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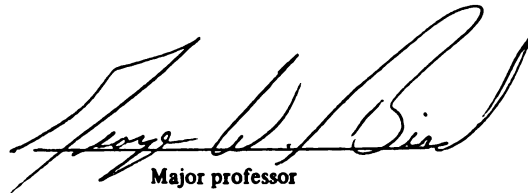
OCCURRENCE, DISTRIBUTION AND MANAGEMENT OF  
HETERODERA SCHACHTII IN MICHIGAN: WITH SPECIAL  
 REFERENCE TO PASTEURIA AS A BIOLOGICAL CONTROL AGENT

presented by

Angela M. Miller

has been accepted towards fulfillment  
 of the requirements for

M.S. degree in Entomology/Nematology

  
 Major professor

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**OCCURRENCE, DISTRIBUTION AND MANAGEMENT OF *HETERODERA*  
*SCHACHTII* IN MICHIGAN: WITH SPECIAL REFERENCE TO *PASTEURIA* AS A  
BIOLOGICAL CONTROL AGENT.**

**By**

**Angela May Miller**

**A THESIS**

**Submitted to  
Michigan State University  
in partial fulfillment of the requirements  
for the degree of**

**MASTER OF SCIENCE**

**Department of Entomology**

**1999**

## **ABSTRACT**

*Heterodera schachtii* Schmidt, 1871 (Nemata) is one of the most important plant-parasitic nematodes affecting sugarbeet production in Michigan. It is responsible for reducing both sugar content and crop yield. This Master of Science Degree thesis consists of the following three components: 1) A survey for the detection of *H. schachtii* in Michigan sugarbeet producing areas. The project was done in collaboration with Monitor and Michigan Sugar Companies. The 1998 survey resulted in the confirmed detection of *H. schachtii* in six Michigan counties. 2) A nematicide trial was used to evaluate the efficacy of two non-fumigant, one fumigant, and one bio-nematicide for the control of *H. schachtii*. The data suggest that there is a potential for DiTera<sup>®</sup>, and Vapam<sup>®</sup> to provide effective control of *H. schachtii*; however, with only one year of data, it was not possible to add any new nematode management recommendations to those currently used in Michigan sugarbeet production. 3) The bacterial parasite of plant-parasitic nematodes, *Pasteuria*, was identified as a potential biological control agent. Research was done to provide a genomic basis for the relationship of species designated in the genus *Pasteuria*, and for comparison of *Pasteuria* to other genera of bacteria. The genomic research was inconclusive. Additional work is needed to access the molecular taxonomy of *Pasteuria*.

## **DEDICATION**

**With love and respect  
and thanks for all her encouragement**

**I would like to dedicate**

**my thesis**

**to my mother and friend**

**Deborah I. Miller**

## **ACKNOWLEDGEMENTS**

I would like to acknowledge my guidance committee, Dr. Elder Paul, Dr. George Garrity, and Dr. Haddish Melakeberhan for their time and interest in my education. I express my gratitude to all the members of my committee for donating their expertise and advice to help me further my education in the field of science.

I would also like to thank Dr. Garrity and Dr. Denise Searles for their help in the microbiology aspect of my project. I greatly appreciate all of the time and input they gave me throughout my experience as a graduate student. I would especially like to thank Dr. Searles for all of the time and effort she put into helping me with my project, and also for the great deal of moral support.

I would also like to acknowledge Dr. D. W. Dickson, Jennifer Anderson, and Tom Hewlett, from the University of Florida, Entomology/Nematology Department for the cultures of *Pasteuria* and all of the useful information. Tom Hewlett was very helpful in assisting me with any important information I needed.

Most importantly I would like to acknowledge all of the members of Dr. George Bird's laboratory, Fred Warner, Mike Berney, Becky Gore, and John Davenport. I worked for Dr. Bird for several years before starting my graduate program and learned a great deal of valuable work experience that helped throughout my graduate career. I owe a great deal of thanks to Fred Warner who got me started in the nematology field as a student worker. Since that time he has been a good mentor and friend. He has taught me a great deal about nematode diagnostics. I would also like to acknowledge John Davenport for teaching me all I know about setting up field experiments. He has been a great

colleague and friend who has always been there to help with many aspects of my research.

I would also like to thank Mike Berney for always answering my countless number of questions dealing with everything from nematode identification to how to fix my

computer. Speaking of computers, very special thanks goes to Becky Gore for helping me with all of my statistical analysis. Without her I would still be trying to analyze all of

my data. Finally, I would like to thank Dr. George Bird for all of his support and

encouragement throughout my graduate program. Dr. Bird has contributed a great deal of time and knowledge to help me succeed as a Master of Science Degree candidate.

## **PREFACE**

The original goal for my Master of Science Program was to obtain training in the area of biological control with a thesis on *Pasteuria*, a bacterial parasite of nematodes. The program quickly identified Dr. George Garrity's Laboratory in the Department of Microbiology as a place to obtain experience in microbiology. The research goal was to develop a molecular probe for *Pasteuria* that could be used in survey work associated with nematode biological control. After about 18 months of technique development and preliminary experiments, it became evident that it would not be possible to complete this goal as part of my M.S. thesis. After discussions with Dr. Bird, it was decided that I should assume complete responsibility for two sugarbeet cyst nematode research projects already in progress. This would allow me to have a complete original research experience beginning with hypothesis development and literature review through data analysis and thesis writing. Based on this decision, my M.S. thesis entitled "Occurrence, Distribution, and Management of *Heterodera schachtii* in Michigan: With Special Reference to *Pasteuria* as a Biological Control Agent" consists of the following three projects: 1) 1998 Michigan sugarbeet survey for the detection of *H. schachtii*, 2) 1998 Nematicide trial for the evaluation of two non-fumigant, one fumigant and one bio-nematicide for the control of *H. schachtii*, and 3) Sequencing the 16s rDNA of *Pasteuria* for phylogenetic analysis. Although it is not possible to integrate these three initiatives in a comprehensive manner within this thesis, all three relate to specific aspects of my original M.S. program goal.

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## INTRODUCTION

The sugarbeet, *Beta vulgaris saccharifera*, was developed by selecting from white strains of fodder beet, originating from the Mediterranean wild beet, *Beta maritima* (Weischer and Steudel 1972). German chemist Andreas Margraff first demonstrated the similar identity of beet-sugar and cane sugar, and in 1747, F. C. Achard started sugar production from beets. Today, around 37% of the world's sugar is produced from beet. In addition to sugar production, sugarbeet is very important as a forage, since not only the crown leaves but also beet pulp are used to feed cattle in many countries (Weischer and Steudel 1972).

In 1997, Michigan ranked fourth in U.S. sugarbeet production. Growers harvested 174,000 acres in 1998, producing 2,871,000 tons, representing 8.9% of the total U.S. production (Anon. 1998). Since 1972, Michigan has had an increase in the number of sugarbeet acres harvested (Figure 1). Michigan sugarbeet yield in tons per acre, however, has decreased over the last 25 years (Figure 2). This decline may be due, in part, to the presence of the sugarbeet cyst nematode (SBCN) *Heterodera schachtii* Schmidt, 1871 (Nemata).

Schacht, first reported *H. schachtii*, in 1859 in Germany (Schacht 1859a,b). The sugarbeet cyst nematode was the first nematode pathogen of sugarbeet to be recognized and has remained one of the crop's most damaging pests, occurring in all major beet-growing areas (Cooke 1993). This nematode reduces both sugar content and total crop yields (Knobloch and Bird 1981). *H. schachtii* was known in western United States as early as 1905, and the first Michigan survey for this nematode was conducted in 1920

(Steele 1984; Knobloch and Bird 1981). This nematode, however was not detected in Michigan until 1948 (Bockstaller 1950). It was subsequently shown to be a major pest under Michigan growing conditions (Knobloch and Bird 1981).

*H. schachtii* is a sedentary endoparasite. Raski (1949) completed a classical description of the life cycle of this nematode. Upon hatching, the nematode will penetrate the root system of a suitable host and migrate through the cortex to permanent feeding sites adjacent to the vascular cylinder (Cooke 1993). The first indication of an infestation of *H. schachtii* in fields of sugarbeet is usually the appearance of one or more well-defined circular to oval areas of reduced growth or poor stands (Steele 1984). Other symptoms may include wilting or yellowing. *H. schachtii* has a rather wide host range that includes weeds, cultivated vegetables, field crops, and ornamentals occurring in 23 plant families (Steele 1965). Sugarbeet is the only major field crop grown in Michigan that is a host for this nematode.

Successful management of *H. schachtii* was achieved for many years through a very strict system of crop rotation (Berney and Bird 1998). More recently the sugarbeet industry has undergone several changes which include an increase in acres planted to sugarbeet and a decrease in crop rotation schemes.

Alternative management strategies, in addition to crop rotation, include; cultural practices, such as sanitation and planting of trap crops; biological control and chemical control. Sanitation is extremely important, especially when dealing with equipment and tare soil. Planting trap crops can stimulate hatching of *H. schachtii* juveniles, which then invade the roots and initiate the formation of syncytia at permanent feeding sites. The syncytia soon break down and prevent most of the nematodes from completing their life

cycles (Wyss et al. 1984). Biological control of *H. schachtii* may be possible in the future through the use of nematode parasitic bacteria and fungi. The genus *Pasteuria* forms a group of endospore-forming bacteria that may have potential to be developed into biological control agents of plant-parasitic nematodes (Stirling 1991). Current research is being done on this group of bacteria to determine its potential for use as a biological control agent against many economically important plant-parasitic nematodes. Pesticides such as soil fumigants and non-fumigant nematicides (carbamates and phosphates) can be used to control not only *H. schachtii*, but also other nematode and arthropod pests (Roberts and Thomason 1981).

Management strategies for *H. schachtii* will ultimately depend on an integrated approach that will include some of the tactics that have been described above. This thesis focuses on three aspects: 1) a Michigan survey designed to determine the extent of *H. schachtii* infestation, 2) a nematicide trial in a field with a low initial population density of *H. schachtii*, and 3) research designed to sequence the 16s rDNA of *Pasteuria*, a potential biological control agent for *H. schachtii*.

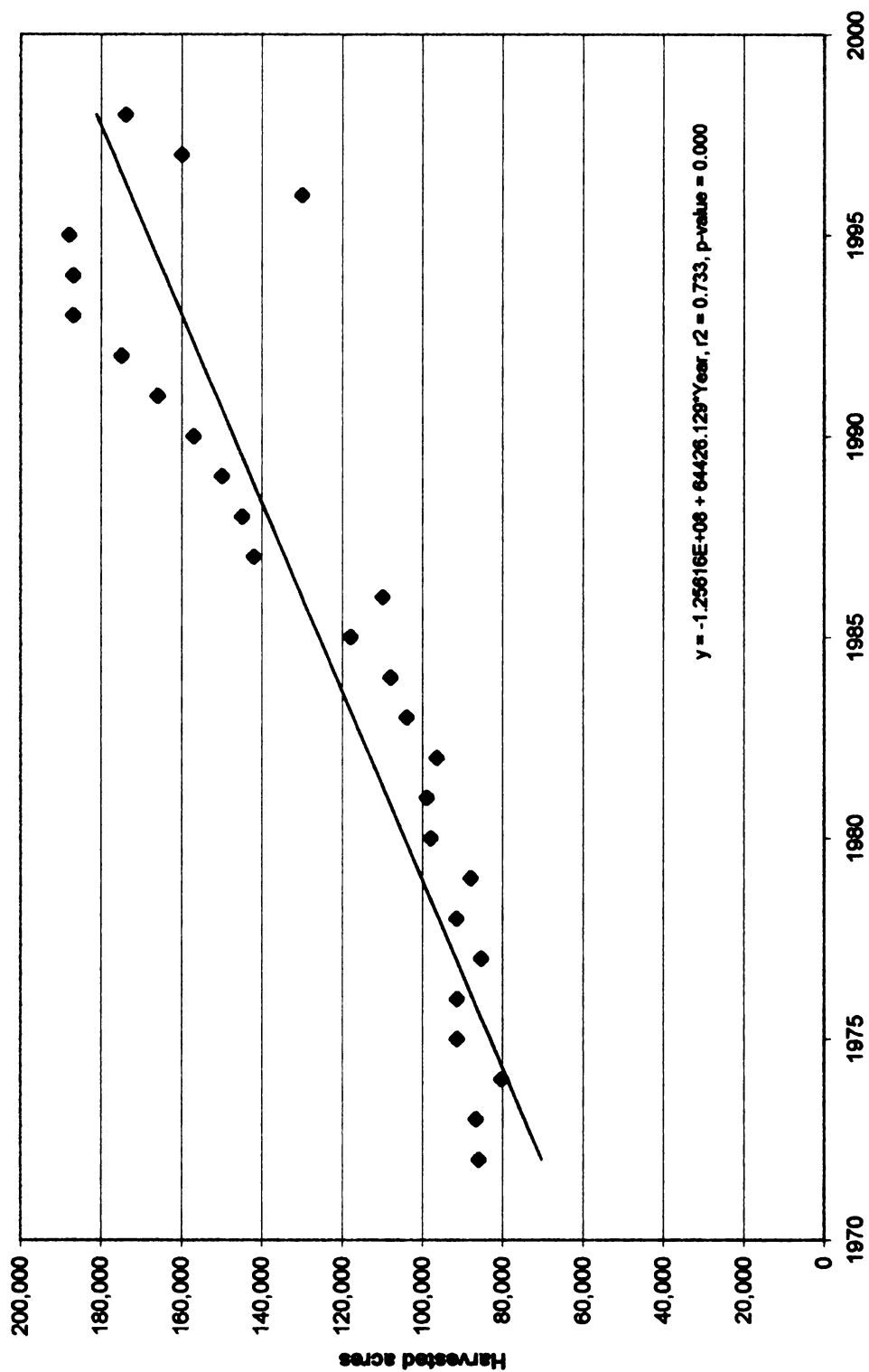


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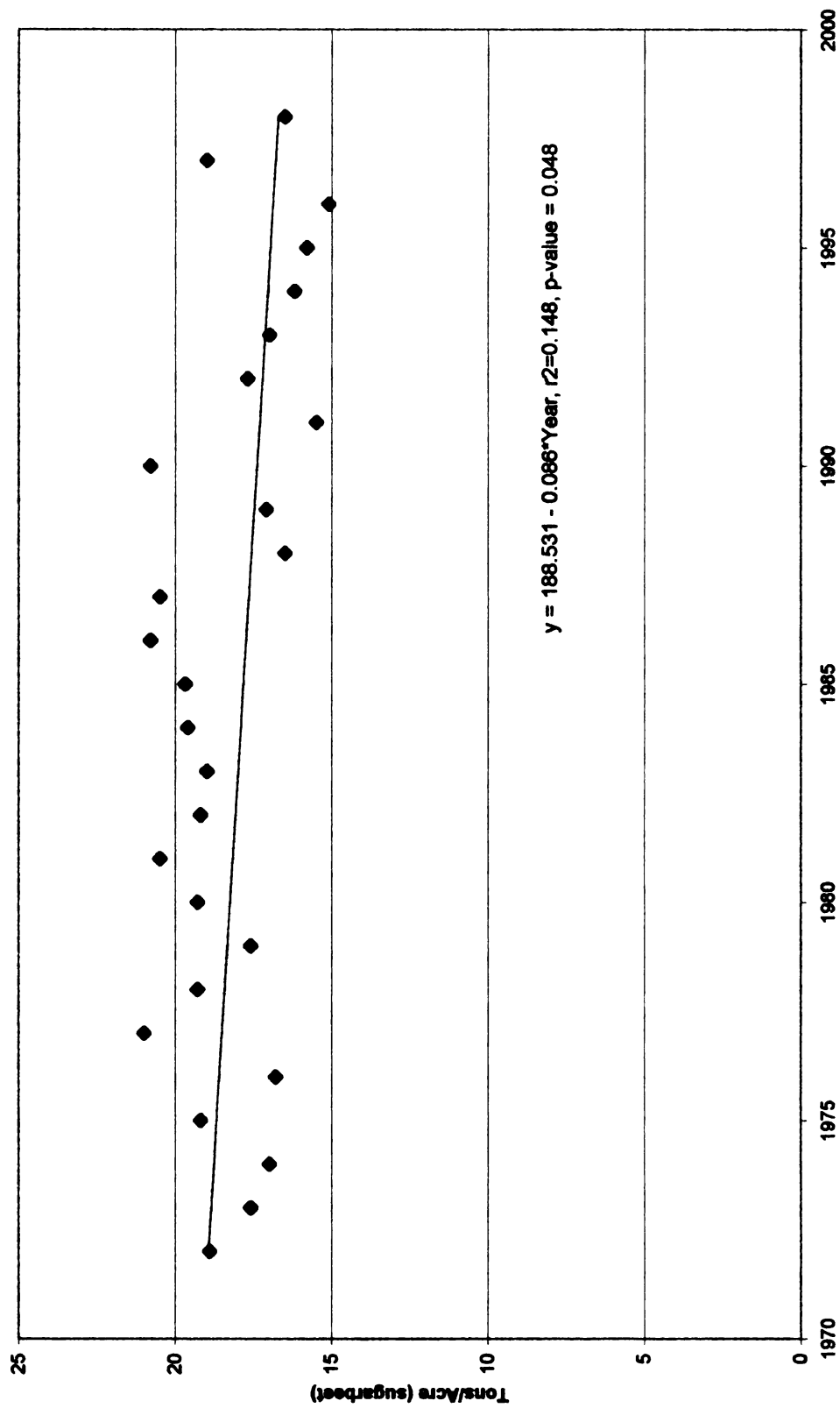


Figure 2. Tons/acre of sugarbeet produced each year from 1972 to 1998 (Michigan Agricultural Statistics).



# **OCCURRENCE AND DISTRIBUTION OF *HETERODERA SCHACHTII* IN MICHIGAN SUGARBEET PRODUCTION**

## **INTRODUCTION**

Sugarbeet, *Beta vulgaris saccharifera*, is grown in the United States under a wide range of soils and climatic conditions (Griffin 1981). The sugarbeet cyst nematode (SBCN), *Heterodera schachtii* Schmidt 1871 (Nemata), is the most important plant-parasitic nematode and one of the most significant plant pathogens affecting sugarbeet (Griffin 1981). As a pathogen, it reduces both sugar content and total crop yields (Knobloch and Bird 1981). *H. schachtii* was the first nematode pathogen of sugarbeet to be recognized (Schacht 1859) and has remained one of the crop's most damaging pests, occurring in all major beet-growing areas (Cooke 1993).

Schacht first reported *H. schachtii* in 1859 in Germany. Schmidt subsequently described the causal agent in 1871. It has been found in at least 40 countries within North and South America, Europe, Africa, and in the Middle East (Steele 1984). It is not known how this nematode gained entry into the United States; however, it may have been introduced with imported sugarbeet seed contaminated with nematode-infested soil (Shaw 1915; and Triffitt 1935). Damage to sugarbeet by *H. schachtii* was first observed in the United States in 1895 (Steele 1984). The causal agent, however, was not identified until 1905. The first detection of *H. schachtii* in Michigan was in 1948. It was subsequently reported from Macomb, Saginaw, and Bay Counties through samples submitted to the Michigan State University Nematode Diagnostic Laboratory (Knobloch and Bird 1981).

It may have been present or spread to several other counties in Michigan due to agronomic practices associated with recent increases in sugarbeet production.

In 1996, Michigan ranked 5th in U.S. sugarbeet production. By 1997, Michigan increased its production to become the 4th largest producer of sugarbeet. Growers planted 160,000 acres and produced 3,040,000 tons, representing 10.2% of the total U.S. production (Anon. 1997). This increase in the amount of sugarbeet production can lead to a greater risk in the spread of *H. schachtii* within the state.

Second-stage juveniles of *H. schachtii* invade the root system of a sugarbeet plant and feed at sites adjacent to the vascular cylinder, interfering with nutrient uptake (Cooke 1993). Nematode damage is most severe during the seedling stage, resulting in either a lack of or delay in seedling emergence (Steele 1984). Nematode-infected plants may exhibit symptoms of wilting or stunting in the field. Populations of *H. schachtii* overwinter in the soil as eggs retained in a protective lemon-shaped cyst formed from the body wall of the dead female. Each cyst contains up to 600 eggs. Some may remain unhatched for many years if a suitable host is unavailable (Cooke 1993).

Currently, there is insufficient information available on the occurrence, distribution, and impact of *H. schachtii* in Michigan. The primary objectives of this project were to determine 1) the geographical distribution of this nematode in the Michigan, 2) nematode population density levels, 3) its prominence in fields infested, 4) how infestation is related to cropping history, and 5) how infestation is related to plant symptomology. A secondary objective was to examine populations of *H. schachtii* for the presence of *Pasteuria* spp., a bacterial parasite, that could possibly be used as a biological control agent of this nematode.

## **MATERIALS AND METHODS**

A cooperative research project with Monitor and Michigan Sugar Companies was initiated in 1998. The objective was to conduct a detection survey for *Heterodera schachtii* throughout 13 Michigan sugarbeet-producing counties. Each of the professional fieldmen from each company collected ten root-soil samples for nematode analysis from 10 different sugarbeet fields. Five of the samples were taken at random from fields not exhibiting shoot system symptoms associated with low sugarbeet yields. The remaining five samples were taken using a stratified sampling method from fields exhibiting symptoms of suspected sugarbeet cyst nematode damage. In this survey, stratified sampling is defined as a method of sampling based on the knowledge of an organisms specific location and behavioral patterns. A total of 214 samples were collected and submitted with a sample form providing information from the grower (Appendix A). Michigan Sugar fieldmen submitted 122 samples, and 92 were submitted by Monitor Sugar fieldmen.

A single bulk sample was prepared by the fieldmen for each of the 10 different sampling sites. Samples taken at random were a composite of nine root-soil cores from the plant-soil rhizosphere. Stratified samples were taken by collecting three different root-soil cores each from three locations in the field exhibiting symptoms. These samples were collected from the plant-soil rhizosphere near the margins of the locations exhibiting symptoms. Soil samples were stored at 5 C° until processed.

Soil from each sample was shaken through a large mesh screen to separate root and soil material. Nematodes were extracted from the root tissue using the flask-shaker method (Bird 1971). Nematodes were extracted from 100 cm<sup>3</sup> of soil taken from each of

the collected samples with a modified centrifugal flotation procedure (625 g of sugar per 1.0 liter of water) with nested sieves with 710  $\mu\text{m}$  and 37  $\mu\text{m}$  openings (Jenkins 1964). Nematodes in both soil and root samples were identified to genus (Mai et al. 1996) and counted using an inverted microscope at 100x magnification. The samples were also examined at this time for the presence of the bacterial nematode parasite, *Pasteuria*. Cysts and juveniles found in the samples were examined microscopically for the detection of *Pasteuria* spores.

*Heterodera schachtii* and *H. glycines* (soybean cyst nematode) have similar taxonomic characteristics. To distinguish between *H. schachtii* and *H. glycines* in samples submitted from Lenawee and Gratiot Counties, bioassays were done on selected samples under greenhouse conditions to verify the identity of the nematodes recovered from the extraction procedures. Samples were selected for bioassays based on the results from the root and soil nematode extractions. Soil was taken from the specified sample and planted to cabbage, a suitable host for *H. schachtii*, and to soybean, a host for *H. glycines*. Cabbage and soybean plants were harvested after 40 days. The entire contents of each pot was placed in a plastic pail and washed with tap water. Nematodes were extracted from this material contained in the pail using the modified centrifugal flotation method with nested sieves with 710  $\mu\text{m}$  and 37  $\mu\text{m}$  openings (Jenkins 1964). Nematodes were identified to species (Mulvey 1985) and counted using a stereoscope microscope at 40x magnification.

Nematode frequency, density and prominence were determined using the procedure from Norton (Norton 1978). Relative and absolute density, relative and absolute frequency, and prominence values were calculated on the data from the samples

collected from counties with a sample population equal to or greater than 20. Prominence value is used to provide a joint indication of both frequency and density.

## RESULTS

Michigan and Monitor Sugar Companies submitted a total of 214 samples from 13 counties. There were 105 samples taken from sites exhibiting symptoms and 109 samples from sites without symptoms. Bioassays for *Heterodera* spp. determination for Lenawee and Gratiot Counties are not complete at this time; therefore, all nematodes were identified to genus only. Samples were confirmed as positive for *H. schachtii* if second-stage juveniles were found in both the root and soil samples. *Heterodera* spp. (cyst nematodes) were recovered from 115 of the samples or 54% of the total sites sampled (Table 1). Population densities ranged from 0 to 49,352 eggs and juveniles per 100 cm<sup>3</sup> of soil (Appendix B). Cyst nematodes were recovered from 50% of the 109 sites that did not exhibit symptoms, with a mean population density of 1088.9 eggs and juveniles (Table 2). In the 105 sites exhibiting symptoms, cyst nematodes were recovered from 58% of the samples, with a mean population density of 2595.8 eggs and juveniles. *Heterodera* spp. were recovered from 9 of the 13 counties sampled in 1998. *H. schachtii* was positively identified in 6 of the thirteen counties involved in this survey (Figure 1).

In addition to *Heterodera* spp., five other important genera of plant-parasitic nematodes were recovered from Michigan sugarbeet fields: *Pratylenchus*; *Paratylenchus*; *Cricconemella*; *Helicotylenchus* and *Tylenchorhynchus*. The distribution of these nematodes varied among counties (Table 3). The importance of these other plant-parasitic

nematodes in sugarbeet production in Michigan is not known at this time. Future research will have to be conducted to determine their impact on sugarbeet production.

Frequency, density, and prominence values were calculated for six counties: Bay, Gratiot; Huron; Saginaw; Sanilac; and Tuscola (Tables 4-9). Huron County had the highest prominence value for *Heterodera* spp. (Table 6). The lowest prominence value of 0.0 came from Gratiot County where *Paratylenchus* spp. were the most prominent. Stratified samples had a higher prominence value for *Heterodera* spp. than random samples (Tables 10 and 11). The data also suggest that the more often you grow sugarbeet the higher the *Heterodera* populations (Table 12).

All of the 214 survey samples submitted from both sugar companies were negative for the presence of *Pasteuria*, a bacterial nematode parasite. Microscopic observations of cysts and juveniles failed to detect spores of *Pasteuria*.

## DISCUSSION

This project has helped to determine the occurrence and distribution of *Heterodera* throughout the major sugarbeet production area in Michigan. There are now six confirmed counties within Michigan that test positive for the presence of *H. schachtii*, Arenac, Bay, Huron, Saginaw, Sanilac, and Tuscola. These six counties accounted for approximately 80% of 1997 Michigan sugarbeet production. With the increase in sugarbeet production in the state there is a risk of further spread to subsequent uninfected fields and neighboring counties. This survey also gives initial information to suggest that infestation from *Heterodera* spp. are related to cropping history and plant symptomatology. The greater the number of sugarbeet crops planted since 1984, the

greater the amount of *Heterodera spp.* recovered. This increase in population densities may be due to the shortening of rotation schemes. Samples taken using a stratified method also appeared to have a greater amount of *Heterodera spp.* recovered. This suggests that symptoms may be an indicator of *H. schachtii* infestations.

Future research will be done in Michigan on the bacterial nematode parasite *Pasteuria*. Although it was not detected in the 1998 sugarbeet survey, it will still be researched as a possible biological control agent for the management of *H. schachtii* in Michigan sugarbeet production.

## CONCLUSIONS

The presence of *H. schachtii* in the top sugarbeet producing counties in Michigan suggests that this nematode may be related to the yield decline that Michigan has been experiencing over the last 15 years. It is necessary that growers use management practices to minimize the risk of spread to uninfected areas. Symptomatology may be an indicator of nematode infestation. Future research may help to provide information on specific symptoms caused by nematode infestation. Growers must also be sure to maintain proper crop rotation schemes to reduce the impact of *H. schachtii* on sugarbeet yields.

**Table 1. Number of *Heterodera* spp. positive samples from the 1998 sugarbeet survey in Michigan for both stratified and random sampling methods.**

| County        | Total County   |                 | Stratified Samples <sup>1</sup> |                 | Random Samples <sup>2</sup> |                 |
|---------------|----------------|-----------------|---------------------------------|-----------------|-----------------------------|-----------------|
|               | No. of samples | No. of positive | No. of samples                  | No. of positive | No. of samples              | No. of positive |
| 1. Arenac     | 9              | 4               | 5                               | 4               | 4                           | 0               |
| 2. Bay        | 51             | 36              | 31                              | 21              | 20                          | 15              |
| 3. Gratiot    | 20             | 2               | 12                              | 1               | 8                           | 1               |
| 4. Huron      | 30             | 21              | 15                              | 11              | 15                          | 10              |
| 5. Isabella   | 1              | 0               | 0                               | 0               | 1                           | 0               |
| 6. Lenawee    | 10             | 1               | 4                               | 1               | 6                           | 0               |
| 7. Mecosta    | 1              | 0               | 1                               | 0               | 0                           | 0               |
| 8. Montcalm   | 3              | 0               | 1                               | 0               | 2                           | 0               |
| 9. Newaygo    | 1              | 1               | 1                               | 1               | 0                           | 0               |
| 10. Saginaw   | 23             | 19              | 10                              | 9               | 13                          | 10              |
| 11. Sanilac   | 29             | 13              | 15                              | 6               | 14                          | 7               |
| 12. St. Clair | 1              | 0               | 0                               | 0               | 1                           | 0               |
| 13. Tuscola   | 35             | 18              | 10                              | 7               | 25                          | 11              |
| Total         | 214            | 115             | 105                             | 61              | 109                         | 54              |

<sup>1</sup>Stratified: Sampling from fields exhibiting foliar symptoms of suspected *H. schachtii* infestation. Stratified samples were taken by collecting three different root-soil cores each from three locations in the field exhibiting symptoms. These samples were collected from the plant-soil rhizosphere near the margins of the locations exhibiting symptoms.

<sup>2</sup>Random: Sampling from fields not exhibiting foliar symptoms of suspected *H. schachtii* infestation. Samples taken at random were a composite of nine root-soil cores from the plant-soil rhizosphere.

**Table 2. 1998 *Heterodera* spp. population densities associated with 214 Michigan sugarbeet fields sampled using a stratified or random sampling method.**

| County        | Stratified <sup>1</sup> |        | Random <sup>2</sup> |        |
|---------------|-------------------------|--------|---------------------|--------|
|               | n                       | Mean   | n                   | Mean   |
| 1. Arenac     | 5                       | 34.0   | 4                   | 0.0    |
| 2. Bay        | 31                      | 2661.9 | 20                  | 461.5  |
| 3. Gratiot    | 12                      | 0.5    | 8                   | 0.3    |
| 4. Huron      | 15                      | 9064.3 | 15                  | 909.9  |
| 5. Isabella   | ---                     | ---    | 1                   | 0.0    |
| 6. Lenawee    | 4                       | 0.5    | 6                   | 0.0    |
| 7. Mecosta    | 1                       | 0.0    | ---                 | ---    |
| 8. Montcalm   | 1                       | 0.0    | 2                   | 0.0    |
| 9. Newaygo    | 1                       | 4.0    | 0                   | 0.0    |
| 10. Saginaw   | 10                      | 1654.0 | 13                  | 1160.0 |
| 11. Sanilac   | 15                      | 746.3  | 14                  | 922.6  |
| 12. St. Clair | ---                     | ---    | 1                   | 0.0    |
| 13. Tuscola   | 10                      | 2616.2 | 25                  | 2712.6 |
| TOTAL         | 105                     | 2595.8 | 109                 | 1088.9 |

<sup>1</sup>Stratified: Sampling from fields exhibiting foliar symptoms of suspected *H. schachtii* infestation. Stratified samples were taken by collecting three different root-soil cores each from three locations in the field exhibiting symptoms. These samples were collected from the plant-soil rhizosphere near the margins of the locations exhibiting symptoms.

<sup>2</sup>Random: Sampling from fields not exhibiting foliar symptoms of suspected *H. schachtii* infestation. Samples taken at random were a composite of nine root-soil cores from the plant-soil rhizosphere.

Table 3. Nematode population density per 100 cm<sup>3</sup> of soil for all 13 counties for *Heterodera*, *Pratylenchus*, *Paratylenchus*, *Helicotylenchus*, *Criconebella* and *Tylenchorhynchus* spp. from 1998 surgarbeet survey.

| County        | <i>Heterodera</i> | <i>Pratylenchus</i> | <i>Paratylenchus</i> | <i>Helicotylenchus</i> | <i>Criconebella</i> | <i>Tylenchorhynchus</i> | Other |
|---------------|-------------------|---------------------|----------------------|------------------------|---------------------|-------------------------|-------|
| 1. Arenac     | 18.9              | 0.2                 | 42.7                 | 15.3                   | 2.3                 | 0.4                     | 0.0   |
| 2. Bay        | 1799.0            | 4.2                 | 20.5                 | 15.4                   | 1.3                 | 0.1                     | 0.2   |
| 3. Gratiot    | 0.4               | 6.5                 | 35.2                 | 21.0                   | 0.9                 | 0.5                     | 3.8   |
| 4. Huron      | 4987.1            | 1.0                 | 9.0                  | 46.0                   | 0.0                 | 0.3                     | 0.0   |
| 5. Isabella   | 0.0               | 0.0                 | 45.0                 | 8.0                    | 0.0                 | 0.0                     | 0.0   |
| 6. Lenawee    | 0.2               | 44.4                | 3.4                  | 87.8                   | 0.0                 | 0.6                     | 0.0   |
| 7. Mecosta    | 0.0               | 1.0                 | 0.0                  | 0.0                    | 0.0                 | 0.0                     | 0.0   |
| 8. Montcalm   | 0.0               | 1.3                 | 1.3                  | 16.0                   | 0.0                 | 1.0                     | 0.7   |
| 9. Newaygo    | 4.0               | 2.0                 | 0.0                  | 0.0                    | 0.0                 | 0.0                     | 0.0   |
| 10. Saginaw   | 1375.0            | 2.7                 | 5.7                  | 47.0                   | 0.0                 | 0.2                     | 0.0   |
| 11. Sanilac   | 831.4             | 9.8                 | 10.4                 | 32.1                   | 9.8                 | 2.3                     | 0.0   |
| 12. St. Clair | 0.0               | 2.0                 | 0.0                  | 8.0                    | 0.0                 | 0.0                     | 0.0   |
| 13. Tuscola   | 2685.1            | 8.1                 | 2.1                  | 10.0                   | 0.2                 | 0.6                     | 0.4   |

**Table 4. Frequency, density, and prominence values for plant-parasitic nematodes collected from Bay County as part of the 1998 sugarbeet survey (n=51).**

| Nematode spp.           | No of Positive Samples | Absolute Frequency <sup>1</sup> | Relative Frequency <sup>2</sup> | Absolute Density <sup>3</sup> | Relative Density <sup>4</sup> | Prominence Value <sup>5</sup> |
|-------------------------|------------------------|---------------------------------|---------------------------------|-------------------------------|-------------------------------|-------------------------------|
| <i>Heterodera</i>       | 36                     | 70.6                            | 33.0                            | 1799.0                        | 97.7                          | 151.1                         |
| <i>Pratylenchus</i>     | 12                     | 23.5                            | 11.0                            | 4.2                           | 0.2                           | 0.2                           |
| <i>Paratylenchus</i>    | 28                     | 54.9                            | 25.7                            | 20.5                          | 1.1                           | 1.5                           |
| <i>Helicotylenchus</i>  | 27                     | 52.9                            | 24.8                            | 15.4                          | 0.8                           | 1.1                           |
| <i>Criconemella</i>     | 4                      | 7.8                             | 3.7                             | 1.3                           | 0.1                           | 0.0                           |
| <i>Tylenchorhynchus</i> | 2                      | 3.9                             | 1.8                             | 0.1                           | 0.0                           | 0.0                           |

<sup>1</sup>Absolute Frequency = (number of samples containing a species)/(number of samples collected) \* 100

<sup>2</sup>Relative Frequency = (frequency of species)/(sum of frequency of all species)\*100

<sup>3</sup>Absolute Density = Sample Mean

<sup>4</sup>Relative Density = (number of individuals of a species in a sample)/(total of all individuals in a sample)\*100

<sup>5</sup>Prominence Value = density \* sqrt(absolute frequency)/100

**Table 5. Frequency, density, and prominence values for plant-parasitic nematodes collected from Gratiot County as part of the 1998 sugarbeet survey (n=20).**

| Nematode spp.           | No of Positive Samples | Absolute Frequency <sup>1</sup> | Relative Frequency <sup>2</sup> | Absolute Density <sup>3</sup> | Relative Density <sup>4</sup> | Prominence Value <sup>5</sup> |
|-------------------------|------------------------|---------------------------------|---------------------------------|-------------------------------|-------------------------------|-------------------------------|
| <i>Heterodera</i>       | 2                      | 10                              | 5.6                             | 0.4                           | 0.6                           | 0.0                           |
| <i>Pratylenchus</i>     | 7                      | 35                              | 19.4                            | 6.5                           | 10.2                          | 0.4                           |
| <i>Paratylenchus</i>    | 11                     | 55                              | 30.6                            | 35.2                          | 55.1                          | 2.6                           |
| <i>Helicotylenchus</i>  | 12                     | 60                              | 33.3                            | 21.0                          | 32.8                          | 1.6                           |
| <i>Criconemella</i>     | 2                      | 10                              | 5.6                             | 0.0                           | 0.0                           | 0.0                           |
| <i>Tylenchorhynchus</i> | 2                      | 10                              | 5.6                             | 0.9                           | 1.3                           | 0.0                           |

<sup>1</sup>Absolute Frequency = (number of samples containing a species)/(number of samples collected) \* 100

<sup>2</sup>Relative Frequency = (frequency of species)/(sum of frequency of all species)\*100

<sup>3</sup>Absolute Density = Sample Mean

<sup>4</sup>Relative Density = (number of individuals of a species in a sample)/(total of all individuals in a sample)\*100

<sup>5</sup>Prominence Value = density \* sqrt(absolute frequency)/100

Table 6. Frequency, density, and prominence values for plant-parasitic nematodes collected from Huron County as part of the 1998 sugarbeet survey (n=30).

| Nematode spp.           | No of Positive Samples | Absolute Frequency <sup>1</sup> | Relative Frequency <sup>2</sup> | Absolute Density <sup>3</sup> | Relative Density <sup>4</sup> | Prominence Value <sup>5</sup> |
|-------------------------|------------------------|---------------------------------|---------------------------------|-------------------------------|-------------------------------|-------------------------------|
| <i>Heterodera</i>       | 21                     | 70.0                            | 29.6                            | 4987.1                        | 98.9                          | 417.3                         |
| <i>Pratylenchus</i>     | 8                      | 26.7                            | 11.3                            | 1.0                           | 0.0                           | 0.1                           |
| <i>Paratylenchus</i>    | 17                     | 56.7                            | 23.9                            | 9.0                           | 0.2                           | 0.7                           |
| <i>Helicotylenchus</i>  | 23                     | 76.7                            | 32.4                            | 46.0                          | 0.9                           | 4.0                           |
| <i>Criconebella</i>     | 0                      | 0.0                             | 0.0                             | 0.0                           | 0.0                           | 0.0                           |
| <i>Tylenchorhynchus</i> | 2                      | 6.7                             | 2.8                             | 0.3                           | 0.0                           | 0.0                           |

<sup>1</sup> Absolute Frequency = (number of samples containing a species)/(number of samples collected) \* 100

<sup>2</sup> Relative Frequency = (frequency of species)/(sum of frequency of all species)\*100

<sup>3</sup> Absolute Density = Sample Mean

<sup>4</sup> Relative Density = (number of individuals of a species in a sample)/(total of all individuals in a sample)\*100

<sup>5</sup> Prominence Value = density \* sqrt(absolute frequency)/100

Table 7. Frequency, density, and prominence values for plant-parasitic nematodes collected from Saginaw County as part of the 1998 sugarbeet survey (n=23).

| Nematode spp.           | No of Positive Samples | Absolute Frequency <sup>1</sup> | Relative Frequency <sup>2</sup> | Absolute Density <sup>3</sup> | Relative Density <sup>4</sup> | Prominence Value <sup>5</sup> |
|-------------------------|------------------------|---------------------------------|---------------------------------|-------------------------------|-------------------------------|-------------------------------|
| <i>Heterodera</i>       | 19                     | 82.6                            | 32.8                            | 1375.0                        | 96.1                          | 125.0                         |
| <i>Pratylenchus</i>     | 11                     | 47.8                            | 19.0                            | 2.7                           | 0.2                           | 0.2                           |
| <i>Paratylenchus</i>    | 10                     | 43.5                            | 17.2                            | 5.7                           | 0.4                           | 0.4                           |
| <i>Helicotylenchus</i>  | 17                     | 73.9                            | 29.3                            | 47.0                          | 3.3                           | 4.0                           |
| <i>Criconebella</i>     | 0                      | 0.0                             | 0.0                             | 0.0                           | 0.0                           | 0.0                           |
| <i>Tylenchorhynchus</i> | 1                      | 4.3                             | 1.7                             | 0.2                           | 0.0                           | 0.0                           |

<sup>1</sup> Absolute Frequency = (number of samples containing a species)/(number of samples collected) \* 100

<sup>2</sup> Relative Frequency = (frequency of species)/(sum of frequency of all species)\*100

<sup>3</sup> Absolute Density = Sample Mean

<sup>4</sup> Relative Density = (number of individuals of a species in a sample)/(total of all individuals in a sample)\*100

<sup>5</sup> Prominence Value = density \* sqrt(absolute frequency)/100

**Table 8. Frequency, density, and prominence values for plant-parasitic nematodes collected from Sanilac County as part of the 1998 sugarbeet survey (n=29).**

| Nematode spp.           | No of Positive Samples | Absolute Frequency <sup>1</sup> | Relative Frequency <sup>2</sup> | Absolute Density <sup>3</sup> | Relative Density <sup>4</sup> | Prominence Value <sup>5</sup> |
|-------------------------|------------------------|---------------------------------|---------------------------------|-------------------------------|-------------------------------|-------------------------------|
| <i>Heterodera</i>       | 13                     | 44.8                            | 18.8                            | 831.4                         | 92.8                          | 55.7                          |
| <i>Pratylenchus</i>     | 14                     | 48.3                            | 20.3                            | 9.8                           | 1.1                           | 0.7                           |
| <i>Paratylenchus</i>    | 18                     | 62.1                            | 26.1                            | 10.4                          | 1.2                           | 0.8                           |
| <i>Helicotylenchus</i>  | 20                     | 69.0                            | 29.0                            | 32.1                          | 3.6                           | 2.7                           |
| <i>Criconemella</i>     | 3                      | 10.3                            | 4.3                             | 9.8                           | 1.1                           | 0.3                           |
| <i>Tylenchorhynchus</i> | 1                      | 3.4                             | 1.4                             | 2.3                           | 0.3                           | 0.0                           |

<sup>1</sup> Absolute Frequency = (number of samples containing a species)/(number of samples collected) \* 100

<sup>2</sup> Relative Frequency = (frequency of species)/(sum of frequency of all species)\*100

<sup>3</sup> Absolute Density = Sample Mean

<sup>4</sup> Relative Density = (number of individuals of a species in a sample)/(total of all individuals in a sample)\*100

<sup>5</sup> Prominence Value = density \* sqrt(absolute frequency)/100

**Table 9. Frequency, density, and prominence values for plant-parasitic nematodes collected from Tuscola County as part of the 1998 sugarbeet survey (n=35).**

| Nematode spp.           | No of Positive Samples | Absolute Frequency <sup>1</sup> | Relative Frequency <sup>2</sup> | Absolute Density <sup>3</sup> | Relative Density <sup>4</sup> | Prominence Value <sup>5</sup> |
|-------------------------|------------------------|---------------------------------|---------------------------------|-------------------------------|-------------------------------|-------------------------------|
| <i>Heterodera</i>       | 18                     | 51.4                            | 24.7                            | 2685.1                        | 99.2                          | 192.6                         |
| <i>Pratylenchus</i>     | 13                     | 37.1                            | 17.8                            | 8.1                           | 0.3                           | 0.5                           |
| <i>Paratylenchus</i>    | 14                     | 40.0                            | 19.2                            | 2.1                           | 0.1                           | 0.1                           |
| <i>Helicotylenchus</i>  | 24                     | 68.6                            | 32.9                            | 10.0                          | 0.4                           | 0.8                           |
| <i>Criconemella</i>     | 1                      | 2.9                             | 1.4                             | 0.2                           | 0.0                           | 0.0                           |
| <i>Tylenchorhynchus</i> | 3                      | 8.6                             | 4.1                             | 0.6                           | 0.0                           | 0.0                           |

<sup>1</sup> Absolute Frequency = (number of samples containing a species)/(number of samples collected) \* 100

<sup>2</sup> Relative Frequency = (frequency of species)/(sum of frequency of all species)\*100

<sup>3</sup> Absolute Density = Sample Mean

<sup>4</sup> Relative Density = (number of individuals of a species in a sample)/(total of all individuals in a sample)\*100

<sup>5</sup> Prominence Value = density \* sqrt(absolute frequency)/100

**Table 10. Frequency, density, and prominence values for plant-parasitic nematodes for all the stratified samples collected from the 1998 sugarbeet survey.**

| Nematode spp.           | No of Positive Samples | Absolute Frequency <sup>1</sup> | Relative Frequency <sup>2</sup> | Absolute Density <sup>3</sup> | Relative Density <sup>4</sup> | Prominence Value <sup>5</sup> |
|-------------------------|------------------------|---------------------------------|---------------------------------|-------------------------------|-------------------------------|-------------------------------|
| <i>Heterodera</i>       | 61                     | 58                              | 24.83                           | 2595.8                        | 98                            | 198                           |
| <i>Pratylenchus</i>     | 36                     | 34                              | 14.65                           | 9.3                           | 0.3                           | 0.5                           |
| <i>Paratylenchus</i>    | 60                     | 57                              | 24.42                           | 22                            | 0.8                           | 1.7                           |
| <i>Helicotylenchus</i>  | 75                     | 71                              | 30.53                           | 29.23                         | 1.1                           | 2.5                           |
| <i>Criconemella</i>     | 8                      | 7.6                             | 3.256                           | 1                             | 0                             | 0                             |
| <i>Tylenchorhynchus</i> | 6                      | 5.7                             | 2.442                           | 0.84                          | 0                             | 0                             |

<sup>1</sup> Absolute Frequency = (number of samples containing a species)/(number of samples collected) \* 100

<sup>2</sup> Relative Frequency = (frequency of species)/(sum of frequency of all species)\*100

<sup>3</sup> Absolute Density = Sample Mean

<sup>4</sup> Relative Density = (number of individuals of a species in a sample)/(total of all individuals in a sample)\*100

<sup>5</sup> Prominence Value = density \* sqrt(absolute frequency)/100

**Table 11. Frequency, density, and prominence values for plant-parasitic nematodes for all the random samples collected from the 1998 sugarbeet survey.**

| Nematode spp.           | No of Positive Samples | Absolute Frequency <sup>1</sup> | Relative Frequency <sup>2</sup> | Absolute Density <sup>3</sup> | Relative Density <sup>4</sup> | Prominence Value <sup>5</sup> |
|-------------------------|------------------------|---------------------------------|---------------------------------|-------------------------------|-------------------------------|-------------------------------|
| <i>Heterodera</i>       | 54                     | 49.541                          | 24                              | 1089                          | 96.201                        | 76.6                          |
| <i>Pratylenchus</i>     | 40                     | 36.697                          | 18                              | 4.4                           | 0.3887                        | 0.27                          |
| <i>Paratylenchus</i>    | 53                     | 48.624                          | 24                              | 6.3                           | 0.5565                        | 0.44                          |
| <i>Helicotylenchus</i>  | 63                     | 57.798                          | 28                              | 27.1                          | 2.394                         | 2.06                          |
| <i>Criconemella</i>     | 3                      | 2.7523                          | 1.3                             | 2.6                           | 0.2297                        | 0.04                          |
| <i>Tylenchorhynchus</i> | 10                     | 9.1743                          | 4.5                             | 2.6                           | 0.2297                        | 0.08                          |

<sup>1</sup> Absolute Frequency = (number of samples containing a species)/(number of samples collected) \* 100

<sup>2</sup> Relative Frequency = (frequency of species)/(sum of frequency of all species)\*100

<sup>3</sup> Absolute Density = Sample Mean

<sup>4</sup> Relative Density = (number of individuals of a species in a sample)/(total of all individuals in a sample)\*100

<sup>5</sup> Prominence Value = density \* sqrt(absolute frequency)/100

Table 12. Number of years in sugarbeet out of the last 14 growing seasons (not including present year).

| Years in<br>sugarbeets | <i>Heterodera</i> | <i>Pratylenchus</i> | <i>Paratylenchus</i> | <i>Helicotylenchus</i> | <i>Criconemella</i> | <i>Tylenchorhynchus</i> | Other |
|------------------------|-------------------|---------------------|----------------------|------------------------|---------------------|-------------------------|-------|
| 0 (n=7)                | 0.6               | 1.9                 | 0.3                  | 35.1                   | 0.0                 | 0.0                     | 0.0   |
| 1 (n=13)               | 1622.0            | 4.8                 | 2.7                  | 20.3                   | 21.5                | 0.5                     | 0.1   |
| 2 (n=27)               | 148.8             | 9.3                 | 27.4                 | 25.7                   | 0.2                 | 0.6                     | 1.1   |
| 3 (n=36)               | 1603.1            | 9.2                 | 18.7                 | 20.6                   | 1.6                 | 0.7                     | 2.0   |
| 4 (n=84)               | 1986.4            | 7.5                 | 11.4                 | 22.8                   | 0.0                 | 0.1                     | 0.0   |
| 5 (n=44)               | 3196.2            | 3.8                 | 12.5                 | 46.7                   | 1.1                 | 1.7                     | 0.0   |
| 6 (n=3)                | 317.3             | 0.0                 | 12.7                 | 38.0                   | 0.0                 | 0.0                     | 0.0   |



# **CHEMICAL MANAGEMENT OF *HETERODERA SCHACHTII* IN MICHIGAN SUGARBEET PRODUCTION**

## **INTRODUCTION**

*Heterodera schachtii* (sugarbeet cyst nematode) was first shown to reduce sugarbeet yields under Michigan growing conditions over 50 years ago. Sugarbeet is the only major field crop host for this nematode. In the past it has been mainly managed with strict crop rotations. Crop rotation has worldwide acceptance as the most practical, economical means of obtaining profitable yields on nematode-infested land (Steele 1984). In nematode-infested fields, recommended rotational schemes may permit growing sugarbeet once in three to seven years depending on the severity of infestation and local conditions that influence population dynamics (Steele 1984). Soil fumigants and non-fumigant nematicides are used for management of *H. schachtii* on only an extremely limited basis.

The Michigan sugarbeet industry has undergone several major changes during the past 10 years, including an increase in the number of acres planted annually, and a shortening of crop rotation schemes. Increased sugarbeet production and shorter crop rotations demand alternate strategies and tactics for managing *H. schachtii* population levels.

Alternative methods of management include various chemical and cultural control practices. Presently, there are no sugarbeet varieties that are resistance to *H. schachtii* available for use in Michigan sugarbeet production. Cultural practices include sanitation, early planting, and the use of trap crops. Sanitation, especially in relation to equipment

and tare soil, is very important to prevent the further spread of *H. schachtii* from infested to non-infested fields. Growing a nematode-resistant cruciferous trap crop prior to planting sugarbeet may decrease populations of *H. schachtii* (Müller 1991). These trap crops are planted after harvesting a summer crop, and in suitable soil conditions their root exudates stimulate hatching of *H. schachtii* juveniles, which then invade the roots and initiate the formation of syncytia at permanent feeding sites. The syncytia will break down and prevent most of the nematodes from completing their life cycles to form viable females (Wyss et al. 1984). The practice of using trap crops in Michigan sugarbeet production has not been extensively evaluated for its effectiveness. Chemical control involves the use of fumigant and non-fumigant nematicides.

The objective of this project was to evaluate two non-fumigant nematicides, one fumigant, and one bio-nematicide for the control of *H. schachtii*. These nematicides include Temik<sup>®</sup>, Counter<sup>®</sup>, DiTera<sup>®</sup>, and Vapam<sup>®</sup>.

## **MATERIALS AND METHODS**

In 1998, a sugarbeet cyst nematode control research trial was set up in Bay City, Michigan, on the Gerald Appold Farm. This particular field site has been in a wheat, soybean, and sugarbeet rotation for the last two decades. The research site used for this trial was 0.96 acres (260 x 160 feet) in size had not been planted to sugarbeet since 1995. This trial was set up as a random complete block design. It consisted of nine treatments (Table 1) in six replicated blocks of four-row by 40 foot plots. Sugarbeet seeds were planted in a silty loam soil in 30-inch rows with seed spacing of 4.5 inches on May 5, 1998. Because of a relatively poor stand establishment, some rows within the blocks were

replanted on June 6, 1998. The nine nematicides were applied at planting according to pre-determined rates (Table 1). This research trial included four treatments for aphid control; however, for the purpose of this thesis the data from treatments 10-13, where no nematicide was applied, will not be included.

Soil samples for nematode analysis were taken three times during the growing season, at- plant (5-06-98), mid-season (07-31-98), and at harvest (11-03-98). Soil samples taken at- plant and at mid-season were collected from the two outside rows of each four row plot. Harvest soil was collected from the two center rows after the beets were removed at harvest. Soil samples consisted of several soil cores placed in one bulk sample. Root samples were also collected at midseason. Three beets were dug from each of the two center rows and root tissue was collected.

Nematodes were extracted from 100 cm<sup>3</sup> of soil from each of the samples with a modified centrifugal flotation procedure (625 g of sugar per 1 l of water) with nested sieves with 710 µm and 37 µm openings (Jenkins 1964). Nematodes were extracted from midseason root tissue using the flask-shaker method (Bird 1971). *H. schachtii* extracted from both soil and roots were identified (Mai et al. 1996) and counted using an inverted microscope at 100x magnification.

Sugarbeet in the two 40-foot center rows of each four-row plot were harvested on November 3, 1998. They were counted for each four-row plot and recorded as number of sugarbeet per 80 ft (Table 5). Monitor Sugar Company used the harvested sugarbeet to determine the amount of raw white sugar per acre (RWSA) and tons per acre.

Statistical analysis of the data was done using SYSTAT (SYSTAT 7.0 1998). Regressions were done on the whole data set, and for DiTera<sup>®</sup>, and Vapam<sup>®</sup>, which were

the only two treatments applied at multiple rates. Cyst population levels determined from collecting soil samples at-plant, mid-season, and harvest, were mapped to show *H. schachtii* dispersal throughout the field over the length of the growing season.

## RESULTS

There were no significant differences between population densities of cysts, second-stage juveniles, and eggs in soil samples taken at planting, midseason, and harvest among the nine treatments (Tables 2-4). Number of cysts in the soil ranged from 0 at-plant (Vapam® 0.5 gal/a) to 29.2 (Check) at harvest per 100 cm<sup>3</sup> of soil. Eggs in the soil were found at levels ranging from 0.3 at-plant to 3341.7 at harvest per 100 cm<sup>3</sup> of soil. Second-stage juvenile (J2) population levels ranged from a low of 1.7 at-plant to 1205 at harvest per 100 cm<sup>3</sup> of soil.

There were significant differences among the nematicide treatments in the number of second-stage juveniles present in the mid-season roots (Table 3). DiTera® 12-inch band, DiTera® 6-inch band, Vapam® 0.5 gal/a, and Vapam® 1.0 gal/a, were significantly different ( $p=0.05$ ) from the check. Second-stage juvenile population levels in the root ranged from 34.3 to 146.4 per 1.0 g of root tissue. The number of second-stage juveniles present in the root compared with the total amount of juveniles present in the mid-seasons samples was calculated for each of the nine treatments (Table 6). Treatment 7 (Vapam® 0.5 gal/a) had the lowest amount of juveniles present in the root tissue.

Simple linear regression models were developed to explain variability in tons of sugarbeet harvested per acre for the entire data set. Three independent variables were analyzed (portion of J2s in root at mid-season, mid-season cyst counts, and harvest cyst

counts), and each had a p-value associated with it less than 0.05 (Table 7). The best regression model used mid-season cysts as an indicator of final tons per acre. This model was:

$$Y_{SB} = 15.032 - 0.715C_{MS}$$

Where:

$Y_{SB}$  = sugarbeet yield (tons/acre)

$C_{MS}$  = mid-season cyst counts (100 cm<sup>3</sup> soil)

This model explained 10.8% of the variability in yield, with a p-value of 0.016. The number of mid-season cysts in the soil was negatively correlated with yield.

Linear regression models for DiTera<sup>®</sup> suggest that the amount of active ingredient applied correlated with the portion of second-stage juveniles found in the root system. This model explained 18% of the variability in yield with a p-value of .039 (Table 8). The DiTera<sup>®</sup> treatments also demonstrated that mid-season cyst populations can still be used as a reasonably good predictor for yield ( $p = .054$ ,  $r^2 = .158$ ). The linear model associated with mid-season and harvest cyst populations indicated that the higher the population of cysts the lower the final tons per acre.

The best linear model for Vapam<sup>®</sup> indicated that the cyst population at harvest explained 19.3% of the variability in the tons per acre with a p-value of .032 (Table 9). Even though mid-season p-value was a little high it can still be used as a reasonably good indicator of final yields.

Mapping the horizontal distribution of *H. schachtii* cysts at planting, mid-season, and harvest demonstrate changes in the distribution of cysts throughout the growing season (Figures 1-3). The at-plant populations were aggregated in the middle of the field.

As the season progressed, the population spread throughout the field, with the highest population density of *H. schachtii* in the northwest corner. Cyst population densities for the growing season ranged from a low of 0.0 cysts per 100 cm<sup>3</sup> of soil at planting to 32.3 at harvest.

## DISCUSSION

The at-plant cyst counts were not significantly different, which is an important factor in setting up this nematicide trial. This shows that there was a relatively uniform distribution of *H. schachtii* throughout the field and that this was a good site for the nematicide trial. However, this site had low initial nematode population densities, which did not explain a lot of the variability in yield. Late planting date and lack of precipitation in the spring of 1998 may help explain the poor sugarbeet emergence and some of the variability in the nematode population densities. DiTera<sup>®</sup> (6 inch and 12 inch band) and Vapam<sup>®</sup> (0.5 and 1.0 gal/acre) had significantly fewer second-stage juveniles in the root than the check. It is possible that these two nematicides are interfering with the nematode's ability to penetrate the root system. The 1999 nematicide trial might provide some additional data to support this theory. None of the nematicides used in this trial provided season-long control of *H. schachtii*. Data suggest that there is a potential for DiTera<sup>®</sup> and Vapam<sup>®</sup> to be effective, but with only one year of data, it was not possible to get a sense of an optimal concentration. At the initial population density of *H. schachtii* associated with this site, neither of the nematicides (Counter<sup>®</sup>, Temik<sup>®</sup>) registered for use in sugarbeet production provided detectable nematode control or sugarbeet yield increases.

## **CONCLUSIONS**

Management of *H. schachtii* must be accomplished using an integrated approach. Data from the 1998 nematicide trial does not provide sufficient evidence to conclude that chemical control alone can effectively reduce cyst populations. In addition to chemical control, it is important to use cultural controls such as rotation with non-host crops and proper seed and soil sanitation practices. It is important to have well established pathogenicity thresholds for *H. schachtii* (Caswell et al. 1986).

**Table 1. Nematicides and application procedures used in a 1998 sugarbeet cyst nematode trial in Bay City, Michigan.**

| <b>Treatment</b>                 | <b>Application procedure and dosage<sup>1</sup></b>         |
|----------------------------------|-------------------------------------------------------------|
| <b>1. Check</b>                  | <b>Non-Treated Control</b>                                  |
| <b>2. Temik 15G<sup>2</sup></b>  | <b>5.0 lb ai/a in-furrow at-planting</b>                    |
| <b>3. Counter CR<sup>3</sup></b> | <b>12 oz/1,000 row ft in-furrow at-planting</b>             |
| <b>4. DiTera ES<sup>4</sup></b>  | <b>11 gal/a 12-inch band at-planting 1:3 water dilution</b> |
| <b>5. DiTera ES</b>              | <b>11 gal/a 6-inch band at-planting 1:3 water dilution</b>  |
| <b>6. DiTera ES</b>              | <b>11 gal/a broadcast at-planting 1:3 water dilution</b>    |
| <b>7. Vapam<sup>5</sup></b>      | <b>0.5 gal/a in-row at planting 1:3 water dilution</b>      |
| <b>8. Vapam</b>                  | <b>1.0 gal/a in-row at-planting 1:3 water dilution</b>      |
| <b>9. Vapam</b>                  | <b>2.0 gal/a in-row at-planting 1:3 water dilution</b>      |

<sup>1</sup>May 5, 1998 planting date.

<sup>2</sup>Trade name: Temik<sup>®</sup>; Common name: aldicarb; Chemical name: 2-Methyl-2-(methylthio) propionaldehyde O-(methylcarbamoyl) oxime; Use: systemic pesticide.

<sup>3</sup>Trade name: Counter<sup>®</sup>; Common name: terbufos; Chemical name: S-[[[(1,1-dimethylethyl)thio]methyl] 0,0-diethyl phosphorodithioate; Use: systemic insecticide and nematicide.

<sup>4</sup>Trade name: DiTera<sup>®</sup>; Common name: ABG-9008; Biological name: *Myrothecium verrucaria*; Use: biological nematicide.

<sup>5</sup>Trade name: Vapam<sup>®</sup>; Common name: metham; Chemical name: Sodium methyldithiocarbamate (anhydrous); Use: soil fumigant (fungicide, insecticide, nematicide and herbicide).

**Table 2. At-plant sugarbeet cyst nematode population densities associated with nine treatments in a 1998 trial in Bay City, Michigan (soil sampling date: May 5, 1998).**

| Treatment <sup>1</sup> | Cysts <sup>2</sup> | J2    | Eggs  |
|------------------------|--------------------|-------|-------|
| 1. Check               | 1.2                | 25.3  | 165.0 |
| 2. Temik 15G           | 1.7                | 30.7  | 75.0  |
| 3. Counter CR          | 1.3                | 8.0   | 46.7  |
| 4. DiTera ES           | 1.7                | 27.7  | 123.3 |
| 5. DiTera ES           | 0.7                | 15.7  | 20.3  |
| 6. DiTera ES           | 1.3                | 18.3  | 148.7 |
| 7. Vapam               | 0.0                | 1.7   | 0.3   |
| 8. Vapam               | 1.0                | 27.7  | 79.3  |
| 9. Vapam               | 2.3                | 55.0  | 212.7 |
| ANOVA                  | 0.724              | 0.533 | 0.561 |

<sup>1</sup>See Table 1 for detailed explanation of treatments.

<sup>2</sup>Cysts, J2s and eggs were extracted and counted per 100 cm<sup>3</sup> of soil.

**Table 3. Mid-season sugarbeet cyst nematode population densities associated with nine treatments in a 1998 trial in Bay City, Michigan (soil and root sampling date: July 31, 1998).**

| Treatment <sup>1</sup> | In Soil <sup>2</sup> |       |       | Root Samples <sup>3</sup> |       |
|------------------------|----------------------|-------|-------|---------------------------|-------|
|                        | Cysts                | J2    | Eggs  | J2 <sup>4</sup>           | Males |
| 1. Check               | 1.7                  | 34.3  | 50.0  | 388.9 bc                  | 22.2  |
| 2. Temik 15G           | 2.2                  | 146.4 | 175.2 | 171.7 abc                 | 20.3  |
| 3. Counter CR          | 2.3                  | 38.3  | 75.0  | 183.3 abc                 | 6.7   |
| 4. DiTera ES           | 2.2                  | 58.7  | 45.0  | 111.7 a                   | 18.3  |
| 5. DiTera ES           | 2.8                  | 99.2  | 190.0 | 33.3 a                    | 3.3   |
| 6. DiTera ES           | 2.3                  | 88.7  | 166.0 | 422.2 c                   | 7.8   |
| 7. Vapam               | 2.7                  | 123.3 | 136.7 | 43.3 a                    | 0.0   |
| 8. Vapam               | 2.2                  | 71.0  | 178.3 | 36.7 a                    | 3.3   |
| 9. Vapam               | 1.2                  | 57.7  | 134.7 | 145.0 ab                  | 13.3  |
| ANOVA                  | 0.867                | 0.477 | 0.559 | 0.042                     | 0.629 |

<sup>1</sup>See Table 1 for detailed explanation of treatments.

<sup>2</sup>Cysts, J2s and eggs were extracted and counted per 100 cm<sup>3</sup> of soil.

<sup>3</sup>Root samples were taken from one gram of root.

<sup>4</sup>Mean Separation: LSD. Those followed by the same letter are not statistically significantly different at 0.05 level.

**Table 4. At harvest sugarbeet cyst nematode population densities associated with nine treatments in a 1998 trial in Bay City, Michigan (soil sampling date: November 3, 1998).**

| Treatment <sup>1</sup> | Cysts <sup>2</sup> | J2     | Eggs   |
|------------------------|--------------------|--------|--------|
| 1. Check               | 29.2               | 991.7  | 2561.7 |
| 2. Temik 15G           | 24.5               | 840.0  | 2236.7 |
| 3. Counter CR          | 22.2               | 826.7  | 2343.3 |
| 4. DiTera ES           | 32.3               | 1106.7 | 3230.0 |
| 5. DiTera ES           | 15.5               | 731.7  | 1436.7 |
| 6. DiTera ES           | 17.0               | 676.7  | 1723.0 |
| 7. Vapam               | 28.5               | 1205.0 | 3341.7 |
| 8. Vapam               | 26.7               | 1168.3 | 2915.0 |
| 9. Vapam               | 18.0               | 725.0  | 1940.0 |
| ANOVA                  | 0.189              | 0.627  | 0.101  |

<sup>1</sup>See Table 1 for detailed explanation of treatments.

<sup>2</sup>Cysts, J2s and eggs were extracted and counted per 100 cm<sup>3</sup> of soil.

**Table 5. Sugarbeet yields from a 1998 nematicide trial in Bay City, Michigan.**

| Treatment <sup>1</sup> | No of beets <sup>2</sup> | Yield (Tons/Acre) | Avg Weight/Beet (lb) |
|------------------------|--------------------------|-------------------|----------------------|
| 1. Check               | 56.2                     | 11.0              | 1.8                  |
| 2. Temik 15G           | 70.8                     | 14.0              | 1.8                  |
| 3. Counter CR          | 71.8                     | 13.6              | 1.7                  |
| 4. DiTera ES           | 63.3                     | 12.1              | 1.8                  |
| 5. DiTera ES           | 60.3                     | 13.0              | 1.8                  |
| 6. DiTera ES           | 76.3                     | 14.7              | 1.8                  |
| 7. Vapam               | 62.3                     | 13.6              | 2.0                  |
| 8. Vapam               | 70.7                     | 16.0              | 2.1                  |
| 9. Vapam               | 70.3                     | 14.4              | 1.9                  |
| ANOVA                  | 0.285                    | 0.579             | 0.543                |

<sup>1</sup>See Table 1 for detailed explanation of treatments.

<sup>2</sup>Number of beets/80 ft row at harvest.

**Table 6. Portion of second-stage juveniles of *Heterodera schachtii* recovered from midseason root samples for the nine different treatments from the 1998 nematicide trial in Bay City, Michigan.**

| <b>Treatment<sup>1</sup></b> | <b>Portion of J2 in Root<sup>2</sup></b> | <b>Yield (tons/a)</b> |
|------------------------------|------------------------------------------|-----------------------|
| 1. Check                     | 0.597                                    | 11.0                  |
| 2. Temik 15G                 | 0.352                                    | 14.0                  |
| 3. Counter CR                | 0.231                                    | 13.6                  |
| 4. DiTera ES                 | 0.322                                    | 12.1                  |
| 5. DiTera ES                 | 0.122                                    | 13.0                  |
| 6. DiTera ES                 | 0.374                                    | 14.7                  |
| 7. Vapam                     | 0.063                                    | 13.6                  |
| 8. Vapam                     | 0.193                                    | 16.0                  |
| 9. Vapam                     | 0.333                                    | 14.4                  |
| ANOVA                        | 0.128                                    | 0.579                 |

<sup>1</sup>See Table 1 for detailed explanation of treatments.

<sup>2</sup>Portion of J2 in root = (J2 in root)/(J2 in root + J2 in soil + Eggs in soil)

**Table 7. Simple linear regression models that explain variability in yield (tons per acre) for the 1998 sugarbeet nematicide trial in Bay City, Michigan.**

| Dependent | Independent      | n  | Constant | Variable | r <sup>2</sup> | p-value |
|-----------|------------------|----|----------|----------|----------------|---------|
| Tons Acre | Portion in Root  | 54 | 14.577   | -3.831   | 0.089          | 0.030   |
| Tons Acre | Mid Season Cysts | 54 | 15.032   | -0.715   | 0.108          | 0.016   |
| Tons Acre | Harvest Cysts    | 54 | 16.176   | -0.108   | 0.097          | 0.022   |

**Table 8. Simple linear regression models that explain variability in yield, number of cysts, and portion of nematode population in roots for the 1998 sugarbeet nematicide trial in Bay City, Michigan for the control and DiTera treatments only.**

| Dependent       | Independent      | n  | Constant | Variable | r <sup>2</sup> | p-value |
|-----------------|------------------|----|----------|----------|----------------|---------|
| Tons Acre       | ai/A             | 24 | 12.410   | 0.143    | 0.003          | 0.796   |
| Midseason Cysts | ai/A             | 24 | 1.832    | 0.197    | 0.023          | 0.478   |
| Harvest Cysts   | ai/A             | 24 | 27.157   | -1.721   | 0.042          | 0.339   |
| Portion in Root | ai/A             | 24 | 0.536    | -0.086   | 0.180          | 0.039   |
| Tons Acre       | Portion in root  | 24 | 13.641   | -2.624   | 0.043          | 0.334   |
| Tons Acre       | J2 in root       | 24 | 13.217   | -0.002   | 0.028          | 0.436   |
| Tons Acre       | Mid Season Cysts | 24 | 14.495   | -0.791   | 0.158          | 0.054   |
| Tons Acre       | Harvest Cysts    | 24 | 16.367   | -0.155   | 0.260          | 0.011   |

**Table 9. Simple linear regression models that explain variability in yields, cyst populations and portion of nematodes in root for the 1998 sugarbeet nematicide trial in Bay City, Michigan for the control and Vapam treatments only.**

| Dependent        | Independent     | n  | Constant | Variable | r <sup>2</sup> | p-value |
|------------------|-----------------|----|----------|----------|----------------|---------|
| Tons Acre        | ai/A            | 24 | 12.382   | 1.567    | 0.071          | 0.209   |
| Mid Season Cysts | ai/A            | 24 | 2.267    | -0.400   | 0.026          | 0.449   |
| Harvest Cysts    | ai/A            | 24 | 30.633   | -5.771   | 0.117          | 0.102   |
| Portion in Root  | ai/A            | 24 | 0.355    | -0.067   | 0.018          | 0.532   |
| Tons Acre        | Portion in root | 24 | 14.812   | -3.569   | 0.092          | 0.150   |
| Tons Acre        | j2 in root      | 24 | 14.288   | 0.003    | 0.048          | 0.302   |
| Tons Acre        | MS Cysts        | 24 | 15.459   | -0.890   | 0.139          | 0.073   |
| Tons Acre        | H Cysts         | 24 | 17.680   | -0.153   | 0.193          | 0.032   |

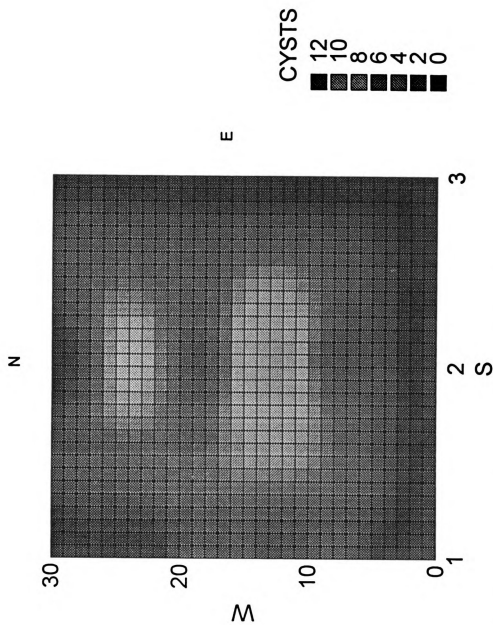


Figure 1. At-plant horizontal distribution of *Heterodera schachtii* cysts per 100 cm<sup>3</sup> of soil.

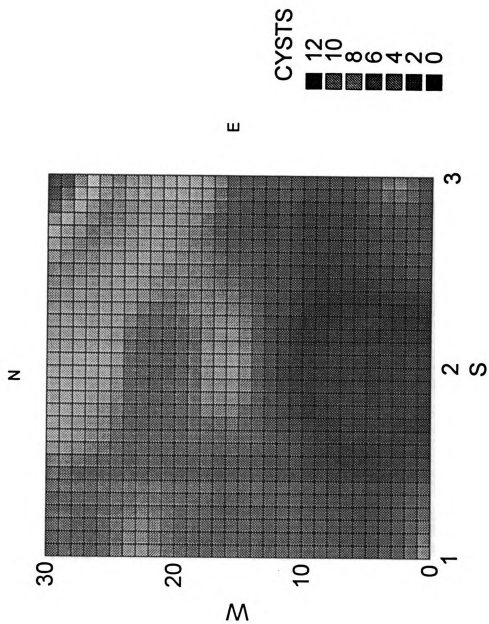


Figure 2. Mid-season horizontal distribution of *Heterodera schachtii* cysts per 100 cm<sup>3</sup> of soil.

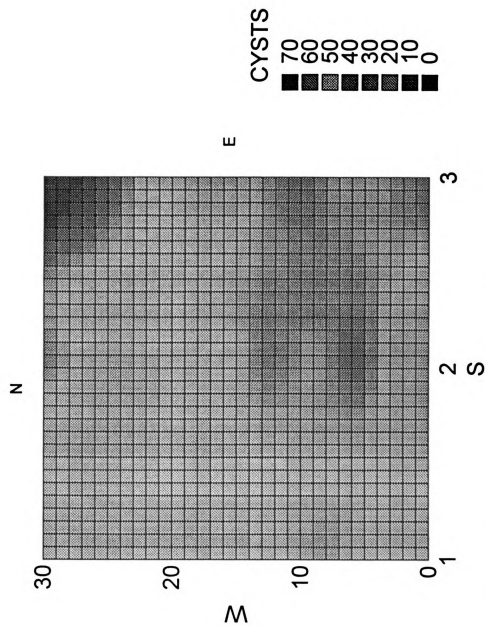


Figure 3. At harvest horizontal distribution of *Heterodera schachtii* cysts per 100 cm<sup>3</sup> of soil.

# PHYLOGENETIC ANALYSIS OF *PASTEURIA* BY SEQUENCING THE 16S rRNA GENE

## INTRODUCTION

The ontogeny and behavior of plant-parasitic nematodes are influenced by temperature, moisture, aeration, and a vast array of living organisms including other nematodes, bacteria, fungi, algae, protozoan, insects and other soil animals (Stirling 1991). Of these soil-borne organisms, the bacterium *Pasteuria* spp. has potential as an economically and environmentally practical biological control agent of specific nematodes (Hewlett et al. 1994). *Pasteuria* is a Gram-positive endospore-forming bacterium. It has been found associated with a variety of nematode hosts and in many different climates and environmental conditions throughout the world and appears to have the ability to suppress plant-parasitic nematode populations in crop production systems (Sayre and Starr 1988).

Metchnikoff described *Pasteuria ramosa* in 1888. It is a parasite of water fleas of the genus *Daphnia*. Three additional species have been described. They are all parasites of nematodes classified in the order Tylenchida and include *P. penetrans* (Sayre and Starr 1985), *P. thornei* (Sayre and Starr 1988), and *P. nishizawae* (Sayre et al. 1991). *P. penetrans* is a parasite of *Meloidogyne* spp. (Heteroderidae), *P. thornei* a parasite of *Pratylenchus* spp. (Pratylenchidae) and *P. nishizawae* a parasite of *Heterodera* spp. (Heteroderidae).

The life cycles, host ranges, and spore morphologies are important characters in the classification of these bacteria (Sayre et al. 1991; Ciancio et al. 1994). The taxonomy of the hyperparasite remains unclear, but it is probably made up of a number of species

and strains which differ in their host range and virulence; many economically important genera of plant-parasitic nematodes have been shown to have an association with these bacteria (Sayre and Starr 1988). The taxonomic relationships within *Pasteuria* are still poorly understood because cultures of various pathotypes are not readily available for biochemical and genetic investigations (Ciancio 1995). With the success in DNA sequencing of the 16s rRNA gene of *P. ramosa* (Ebert et al. 1996) and current genetic work being done with *P. penetrans* and an undescribed Illinois species, might help answer some of the questions concerning the molecular taxonomy of *Pasteuria* species.

*Pasteuria* spp. are Gram-positive with dichotomously branching, septate hyphae. The terminal hyphae enlarge to form sporangia and eventually endospores (Sayre and Starr 1985). Endospores occur in soil. As nematodes migrate through the soil, they encounter endospores and when a suitable nematode host is present, endospores become attached to the nematode cuticle. Attachment appears to be species specific. Penetration of the nematode host is accomplished by the use of a germ tube produced by the bacterium. This tube emerges through the central opening of the basal ring, penetrates the cuticle and enters into the host's hypodermal tissue. Eventually, hyphae grow into the nematode pseudocoelom. Sporulation occurs when the nematode host has been almost completely invaded by the vegetative growth (Ciancio 1995). The fecundity of infected females is greatly reduced. Eventually the nematode body becomes completely filled with mature endospores. These endospores are released back into the soil through decomposition of the nematode cadaver. Endospores are resistant to heat and desiccation, and remain viable in soil until a suitable host is encountered (Sayre and Starr 1985).

*Pasteuria* spp. have not been cultured *in vitro*. They are currently cultured on their respective hosts under greenhouse or laboratory conditions. The inability to mass-produce this bacterium under controlled conditions is partially responsible for its limited development as a biological control agent.

The objective of this project was to sequence the 16s rDNA of *Pasteuria* for phylogenetic analysis. This research may help to provide a genomic basis for the relationship of species designated in the bacterial genus *Pasteuria*, and for comparison to other bacterial genera.

## **MATERIALS AND METHODS**

A culture of *Meloidogyne arenaria* females infected with an isolate of *P. penetrans* B-4 was obtained from Dr. Don Dickson, University of Florida, Entomology/Nematology Department. Approximately 15 endospore-filled females were handpicked with forceps and placed in a saline treated microfuge tube containing 50 µl of sterile water. The females were then gently ground using a sterile Teflon microcentrifuge tube sample pestle to release endospores, which were then enumerated on a hemocytometer at 40x magnification.

To eliminate DNA from any external contaminating microorganisms, spores were treated with lysozyme, sodium dodecyl sulphate (SDS) solution, DNase, RNase, and proteinase K (Ebert et al. 1996). The spores were then lysed by adding 1 volume of phenol and 1 volume of 100 µm glass beads to the microfuge tube containing the spores and bead beating (Mini Beadbeater 8, Biospec Products) for 1 minute at 5,000 rpm (Anderson, In press). The spore suspension was centrifuged for 5 minutes (Labnet

Hermle Z180, Fisher) and the aqueous layer was transferred to a new microfuge tube. Adding 0.1 volume of 3M sodium acetate and 2 volumes of 95% ethanol to the microfuge tube and placing the tube on ice for 10 minutes precipitated the DNA. The DNA was collected by centrifugation, air-dried and resuspended in 20 µl of TE buffer (pH 8.0). The extracted DNA was then used as template for PCR amplification.

The 16s rDNA was amplified using the following primers (Rainey et al 1996): 27F (5' GAGTTTGATCCTGG CTCAG 3'), and 1385R (5' CGGTGTGTRCAAGGCCC 3'), which are both bacterial universal primers. Each reaction (25 µl) contained 1.0 µl of purified DNA, 15 pmol of each primer, and 2.5 units of Taq polymerase (Gibco, Grand Island, NY). The template DNA was amplified using a Gene Amp PCR System 2400 Thermo Cycler (Perkin Elmer, Norwalk, CT). The temperature conditions and cycles were as follows: 4 minutes at 94 °C to activate the polymerase, 35 cycles of denaturation (94 °C, 1 minute), annealing (48 °C, 1 minute), and extension (72 °C, 1 minute), and a final extension (72 °C, 6 minutes). The final PCR products were treated with the Prep-A-Gene DNA Purification Kit (Biorad) prior to sequencing. The PCR products were checked using a 7% agarose gel stained with ethidium bromide and viewed under an ultra violet light.

Sequencing reactions were performed using the Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer Applied Biosystems) following the manufacturers instructions, using approximately 90 ng of purified PCR product per reaction. Primers included 27F (5' GAGTTTGATCCTGG 3'), 343R (5' CTGCTGCCTCCCGTA 3'), and 1385R (5' CGGTGTGTRCAAGGCCC 3') (Rainey et al. 1996). Reactions were precipitated in ethanol according to manufacturer's instructions, resuspended in template

1385R (5' CGGTGTGTRCAAGGCCC 3') (Rainey et al. 1996). Reactions were precipitated in ethanol according to manufacturer's instructions, resuspended in template suppression reagent (Perkin Elmer Applied Biosystems) and analyzed on a Perkin Elmer ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Once sequences were obtained, a BLAST net search of GenBank Sequence database was done for phylogenetic analysis.

## RESULTS

Partial 16s rDNA sequences were obtained for primer 27F and 1385R (Figure 1). Primer 343R did not produce a usable sequence. The BLAST net search of the 16s rDNA gene indicated that the sequence produced from *P. penetrans* B-4 was closely related to *Pseudomonas* spp.

Although we were not successful in obtaining the sequence for the 16s gene, several months of technique development and preliminary experiments did yield some important information on how to work with *Pasteuria* (Appendix C). This information will be useful in future research done on this bacterial parasite of plant-parasitic nematodes.

## DISCUSSION

The results of this *Pasteuria* sequencing research were inconclusive. The amplified DNA did not produce a high quality sequence for phylogenetic analysis. It is possible that the DNA extraction process was not effective. We were not able to successfully establish a culture of this organism to use in this research. Because of this,

## **CONCLUSION**

Additional research is needed to access the molecular taxonomy of the genus *Pasteuria*. This is also important for development of a molecular probe for *Pasteuria* for the use in survey work associated with biological control. A Ph.D. student in Microbiology will continue this project, beginning in the summer of 1999. Success in the development of a molecular probe may prove to be very effective in the detection of *Pasteuria* in soil survey work done for the study of plant-parasitic nematodes in Michigan crop production systems.

**A. Primer 27F**

GGCCTATCTGCAGTCGACCGGNAGAGAGGTGCTNGCNCCTCTCGAGAGCGNC  
NGACGGGNGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACNGCTN  
GGAAANGGACGCTAATACCGCATACCGTNTACGGGAGAAAGCAGGGGACCT  
TCGGGCCTTGCACACAC

**B. Primer 1385R**

CCCTCTCGACTAACCAGTGCCAGTANGCCTCAGNNGCATTACTCACCCGTGC  
CGCTCGCTCTCAAGTAGGTGCAAGCACCTCGTCTACCGCTCGACTTGCATGT  
GTTAGGCCTGCGCAGCGTNAANCTGAGCCGGATAAACTA

**Figure 1. Partial 16s rDNA sequences from *Pasteuria penetrans*. Using primers 27F and 1385R.**

## CONCLUDING DISCUSSION

*Heterodera schachtii* was detected in six of the top sugarbeet producing counties in Michigan. The presence of this nematode may be a factor in the yield decline Michigan has been experiencing over recent years. Increased sugarbeet production and shorter crop rotations demand alternate strategies and tactics for managing *H. schachtii* population levels. The research involved in this M.S. program investigated two alternative methods of control, chemical and biological.

The nematicide trial demonstrated, after one year of data, that nematicides alone will not provide sufficient control of *H. schachtii*. This indicates that management of *H. schachtii* must be accomplished using an integrated approach, which is why I also looked at the practice of using *Pasteuria* as possible biological control agent.

*Pasteuria* was not detected in Michigan populations of *Heterodera schachtii* during the course of my M.S. program. However, with future research being done on this bacterial parasite it may be possible to develop a molecular probe to aid in the detection of *Pasteuria* in future soil survey work associated with plant-parasitic nematodes in Michigan. *Pasteuria* may prove to have the ability to suppress nematode populations in Michigan crop production systems.

Future research will be done to provide more information on the effectiveness of nematicides for controlling *H. schachtii* populations. It is hopeful that more work will be done in Michigan to help provide more information on the use of *Pasteuria* as a biological control agent against plant-parasitic nematodes.

## **LITERATURE CITED**

## LITERATURE CITED

- Anderson, J. M., J. E. Maruniak, J. F. Preston, D. W. Dickson, and T. E. Hewlett. 1999. Phylogenetic analysis of *Pasteuria penetrans*, a parasitic bacterium of root-knot nematodes, by 16s r RNA Gene Cloning and Sequencing. *Journal of Nematology*: 31 (In Press).
- Anonymous. 1997. USDA Agricultural Statistics. Washington D.C.: U.S. Government Printing Office.
- Anonymous. 1998. USDA Agricultural Statistics. Washington D.C.: U.S. Government Printing Office.
- Berney, M. and G. W. Bird. 1998. Nematodes, pp. 71-79; IN: Cavigelli, M. A., S. R. Deming, L. K. Probyn and R. R. Harwood (Eds.) Michigan Field Crop Ecology. Michigan State University Extension Bulletin E-2646. 86 pp.
- Bird, G. W. 1971. Influence of incubation solution on the rate of recovery of *Pratylenchus brachyurus* from cotton roots. *Journal of Nematology* 3:378-385.
- Bockstahler, H. W. 1950. The sugarbeet nematode in Michigan. Proc. 6<sup>th</sup> General Meeting of the Sugarbeet Technologists. pp. 479-480.
- Caswell, E. P., A. E. MacGuidwin, K. Milne, C. E. Nelsen, I. J. Thomason, and G. W. Bird. 1986. A simulation model of *Heterodera schachtii* infecting *Beta vulgaris*. *Journal of Nematology* 18(4):512-519.
- Ciancio, A. 1995. Phenotypic adaptations in *Pasteuria* spp. nematode parasites. *Journal of Nematology* 27:328-338.
- Ciancio, A., R. Bonsignore, N. Vovlas, and F. Lamberti. 1994. Host records and spore morphometrics of *Pasteuria penetrans* group parasites of nematodes. *Journal of Invertebrate Pathology* 63:260-267.

- Cooke, D. 1993. Chapter 4: Nematode parasites of sugarbeet IN: Evans, K. D. L. Trudgill and J. M. Webster (Eds.) *Plant Parasitic Nematodes in Temperate Agriculture*. CAB International, Wallingford. 648 pp.
- Ebert, D., P. Rainey, T. M. Embley, and D. Scholz. 1996. Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: Rediscovery of an obligate endoparasite of *Daphnia magna* Straus. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 351:1689-1701.
- Griffin, G. D. 1981. The relationship of plant age, soil temperature, and population density of *Heterodera schachtii* on the growth of sugarbeet. *Journal of Nematology* 13:184-190.
- Hewlett, T. E., R. Cox, D. W. Dickson, and R. A. Dunn. 1994. Occurrence of *Pasteuria* spp. in Florida. *Journal of Nematology* 26:616-619.
- Jenkins, W. F. 1964. A rapid centrifugal-flotation technique for extracting nematodes from soil. *Plant Disease Reporter* 48:692.
- Knobloch, N. and G. W. Bird. 1981. Plant-parasitic nematodes of Michigan: With special reference to the genera of the Tylenchorhynchinae (Nematoda). Michigan Agricultural Experiment Station Research Report No. 419. 35pp.
- Mai W. F., P. G. Mullin, H. H. Lyon, and K. Loeffler. 1996. *Plant-parasitic Nematodes A Pictorial Key to Genera* (fifth edition). Cornell University Press, Ithaca, N.Y. 277 pp.
- Müller, J. 1991. Catch cropping for population control of *Heterodera schachtii*. *Proceedings of the 54<sup>th</sup> Winter Congress of the International Institute for Sugar Beet Research*, pp. 179-196.
- Mulvey, R. H. and A. M. Golden. 1985. An illustrated key to the cyst-forming genera and species of Heteroderidae in the western hemisphere with species morphometrics and distribution. *Journal of Nematology* 15:1-59.

- Norton, D. C. 1978. Chapter 4: Communities IN: Ecology of Plant-parasitic Nematodes. John Wiley & Sons, New York. 268 pp.
- Rainey, F. A., N. Ward-Rainey, R. M. Kroppenstedt, and E. Stackenbrandt. 1996. The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. Nov. International Journal of Systematic Bacteriology 46(4):1088-1092.
- Raski, D. J. 1949. The life history and morphology of the sugarbeet nematode, *Heterodera schachtii* Schmidt. Phytopathology 40:135-152.
- Roberts, P. A. and I. J. Thomason. 1981. Sugarbeet Pest Management: Nematodes. Special Publication 3272, Division of Agricultural Sciences, University of California.
- Sayre, R. M. and M. P. Starr. 1985. *Pasteuria penetrans* (ex Thorne, 1940) nom. Rev., comb. N., sp. n., a mycelial and endospore-forming bacterium parasitic in plant-parasitic nematodes. Proceedings of the Helminthological Society of Washington 52:149-165.
- Sayre, R. M. and M. P. Starr. 1988. Bacterial diseases and antagonisms of nematodes, pp. 69-101; IN: G. O. Poinar, Jr., and H.-B. Jansson (Eds.) Diseases of Nematodes. CRC Press, Boca Raton, Florida.
- Sayre, R. M., W. P. Wergin, T. Nishizawa, and M. P. Starr. 1991. Light and electron microscopical study of a bacterial parasite from the cyst nematode, *Heterodera glycines*. Journal Helmenthol. Soc. Wash. 58(1):69-81.
- Schact, H. 1859a. Ueber einige Feinde der Rübenfelder. Ztschr. Ver. Rübenzucker-Ind. Zollver. 9:175-179.
- Schact, H. 1859b. Ueber einige feinde and Krankheiten der Zucherrube. Ztschr. Ver. Rübenzucker-Ind. Zollver. 9:239-250.
- Shaw, H. B. 1915. The sugarbeet nematode and its control. Sugar Chicago, vol 17.

- Steele, A. E. 1965. The host range of the sugar beet nematode *Heterodera schachtii* Schmidt. *Journal Am. Soc. Sugar Beet Technol.* 30:573-603.
- Steele, A. E. 1984. Chapter 14: Nematode parasites of sugarbeet IN: Nickle, W.R. (Ed.) *Plant and Insect Nematodes*. Marcel Dekker, Inc., New York. 925 pp.
- Stirling, G. R. 1991. Biological control of plant parasitic nematodes: Progress, problems and prospects. Wallingford, UK: CAB International.
- Thorne, G. 1961. *Principles of Nematology*. McGraw-Hill Book Co. N.Y. 553 pp.
- Tiffit, M. J. 1935. The origin of strains of *Heterodera schachtii* occurring in Britain, with special reference to the beet-strain. *Journal Helm.* 13:149-158.
- Weischer, B. and W. Steudel. 1972. Chapter 3: Nematode diseases of sugarbeet IN: Webster J. M. (Ed.) *Economic Nematology*. Academic Press, London. 563pp.
- Wyss, U., C. Stender, and H. Lehmann. 1984. Ultrastructure of feeding sites of the cyst nematode *Heterodera schachtii* Schmidt in roots of susceptible and resistant *Raphanus sativus* L. var *oleiformis* Pers. cultivars. *Physiological Plant Pathology* 25:21-37.

## **APPENDICES**

## Appendix A

### 1998 SUGARBEET CYST NEMATODE SURVEY

*Michigan Sugar Company, Monitor Sugar Company and Michigan State University Cooperating*

#### Site Information

Grower Name: \_\_\_\_\_ 1997 Crop: \_\_\_\_\_  
Address: \_\_\_\_\_ 1996 Crop: \_\_\_\_\_  
County: \_\_\_\_\_ Township: \_\_\_\_\_ 1995 Crop: \_\_\_\_\_  
Section: \_\_\_\_\_ Field ID: \_\_\_\_\_ Number of sugarbeet  
Soil Texture \_\_\_\_\_ crops since 1984 \_\_\_\_\_

#### Sample Method Used (circle method)

Stratified (No. 1)

Random (No. 2)

Fieldman Name: \_\_\_\_\_

Company: \_\_\_\_\_

#### Symptoms-Signs Observed (check symptom-sign)

☐ Poor sugarbeet emergence or stand.  
☐ Stunted or off-color shoot system.  
☐ Underdeveloped or deformed taproots.  
☐ Spots of wilted plants.  
☐ Hairy-roots  
☐ Low sugarbeet yield  
☐ Sugarbeet cyst females on root tissue  
☐ None

#### Nematode Sample Results

100 cm<sup>3</sup> soil

1.0 g root tissue

Sugarbeet cyst nematode cysts

\_\_\_\_\_

\_\_\_\_\_

Sugarbeet cyst nematode eggs and juveniles

\_\_\_\_\_

\_\_\_\_\_

Sugarbeet cyst nematode males

\_\_\_\_\_

\_\_\_\_\_

Other nematodes \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Comments (Fieldman or MSU Nematode Diagnostician)

Appendix B. 1998 *Heterodera* spp. Survey Data

| No. | Company | County   | CountyNo | Twp.        | Sect. | No. Crops | Method     | Cysts | Lesion Pin | Spiral | Ring | Stunt | Other |
|-----|---------|----------|----------|-------------|-------|-----------|------------|-------|------------|--------|------|-------|-------|
| 1   | Monitor | Bay      | 2        | Hampton     | 22    | 4         | Random     | 4470  | 0          | 11     | 0    | 0     | 0     |
| 2   | Monitor | Bay      | 2        | Hampton     | 16    | 3         | Stratified | 14277 | 0          | 4      | 7    | 0     | 0     |
| 3   | Monitor | Tuscola  | 13       | Wisner      | 27    | 4         | Stratified | 9280  | 0          | 0      | 0    | 0     | 0     |
| 4   | Monitor | Bay      | 2        | Hampton     | 22    | 4         | Stratified | 4000  | 0          | 5      | 0    | 0     | 0     |
| 5   | Monitor | Arenac   | 1        | Turner      | 26    | 4         | Stratified | 12    | 0          | 250    | 0    | 0     | 0     |
| 6   | Monitor | Arenac   | 1        | Turner      | 26    | 4         | Stratified | 0     | 0          | 7      | 32   | 0     | 0     |
| 7   | Monitor | Arenac   | 1        | Turner      | 14    | 5         | Stratified | 110   | 0          | 11     | 24   | 21    | 0     |
| 8   | Monitor | Arenac   | 1        | Turner      | 11    | 4         | Stratified | 3     | 0          | 36     | 0    | 0     | 0     |
| 9   | Monitor | Arenac   | 1        | Turner      | 11    | 4         | Stratified | 45    | 0          | 0      | 0    | 0     | 0     |
| 10  | Monitor | Arenac   | 1        | Turner      | 10    | 5         | Random     | 0     | 0          | 50     | 0    | 0     | 0     |
| 11  | Monitor | Bay      | 2        | KawKawlin   | 28    | 4         | Stratified | 0     | 1          | 12     | 3    | 0     | 0     |
| 12  | Monitor | Bay      | 2        | KawKawlin   | 34    | 3         | Stratified | 0     | 0          | 0      | 16   | 0     | 0     |
| 13  | Monitor | Bay      | 2        | Monitor     | 11    | 4         | Random     | 86    | 0          | 3      | 15   | 0     | 0     |
| 14  | Monitor | Bay      | 2        | Monitor     | 6     | 3         | Stratified | 242   | 0          | 27     | 28   | 0     | 0     |
| 15  | Monitor | Bay      | 2        | KawKawlin   | 32    | 4         | Stratified | 0     | 0          | 0      | 0    | 0     | 0     |
| 16  | Monitor | Bay      | 2        | Monitor     | 5     | 3         | Random     | 400   | 0          | 0      | 0    | 0     | 0     |
| 17  | Monitor | Bay      | 2        | KawKawlin   | 32    | 4         | Random     | 1     | 0          | 0      | 0    | 0     | 0     |
| 18  | Monitor | Bay      | 2        | KawKawlin   | 28    | 3         | Stratified | 2280  | 0          | 17     | 0    | 0     | 0     |
| 19  | Monitor | Bay      | 2        | KawKawlin   | 27    | 4         | Stratified | 1280  | 0          | 75     | 0    | 0     | 0     |
| 20  | Monitor | Bay      | 2        | KawKawlin   | 22    | 4         | Random     | 180   | 0          | 0      | 0    | 0     | 0     |
| 21  | Monitor | Arenac   | 1        | Turner      | 9     | 4         | Random     | 0     | 0          | 3      | 2    | 0     | 4     |
| 22  | Monitor | Gratiot  | 3        | North Shade | 9     | 2         | Stratified | 0     | 2          | 31     | 0    | 0     | 2     |
| 23  | Monitor | Gratiot  | 3        | New Haven   | 28    | 2         | Stratified | 0     | 0          | 61     | 18   | 0     | 8     |
| 24  | Monitor | Gratiot  | 3        | Hamilton    | 6     | 2         | Stratified | 0     | 6          | 511    | 4    | 0     | 0     |
| 25  | Monitor | Gratiot  | 3        | North Star  | 12    | 2         | Stratified | 0     | 0          | 30     | 0    | 0     | 0     |
| 26  | Monitor | Montcalm | 8        | Bloomer     | 20    | 2         | Random     | 0     | 0          | 0      | 0    | 2     | 1     |
| 27  | Monitor | Montcalm | 8        | Bloomer     | 20    | 2         | Random     | 0     | 4          | 1      | 0    | 0     | 1     |
| 28  | Monitor | Montcalm | 8        | Crystal     | 34    | 1         | Stratified | 0     | 0          | 3      | 48   | 0     | 0     |
| 29  | Monitor | Gratiot  | 3        | North Star  | 9     | 2         | Stratified | 0     | 0          | 3      | 1    | 0     | 0     |
| 30  | Monitor | Mecosta  | 7        | Hinton      | 11    | 0         | Stratified | 0     | 1          | 0      | 0    | 0     | 0     |

Appendix B. 1998 *Heterodera* spp. Survey Data

| No. | Company | County   | CountyNo | Twp.        | Sect. | No. | Crops | Method     | Cysts | Lesion | Pin | Spiral | Ring | Stunt | Other |
|-----|---------|----------|----------|-------------|-------|-----|-------|------------|-------|--------|-----|--------|------|-------|-------|
| 1   | Monitor | Bay      | 2        | Hampton     | 22    |     | 4     | Random     | 4470  | 0      | 11  | 0      | 0    | 0     | 0     |
| 2   | Monitor | Bay      | 2        | Hampton     | 16    |     | 3     | Stratified | 14277 | 0      | 4   | 7      | 0    | 0     | 0     |
| 3   | Monitor | Tuscola  | 13       | Wisner      | 27    |     | 4     | Stratified | 9280  | 0      | 0   | 0      | 0    | 0     | 0     |
| 4   | Monitor | Bay      | 2        | Hampton     | 22    |     | 4     | Stratified | 4000  | 0      | 5   | 0      | 0    | 0     | 0     |
| 5   | Monitor | Arenac   | 1        | Turner      | 26    |     | 4     | Stratified | 12    | 0      | 250 | 0      | 0    | 0     | 0     |
| 6   | Monitor | Arenac   | 1        | Turner      | 26    |     | 4     | Stratified | 0     | 0      | 7   | 32     | 0    | 0     | 0     |
| 7   | Monitor | Arenac   | 1        | Turner      | 14    |     | 5     | Stratified | 110   | 0      | 11  | 24     | 21   | 0     | 0     |
| 8   | Monitor | Arenac   | 1        | Turner      | 11    |     | 4     | Stratified | 3     | 0      | 36  | 0      | 0    | 0     | 0     |
| 9   | Monitor | Arenac   | 1        | Turner      | 11    |     | 4     | Stratified | 45    | 0      | 0   | 0      | 0    | 0     | 0     |
| 10  | Monitor | Arenac   | 1        | Turner      | 10    |     | 5     | Random     | 0     | 0      | 50  | 0      | 0    | 0     | 0     |
| 11  | Monitor | Bay      | 2        | KawKawlin   | 28    |     | 4     | Stratified | 0     | 1      | 12  | 3      | 0    | 0     | 0     |
| 12  | Monitor | Bay      | 2        | KawKawlin   | 34    |     | 3     | Stratified | 0     | 0      | 0   | 16     | 0    | 0     | 0     |
| 13  | Monitor | Bay      | 2        | Monitor     | 11    |     | 4     | Random     | 86    | 0      | 3   | 15     | 0    | 0     | 0     |
| 14  | Monitor | Bay      | 2        | Monitor     | 6     |     | 3     | Stratified | 242   | 0      | 27  | 28     | 0    | 0     | 0     |
| 15  | Monitor | Bay      | 2        | KawKawlin   | 32    |     | 4     | Stratified | 0     | 0      | 0   | 0      | 0    | 0     | 0     |
| 16  | Monitor | Bay      | 2        | Monitor     | 5     |     | 3     | Random     | 400   | 0      | 0   | 0      | 0    | 0     | 0     |
| 17  | Monitor | Bay      | 2        | KawKawlin   | 32    |     | 4     | Random     | 1     | 0      | 0   | 0      | 0    | 0     | 0     |
| 18  | Monitor | Bay      | 2        | KawKawlin   | 28    |     | 3     | Stratified | 2280  | 0      | 17  | 0      | 0    | 0     | 0     |
| 19  | Monitor | Bay      | 2        | KawKawlin   | 27    |     | 4     | Stratified | 1280  | 0      | 75  | 0      | 0    | 0     | 0     |
| 20  | Monitor | Bay      | 2        | KawKawlin   | 22    |     | 4     | Random     | 180   | 0      | 0   | 0      | 0    | 0     | 0     |
| 21  | Monitor | Arenac   | 1        | Turner      | 9     |     | 4     | Random     | 0     | 0      | 3   | 2      | 0    | 4     | 0     |
| 22  | Monitor | Gratiot  | 3        | North Shade | 9     |     | 2     | Stratified | 0     | 2      | 31  | 0      | 0    | 2     | 0     |
| 23  | Monitor | Gratiot  | 3        | New Haven   | 28    |     | 2     | Stratified | 0     | 0      | 61  | 18     | 0    | 8     | 0     |
| 24  | Monitor | Gratiot  | 3        | Hamilton    | 6     |     | 2     | Stratified | 0     | 6      | 511 | 4      | 0    | 0     | 0     |
| 25  | Monitor | Gratiot  | 3        | North Star  | 12    |     | 2     | Stratified | 0     | 0      | 30  | 0      | 0    | 0     | 0     |
| 26  | Monitor | Montcalm | 8        | Bloomer     | 20    |     | 2     | Random     | 0     | 0      | 0   | 0      | 0    | 2     | 1     |
| 27  | Monitor | Montcalm | 8        | Bloomer     | 20    |     | 2     | Random     | 0     | 4      | 1   | 0      | 0    | 1     | 0     |
| 28  | Monitor | Montcalm | 8        | Crystal     | 34    |     | 1     | Stratified | 0     | 0      | 3   | 48     | 0    | 0     | 1     |
| 29  | Monitor | Gratiot  | 3        | North Star  | 9     |     | 2     | Stratified | 0     | 0      | 3   | 1      | 0    | 0     | 0     |
| 30  | Monitor | Mecosta  | 7        | Hinton      | 11    |     | 0     | Stratified | 0     | 1      | 0   | 0      | 0    | 0     | 0     |

| No. | Company | County   | CountyNo | Twp.           | Sect. | No. | Crops | Method     | Cysts | Lesion Pin | Spiral | Ring | Stunt | Other |
|-----|---------|----------|----------|----------------|-------|-----|-------|------------|-------|------------|--------|------|-------|-------|
| 31  | Monitor | Bay      |          | 2 Fraser       | 28    |     | 3     | Random     | 0     | 0          | 55     | 0    | 0     | 0     |
| 32  | Monitor | Bay      |          | 2 Fraser       | 28    |     | 4     | Stratified | 0     | 0          | 106    | 0    | 3     | 0     |
| 33  | Monitor | Bay      |          | 2 Fraser       | 18    |     | 4     | Random     | 0     | 2          | 18     | 8    | 0     | 0     |
| 34  | Monitor | Bay      |          | 2 Garfield     | 13    |     | 5     | Random     | 0     | 0          | 19     | 0    | 0     | 0     |
| 35  | Monitor | Bay      |          | 2 Garfield     | 28    |     | 3     | Stratified | 0     | 0          | 320    | 10   | 41    | 0     |
| 36  | Monitor | Bay      |          | 2 Beaver       | 15    |     | 5     | Stratified | 0     | 4          | 50     | 62   | 11    | 0     |
| 37  | Monitor | Bay      |          | 2 Beaver       | 8     |     | 4     | Random     | 0     | 0          | 0      | 22   | 0     | 0     |
| 38  | Monitor | Bay      |          | 2 Beaver       | 26    |     | 5     | Stratified | 0     | 0          | 0      | 6    | 0     | 0     |
| 39  | Monitor | Isabella |          | 5 Union        | 24    |     | 5     | Random     | 0     | 0          | 45     | 8    | 0     | 0     |
| 40  | Monitor | Bay      |          | 2 KawKawlin    | 7     |     | 5     | Stratified | 0     | 0          | 35     | 2    | 9     | 4     |
| 41  | Monitor | Arenac   |          | 1 Lincoln      | 30    |     | 4     | Random     | 0     | 0          | 12     | 0    | 0     | 0     |
| 42  | Monitor | Arenac   |          | 1              | 7     |     | 4     | Random     | 0     | 2          | 15     | 80   | 0     | 0     |
| 43  | Monitor | Bay      |          | 2 Gibson       | 12    |     | 4     | Random     | 0     | 0          | 0      | 275  | 0     | 0     |
| 44  | Monitor | Gratiot  |          | 3 Emerson      | 12    |     | 5     | Random     | 0     | 0          | 0      | 0    | 0     | 0     |
| 45  | Monitor | Gratiot  |          | 3 Lafayette    | 5     |     | 2     | Stratified | 0     | 0          | 0      | 0    | 0     | 0     |
| 46  | Monitor | Gratiot  |          | 3 Bethany      | 32    |     | 5     | Random     | 0     | 28         | 0      | 0    | 0     | 0     |
| 47  | Monitor | Gratiot  |          | 3 Bethany      | 15    |     | 2     | Random     | 0     | 0          | 0      | 0    | 0     | 15    |
| 48  | Monitor | Gratiot  |          | 3 Wheeler      | 17    |     | 3     | Stratified | 0     | 0          | 0      | 0    | 15    | 0     |
| 49  | Monitor | Bay      |          | 2 Merritt      | 21    |     | 3     | Stratified | 0     | 142        | 0      | 0    | 0     | 11    |
| 50  | Monitor | Bay      |          | 2 Hampton      | 25    |     | 2     | Stratified | 1875  | 0          | 1      | 20   | 0     | 0     |
| 51  | Monitor | Bay      |          | 2 Portsmouth   | 14    |     | 3     | Stratified | 5150  | 0          | 160    | 55   | 0     | 0     |
| 52  | Monitor | Bay      |          | 2 Portsmouth   | 14    |     | 4     | Stratified | 6724  | 0          | 3      | 30   | 0     | 0     |
| 53  | Monitor | Bay      |          | 2 Portsmouth   | 12    |     | 5     | Stratified | 4360  | 0          | 1      | 5    | 0     | 0     |
| 54  | Monitor | Saginaw  |          | 10 Buena Vista | 25    |     | 4     | Random     | 9680  | 0          | 40     | 0    | 0     | 0     |
| 55  | Monitor | Bay      |          | 2 Merritt      | 2     |     | 4     | Random     | 11    | 17         | 0      | 8    | 0     | 0     |
| 56  | Monitor | Saginaw  |          | 10 Buena Vista | 12    |     | 2     | Random     | 1     | 0          | 0      | 0    | 0     | 0     |
| 57  | Monitor | Bay      |          | 2 Portsmouth   | 15    |     | 2     | Random     | 1     | 0          | 0      | 0    | 0     | 0     |
| 58  | Monitor | Bay      |          | 2 Merritt      | 5     |     | 5     | Random     | 23    | 0          | 0      | 0    | 0     | 0     |
| 59  | Monitor | Bay      |          | 2 Monitor      | 36    |     | 6     | Random     | 830   | 0          | 0      | 0    | 0     | 0     |
| 60  | Monitor | Bay      |          | 2 Frankenlust  | 12    |     | 4     | Stratified | 12620 | 0          | 0      | 0    | 0     | 0     |
| 61  | Monitor | Bay      |          | 2 Frankenlust  | 7     |     | 2     | Random     | 1540  | 2          | 3      | 0    | 0     | 0     |
| 62  | Monitor | Bay      |          | 2 Frankenlust  | 7     |     | 1     | Stratified | 7820  | 0          | 0      | 0    | 0     | 0     |

| No. | Company | County  | CountyNo | Twp.             | Sect. No. | Crops | Method       | Cysts | Lesion Pin | Spiral | Ring | Stunt | Other |
|-----|---------|---------|----------|------------------|-----------|-------|--------------|-------|------------|--------|------|-------|-------|
| 63  | Monitor | Bay     |          | 2 Frankenlust    | 5         |       | 4 Stratified | 702   | 0          | 0      | 2    | 0     | 0     |
| 64  | Monitor | Bay     |          | 2 Frankenlust    | 1         |       | 5 Random     | 11    | 0          | 5      | 0    | 0     | 0     |
| 65  | Monitor | Bay     |          | 2 Frankenlust    | 5         |       | 4 Random     | 745   | 17         | 70     | 20   | 0     | 0     |
| 66  | Monitor | Bay     |          | 2 Frankenlust    | 5         |       | 4 Stratified | 4575  | 0          | 0      | 6    | 0     | 0     |
| 67  | Monitor | Bay     |          | 2 Monitor        | 36        |       | 4 Random     | 299   | 0          | 20     | 0    | 0     | 0     |
| 68  | Monitor | Bay     |          | 2 Frankenlust    | 12        |       | 3 Stratified | 1041  | 0          | 0      | 0    | 0     | 2     |
| 69  | MI      | Sanilac |          | 11 Wheatland     | 12        |       | 5 Random     | 12718 | 0          | 0      | 0    | 0     | 0     |
| 70  | MI      | Sanilac |          | 11 Wheatland     | 1         |       | 1 Random     | 8     | 10         | 0      | 32   | 6     | 0     |
| 71  | MI      | Sanilac |          | 11 Wheatland     | 1         |       | 3 Stratified | 0     | 2          | 9      | 84   | 0     | 0     |
| 72  | MI      | Sanilac |          | 11 Marion        | 6         |       | 3 Random     | 6     | 0          | 16     | 4    | 0     | 0     |
| 73  | MI      | Sanilac |          | 11 Custer        | 2         |       | 4 Stratified | 0     | 0          | 0      | 50   | 0     | 0     |
| 74  | MI      | Sanilac |          | 11 Bridgehampton | 1         |       | 5 Stratified | 0     | 0          | 0      | 14   | 4     | 68    |
| 75  | MI      | Sanilac |          | 11 Custer        | 18        |       | 1 Random     | 0     | 32         | 0      | 4    | 274   | 0     |
| 76  | MI      | Sanilac |          | 11 Custer        | 24        |       | 5 Stratified | 10760 | 0          | 18     | 244  | 0     | 0     |
| 77  | MI      | Sanilac |          | 11 Bridgehampton | 7         |       | 2 Stratified | 120   | 0          | 10     | 78   | 0     | 0     |
| 78  | MI      | Huron   |          | 4 Bloomfield     | 15        |       | 3 Stratified | 120   | 0          | 10     | 26   | 0     | 4     |
| 79  | MI      | Huron   |          | 4 Bloomfield     | 3         |       | 2 Stratified | 0     | 4          | 36     | 152  | 0     | 0     |
| 80  | MI      | Huron   |          | 4 Meade          | 1         |       | 4 Stratified | 0     | 4          | 78     | 120  | 0     | 0     |
| 81  | MI      | Huron   |          | 4 Bloomfield     | 17        |       | 2 Stratified | 0     | 0          | 24     | 38   | 0     | 0     |
| 82  | MI      | Huron   |          | 4 Bloomfield     | 10        |       | 3 Stratified | 0     | 0          | 4      | 0    | 0     | 0     |
| 83  | MI      | Huron   |          | 4 Bloomfield     | 11        |       | 0 Random     | 0     | 0          | 0      | 2    | 0     | 0     |
| 84  | MI      | Huron   |          | 4 Lincoln        | 27        |       | 1 Random     | 2     | 0          | 0      | 0    | 0     | 6     |
| 85  | MI      | Huron   |          | 4 Lincoln        | 30        |       | 0 Random     | 0     | 0          | 0      | 2    | 0     | 0     |
| 86  | MI      | Huron   |          | 4 Bloomfield     | 4         |       | 0 Random     | 0     | 6          | 2      | 120  | 0     | 0     |
| 87  | MI      | Huron   |          | 4 Lincoln        | 7         |       | 2 Random     | 0     | 0          | 2      | 0    | 0     | 0     |
| 88  | MI      | Huron   |          | 4 Brookfield     | 18        |       | 4 Stratified | 710   | 0          | 10     | 18   | 0     | 0     |
| 89  | MI      | Huron   |          | 4 Fairhaven      | 27        |       | 4 Stratified | 18    | 0          | 50     | 254  | 0     | 0     |
| 90  | MI      | Tuscola |          | 13 S. Akron      | 7         |       | 4 Random     | 0     | 10         | 0      | 12   | 0     | 0     |
| 91  | MI      | Huron   |          | 4 Fairhaven      | 34        |       | 5 Random     | 1060  | 0          | 2      | 6    | 0     | 0     |
| 92  | MI      | Tuscola |          | 13 N. Akron      | 33        |       | 3 Random     | 940   | 0          | 0      | 0    | 0     | 16    |
| 93  | MI      | Tuscola |          | 13 S. Akron      | 2         |       | 4 Random     | 72    | 0          | 0      | 4    | 0     | 2     |
| 94  | MI      | Tuscola |          | 13 S. Akron      | 20        |       | 4 Random     | 11310 | 0          | 0      | 0    | 0     | 2     |

| No.    | Company | County    | CountyNo       | Twp. | Sect. | No. Crops  | Method | Cysts | Lesion Pin | Spiral | Ring | Stunt | Other |
|--------|---------|-----------|----------------|------|-------|------------|--------|-------|------------|--------|------|-------|-------|
| 95 MI  |         | Tuscola   | 13 S. Akron    | 20   | 4     | Random     |        | 54    | 0          | 0      | 0    | 0     | 0     |
| 96 MI  |         | Huron     | 4 Brookfield   | 18   | 4     | Stratified |        | 440   | 0          | 8      | 12   | 0     | 0     |
| 97 MI  |         | Huron     | 4 Winsor       | 19   | 5     | Stratified |        | 6050  | 0          | 0      | 0    | 0     | 0     |
| 98 MI  |         | Huron     | 4 Sebewaing    | 3    | 4     | Random     |        | 360   | 0          | 8      | 8    | 0     | 0     |
| 99 MI  |         | Tuscola   | 13 N. Akron    | 27   | 4     | Random     |        | 4     | 72         | 0      | 3    | 0     | 0     |
| 100 MI |         | Tuscola   | 13 Wisner      | 7    | 4     | Random     |        | 16    | 0          | 2      | 6    | 0     | 0     |
| 101 MI |         | Tuscola   | 13 N. Akron    | 26   | 4     | Random     |        | 0     | 8          | 14     | 74   | 0     | 0     |
| 102 MI |         | Tuscola   | 13 N. Akron    | 31   | 4     | Random     |        | 6305  | 0          | 0      | 2    | 0     | 0     |
| 103 MI |         | Huron     | 4 Sebewaing    | 3    | 5     | Random     |        | 58    | 0          | 0      | 0    | 0     | 0     |
| 104 MI |         | Huron     | 4 Sebewaing    | 4    | 4     | Random     |        | 470   | 0          | 0      | 2    | 0     | 0     |
| 105 MI |         | Tuscola   | 13 N. Akron    | 28   | 3     | Random     |        | 30880 | 0          | 0      | 6    | 0     | 0     |
| 106 MI |         | Huron     | 4 Brookfield   | 7    | 4     | Stratified |        | 29800 | 0          | 0      | 42   | 0     | 0     |
| 107 MI |         | Saginaw   | 10 Spaulding   | 36   | 4     | Stratified |        | 1036  | 0          | 2      | 6    | 0     | 0     |
| 108 MI |         | Saginaw   | 10 Taymouth    | 7    | 5     | Random     |        | 420   | 2          | 6      | 6    | 0     | 0     |
| 109 MI |         | Saginaw   | 10 Albee       | 8    | 5     | Random     |        | 1446  | 2          | 2      | 10   | 0     | 4     |
| 110 MI |         | Saginaw   | 10 Albee       | 8,9  | 5     | Random     |        | 2725  | 4          | 0      | 14   | 0     | 0     |
| 111 MI |         | Saginaw   | 10 St. Charles | 1    | 5     | Stratified |        | 1582  | 0          | 0      | 2    | 0     | 0     |
| 112 MI |         | Saginaw   | 10 Spaulding   | 31   | 5     | Random     |        | 4     | 2          | 15     | 328  | 0     | 0     |
| 113 MI |         | Saginaw   | 10 Albee       | 5    | 5     | Random     |        | 18    | 10         | 56     | 468  | 0     | 0     |
| 114 MI |         | Saginaw   | 10 Spaulding   | 28   | 2     | Stratified |        | 460   | 28         | 0      | 28   | 0     | 0     |
| 115 MI |         | Sanilac   | 11 Buel        | 31   | 5     | Stratified |        | 0     | 20         | 0      | 2    | 0     | 0     |
| 116 MI |         | Sanilac   | 11 Buel        | 27   | 4     | Random     |        | 0     | 0          | 0      | 0    | 0     | 0     |
| 117 MI |         | Sanilac   | 11 Lexington   | 5    | 2     | Stratified |        | 0     | 106        | 2      | 4    | 0     | 0     |
| 118 MI |         | Sanilac   | 11 Buel        | 5    | 5     | Stratified |        | 0     | 64         | 4      | 0    | 0     | 0     |
| 119 MI |         | St. Clair | 12 Clyde       | 1    | 5     | Random     |        | 0     | 2          | 0      | 8    | 0     | 0     |
| 120 MI |         | Sanilac   | 11 Watertown   | 9    | 2     | Random     |        | 0     | 20         | 10     | 0    | 0     | 0     |
| 121 MI |         | Sanilac   | 11 Custer      | 34   | 5     | Random     |        | 0     | 0          | 0      | 0    | 0     | 0     |
| 122 MI |         | Sanilac   | 11 Speaker     | 29   | 1     | Random     |        | 0     | 4          | 0      | 8    | 0     | 0     |
| 123 MI |         | Sanilac   | 11 Worth       | 9    | 1     | Stratified |        | 6     | 4          | 2      | 2    | 0     | 0     |
| 124 MI |         | Sanilac   | 11 Buel        | 27   | 3     | Stratified |        | 0     | 0          | 0      | 0    | 0     | 0     |
| 125 MI |         | Huron     | 4 Fairhaven    | 34   | 5     | Random     |        | 6     | 0          | 0      | 0    | 0     | 0     |
| 126 MI |         | Gratiot   | 3 Wheeler      | 20   | 1     | Stratified |        | 6     | 0          | 0      | 14   | 0     | 0     |

| No.    | Company | County  | CountyNo | Twp.         | Sect. No. | Crops | Method       | Cysts | Lesion Pin | Spiral | Ring | Stunt | Other |
|--------|---------|---------|----------|--------------|-----------|-------|--------------|-------|------------|--------|------|-------|-------|
| 127 MI |         | Gratiot |          | 3 Wheeler    | 9         |       | 3 Stratified | 0     | 6          | 0      | 34   | 0     | 0     |
| 128 MI |         | Gratiot |          | 3 Bethany    | 2         |       | 5 Stratified | 0     | 0          | 8      | 28   | 2     | 0     |
| 129 MI |         | Gratiot |          | 3 Bethany    | 24        |       | 4 Stratified | 0     | 0          | 0      | 60   | 0     | 0     |
| 130 MI |         | Gratiot |          | 3 Bethany    | 22        |       | 4 Stratified | 0     | 84         | 28     | 108  | 0     | 0     |
| 131 MI |         | Gratiot |          | 3 Wheeler    | 33        |       | 4 Random     | 0     | 0          | 14     | 80   | 0     | 0     |
| 132 MI |         | Gratiot |          | 3 Lafayette  | 3         |       | 3 Random     | 0     | 0          | 0      | 0    | 0     | 0     |
| 133 MI |         | Gratiot |          | 3 Lafayette  | 27        |       | 5 Random     | 0     | 2          | 8      | 14   | 0     | 0     |
| 134 MI |         | Gratiot |          | 3 Lafayette  | 28        |       | 4 Random     | 2     | 0          | 2      | 4    | 0     | 0     |
| 135 MI |         | Gratiot |          | 3 Lafayette  | 5         |       | 3 Random     | 0     | 2          | 8      | 54   | 0     | 0     |
| 136 MI |         | Lenawee |          | 6 Riga       |           |       | 0 Stratified | 0     | 0          | 0      | 0    | 0     | 0     |
| 137 MI |         | Lenawee |          | 6 Riga       |           |       | 1 Random     | 0     | 6          | 16     | 86   | 0     | 0     |
| 138 MI |         | Lenawee |          | 6 Blissfield |           |       | 3 Stratified | 0     | 158        | 2      | 34   | 0     | 0     |
| 139 MI |         | Lenawee |          | 6 Blissfield |           |       | 2 Random     | 0     | 70         | 10     | 206  | 0     | 4     |
| 140 MI |         | Lenawee |          | 6 Palmyra    |           |       | 4 Stratified | 0     | 200        | 0      | 94   | 0     | 0     |
| 141 MI |         | Lenawee |          | 6 Blissfield |           |       | 0 Random     | 0     | 4          | 0      | 122  | 0     | 0     |
| 142 MI |         | Lenawee |          | 6 Riga       |           |       | 3 Random     | 0     | 6          | 0      | 146  | 0     | 2     |
| 143 MI |         | Lenawee |          | 6 Palmyra    |           |       | 2 Random     | 0     | 0          | 6      | 142  | 0     | 0     |
| 144 MI |         | Lenawee |          | 6 Palmyra    |           |       | 4 Stratified | 2     | 0          | 0      | 48   | 0     | 0     |
| 145 MI |         | Lenawee |          | 6 Riga       |           |       | 3 Random     | 0     | 0          | 0      | 0    | 0     | 0     |
| 146 MI |         | Tuscola |          | 13 Denmark   | 2         |       | 4 Random     | 0     | 0          | 0      | 12   | 0     | 0     |
| 147 MI |         | Bay     |          | 2 Merit      | 14        |       | 4 Stratified | 5628  | 2          | 2      | 18   | 0     | 0     |
| 148 MI |         | Tuscola |          | 13 Akron     | 32        |       | 2 Random     | 20    | 0          | 0      | 0    | 0     | 0     |
| 149 MI |         | Bay     |          | 2 Merit      | 36        |       | 3 Stratified | 790   | 4          | 2      | 0    | 0     | 0     |
| 150 MI |         | Bay     |          | 2 Merit      | 11        |       | 4 Stratified | 20    | 20         | 0      | 64   | 0     | 0     |
| 151 MI |         | Tuscola |          | 13 Akron     | 32        |       | 2 Random     | 0     | 0          | 0      | 0    | 0     | 0     |
| 152 MI |         | Tuscola |          | 13 Akron     | 32        |       | 2 Random     | 0     | 0          | 0      | 2    | 0     | 0     |
| 153 MI |         | Tuscola |          | 13 Akron     | 32        |       | 2 Random     | 0     | 0          | 0      | 0    | 0     | 0     |
| 154 MI |         | Tuscola |          | 13 Tuscola   | 17        |       | 3 Random     | 0     | 0          | 8      | 6    | 0     | 0     |
| 155 MI |         | Tuscola |          | 13 Tuscola   | 3         |       | 4 Random     | 17949 | 0          | 0      | 0    | 0     | 0     |
| 156 MI |         | Tuscola |          | 13 Fairgrove | 28        |       | 3 Stratified | 114   | 0          | 0      | 0    | 0     | 0     |
| 157 MI |         | Bay     |          | 2 Merit      | 25        |       | 1 Stratified | 0     | 2          | 10     | 20   | 0     | 0     |
| 158 MI |         | Tuscola |          | 13 Tuscola   | 29        |       | 4 Random     | 0     | 88         | 8      | 18   | 0     | 0     |

| No.    | Company | County  | CountyNo | Twp.        | Sect. | No. Crops | Method     | Cysts | Lesion Pin | Spiral | Ring | Stunt | Other |
|--------|---------|---------|----------|-------------|-------|-----------|------------|-------|------------|--------|------|-------|-------|
| 159 MI | Tuscola | Tuscola | 13       | Tuscola     | 28    | 2         | Random     | 0     | 10         | 0      | 0    | 6     | 0     |
| 160 MI | Tuscola | Tuscola | 13       | Alner       | 13    | 4         | Stratified | 6     | 6          | 2      | 16   | 0     | 0     |
| 161 MI | Sanilac | Sanilac | 11       | Marion      | 17    | 1         | Random     | 4     | 0          | 2      | 0    | 0     | 0     |
| 162 MI | Tuscola | Tuscola | 13       | Ellington   | 10    | 4         | Stratified | 16754 | 18         | 0      | 10   | 0     | 0     |
| 163 MI | Tuscola | Tuscola | 13       | Elkland     | 29    | 3         | Random     | 266   | 4          | 2      | 16   | 0     | 0     |
| 164 MI | Tuscola | Tuscola | 13       | Ellington   | 11    | 4         | Stratified | 4     | 0          | 2      | 22   | 0     | 0     |
| 165 MI | Tuscola | Tuscola | 13       | Almer       | 24    | 3         | Random     | 0     | 3          | 0      | 34   | 0     | 0     |
| 166 MI | Tuscola | Tuscola | 13       | Elmwood     | 16    | 4         | Random     | 0     | 2          | 8      | 4    | 0     | 0     |
| 167 MI | Tuscola | Tuscola | 13       | Columbia    | 33    | 4         | Stratified | 0     | 0          | 6      | 6    | 0     | 0     |
| 168 MI | Tuscola | Tuscola | 13       | Elmwood     | 16    | 4         | Stratified | 2     | 0          | 0      | 0    | 0     | 0     |
| 169 MI | Tuscola | Tuscola | 13       | Elmwood     | 19    | 4         | Random     | 0     | 6          | 10     | 64   | 0     | 0     |
| 170 MI | Tuscola | Tuscola | 13       | Columbia    | 14    | 4         | Random     | 0     | 2          | 2      | 8    | 0     | 0     |
| 171 MI | Huron   | Huron   | 4        | Winsor      | 2     | 4         | Random     | 10300 | 0          | 0      | 6    | 0     | 0     |
| 172 MI | Huron   | Huron   | 4        | Fairhaven   | 24    | 5         | Stratified | 25552 | 0          | 10     | 476  | 0     | 0     |
| 173 MI | Huron   | Huron   | 4        | McKinley    | 34    | 5         | Stratified | 148   | 3          | 8      | 6    | 0     | 0     |
| 174 MI | Huron   | Huron   | 4        | Caseville   | 2     | 4         | Stratified | 340   | 0          | 0      | 16   | 0     | 0     |
| 175 MI | Huron   | Huron   | 4        | McKinley    | 27    | 5         | Random     | 443   | 2          | 4      | 22   | 0     | 0     |
| 176 MI | Huron   | Huron   | 4        | Chandler    | 9     | 5         | Random     | 0     | 0          | 4      | 0    | 0     | 0     |
| 177 MI | Huron   | Huron   | 4        | Fairhaven   | 13    | 5         | Stratified | 23435 | 6          | 0      | 20   | 0     | 0     |
| 178 MI | Huron   | Huron   | 4        | Chandler    | 3     | 3         | Random     | 378   | 0          | 10     | 14   | 0     | 0     |
| 179 MI | Huron   | Huron   | 4        | Winsor      | 18    | 5         | Stratified | 49352 | 4          | 0      | 8    | 0     | 0     |
| 180 MI | Huron   | Huron   | 4        | McKinley    | 15    | 1         | Random     | 572   | 2          | 0      | 10   | 0     | 0     |
| 181 MI | Saginaw | Saginaw | 10       | Buena Vista | 26    | 4         | Random     | 470   | 0          | 0      | 62   | 0     | 0     |
| 182 MI | Saginaw | Saginaw | 10       | Bloomfield  | 34    | 4         | Stratified | 492   | 2          | 0      | 2    | 0     | 0     |
| 183 MI | Saginaw | Saginaw | 10       | Taymouth    | 27    | 3         | Stratified | 192   | 2          | 2      | 0    | 0     | 0     |
| 184 MI | Bay     | Bay     | 2        | Frankenlost | 16,1  | 4         | Stratified | 1688  | 0          | 0      | 4    | 0     | 0     |
| 185 MI | Saginaw | Saginaw | 10       | Ziwaukeee   | 21    | 4         | Stratified | 94    | 0          | 0      | 0    | 0     | 0     |
| 186 MI | Saginaw | Saginaw | 10       | Frankenmuth | 18    | 4         | Random     | 0     | 6          | 2      | 0    | 0     | 0     |
| 187 MI | Saginaw | Saginaw | 10       | Bloomfield  | 27    | 4         | Random     | 0     | 0          | 0      | 20   | 0     | 0     |
| 188 MI | Saginaw | Saginaw | 10       | Bridgeport  | 1     | 3         | Random     | 0     | 0          | 4      | 72   | 0     | 0     |
| 189 MI | Saginaw | Saginaw | 10       | Buena Vista | 24    | 1         | Stratified | 12668 | 2          | 2      | 40   | 0     | 0     |
| 190 MI | Saginaw | Saginaw | 10       | Frankenmuth | 14    | 4         | Random     | 206   | 0          | 0      | 16   | 0     | 0     |

| No. | Company | County  | CountyNo | Twp.           | Sect. No. | Crops | Method       | Cysts | Lesion Pin | Spiral | Ring | Stunt | Other |
|-----|---------|---------|----------|----------------|-----------|-------|--------------|-------|------------|--------|------|-------|-------|
| 191 | Monitor | Bay     |          | 2 Hampton      |           |       | 3 Random     | 622   | 2          | 6      | 24   | 0     | 0     |
| 192 | Monitor | Tuscola |          | 13 Akron       | 11        |       | 4 Stratified | 0     | 54         | 0      | 4    | 0     | 0     |
| 193 | Monitor | Tuscola |          | 13 Akron       | 11        |       | 4 Random     | 0     | 0          | 6      | 6    | 0     | 0     |
| 194 | Monitor | Newaygo |          | 9 Sheridan     | 25        |       | 0 Stratified | 4     | 2          | 0      | 0    | 0     | 0     |
| 195 | Monitor | Sanilac |          | 11 Custer      | 8         |       | 6 Random     | 114   | 0          | 18     | 86   | 0     | 0     |
| 196 | Monitor | Sanilac |          | 11 Marion      | 21        |       | 4 Random     | 0     | 8          | 0      | 0    | 0     | 0     |
| 197 | Monitor | Sanilac |          | 11 Argyle      | 24        |       | 5 Stratified | 0     | 8          | 126    | 210  | 0     | 0     |
| 198 | Monitor | Sanilac |          | 11 Marion      | 35        |       | 5 Random     | 0     | 0          | 2      | 6    | 0     | 0     |
| 199 | Monitor | Bay     |          | 2 Merritt      | 30        |       | 3 Stratified | 2     | 0          | 4      | 54   | 0     | 0     |
| 200 | Monitor | Saginaw |          | 10 Bloomfield  | 26        |       | 3 Stratified | 10    | 0          | 0      | 4    | 0     | 0     |
| 201 | Monitor | Bay     |          | 2 Merritt      | 18        |       | 4 Stratified | 6     | 0          | 0      | 0    | 0     | 0     |
| 202 | Monitor | Saginaw |          | 10 Buena Vista | 12        |       | 4 Stratified | 6     | 0          | 0      | 2    | 0     | 0     |
| 203 | Monitor | Bay     |          | 2 Merritt      | 20        |       | 4 Random     | 10    | 0          | 0      | 0    | 0     | 0     |
| 204 | Monitor | Tuscola |          | 13 Denmark     | 2         |       | 3 Stratified | 0     | 0          | 2      | 12   | 0     | 0     |
| 205 | Monitor | Saginaw |          | 10 Bloomfield  | 7         |       | 5 Random     | 114   | 2          | 0      | 0    | 0     | 0     |
| 206 | Monitor | Tuscola |          | 13 Tuscola     | 4         |       | 4 Stratified | 2     | 0          | 2      | 2    | 0     | 0     |
| 207 | Monitor | Saginaw |          | 10 Bloomfield  | 22        |       | 4 Stratified | 0     | 0          | 0      | 2    | 0     | 0     |
| 208 | Monitor | Bay     |          | 2 Merritt      | 26        |       | 4 Stratified | 7440  | 0          | 0      | 2    | 0     | 0     |
| 209 | Monitor | Sanilac |          | 11 Wheatland   | 28        |       | 5 Stratified | 68    | 2          | 16     | 2    | 0     | 0     |
| 210 | Monitor | Sanilac |          | 11 Argyle      | 13        |       | 6 Random     | 8     | 0          | 20     | 28   | 0     | 0     |
| 211 | Monitor | Sanilac |          | 11 Wheatland   | 12        |       | 5 Random     | 58    | 0          | 2      | 0    | 0     | 0     |
| 212 | Monitor | Sanilac |          | 11 Custer      | 2         |       | 5 Stratified | 0     | 0          | 2      | 6    | 0     | 0     |
| 213 | Monitor | Sanilac |          | 11 Custer      | 12        |       | 5 Stratified | 114   | 2          | 40     | 46   | 0     | 0     |
| 214 | Monitor | Sanilac |          | 11 Custer      | 12        |       | 4 Stratified | 126   | 2          | 4      | 20   | 0     | 0     |

## **Appendix C.**

### **Preliminary Experiments and Techniques developed for work with *Pasteuria***

Cultures of *Pasteuria penetrans* were obtained from the following researchers:

Dr. D. W. Dickson, Department of Entomology and Nematology at the University of Florida, Gainesville.

Dr. Ken Barker, Department of Plant Pathology at North Carolina State University, Raleigh, North Carolina.

Dr. Greg Noel, Department of Crop Sciences at the University of Illinois, Urbana, Illinois.

### **Enzyme solution for dissolving plant root tissue to release infected females**

5 ml 0.5 M sodium acetate pH 4.5

50  $\mu$ l 1.0 M  $\text{CaCl}_2$

45 ml distilled water

.034 g cellulase

2.5 g pectinase

4 grams root tissue

Solution was added to 4 g of root tissue and place on a mechanical shaker for 24 hours at room temperature. After 24 hours, remove from shaker and wash with a steady stream of water over nested sieves with 710  $\mu$ m and 38  $\mu$ m openings to release and catch infected females from degraded root tissue.

### **Culturing Methods**

Crushed 3 infected females to release spores in a 1.5 ml microfuge tube, then added 1.25 ml of distilled water contained approximately 1500 juveniles of *Meloidogyne arenaria*.

Placed microfuge tube on rotating machine to attach spores to juveniles.

This technique was not successful. The volume was too high and the spore concentration was too low. There needed to be a smaller volume of spores, juveniles and water to ensure a higher rate of attachment. Also, the spore concentration needed to be much higher, but with limited material this was not possible.

### **DNA Extraction Attempts**

Alkaline Lysis Na OH 60 °C for 5 minutes

Microwave spores for 5 minutes

Mortar and Pestle with glass beads

Saline treated microfuge tube containing 50 µl of sterile water and ground up glass beads

Mini beadbeater with glass beads and phenol for 1 minute

All of these techniques were unsuccessful, however the mini beadbeater was the recommended way to break open spores. It is possible that this technique could work with some modifications.

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