Acknowledgments



Figure 5. Principal components analysis of all soil variables in fairways and rough areas. The plot is based on vector loadings of the variables for the first three components. The closed circles are positive for nematodes. Hb = H. bacteriophora, Sc = S. carpocapsae, and Sg = S. glaseri.

□ The majority of EPN-positive sites were on the positive side of both of the first two component axes where sand content, potassium and phosphorus are relatively high, and pH, calcium silt and clay contents are low (Fig. 5). Soils high in sand content are known to favor mobility and survival of EPNs compared to soils high in clay content (Barbercheck and Kaya, 1991).

The absence of EPNs on putting greens may be due to the high management intensity that involves low mowing heights and frequent pesticide applications (Wu et al., 2002).

□ Fairways and rough areas are less intensively managed and are more suited to the nematodes in terms of protection from severe environmental conditions and host provision for nematodes to recycle in.

### Conclusions

□ EPNs occur in almost half of the golf courses sampled in Ohio.

□ Surface type and soil texture are important factors in predicting nematode occurrence on golf courses, suggesting that management plays an important role in influencing the natural occurrence of EPNs in turfgrass.

EPNs are more likely to be found in less intensively managed sites that have relatively high sand content, low pH, and moderate soil fertility.

This information is useful in developing turfgrass integrated pest management programs with EPNs as components.

□ In addition, this information is critical to develop conservation approaches for both inoculative releases and natural populations of EPNs.

|    | (%)          | (ug/g)       | (%)         | Kelerences   |  |  |  |  |  |
|----|--------------|--------------|-------------|--|--|--|--|--|--|
|    |              |              |             | • Barbercheck, M.E., Kaya, H.K., 1991. Effect of host condition and soil   |  |  |  |  |  |
| ja | 12.2 ± 0.86a | 54.0 ± 5.60a | 2.9 ± 0.31a | texture on host finding by the entomopathogenous nematodes <i>Heterorhabditis</i><br>bacteriophora (Rhabditida: Heterorhabditidae) and <i>Steinernema carpocapsaa</i><br>(Rhabditida: Steinernematidae). Environ. Entomol. 20, 582-589.  |  |  |  |  |  |
| 6b | 15.3 ± 0.90a | 47.1 ± 6.10b | 3.2 ± 0.15a | <ul> <li>Grewal, P.S., Ehlers, R.U., Shapiro-Ian, D., 2005. "Nematodes as Biocontrol<br/>Agents". CABI Publishing, Wallingford, UK (in press).</li> <li>Kaya, H.K., Stock, S.P., 1997. Techniques in insect nematology. <i>In</i>: Lacey,<br/>L.A. (Ed), "Manual of Techniques in Insect Pathology." Academic Press, New<br/>York, pp. 281-324.</li> <li>SAS 2001. SAS Institute Inc. Cary, NC, USA</li> </ul> |  |  |  |  |  |
| ßb | 13.8 ± 0.87a | 47.8 ± 6.96b | 3.1 ± 0.15a |  |  |  |  |  |  |

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