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BIONOMICS AND CONTROL OF TWO *HETERODERA* SPP.
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CASSANDRA LEE BATES

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BIONOMICS AND CONTROL OF TWO *HETERODERA* SPP. IN MICHIGAN

By

Cassandra Lee Bates

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Abstract

BIONOMICS AND CONTROL OF
TWO *HETERODERA* SPP. IN MICHIGAN

By

Cassandra Lee Bates

Heterodera glycines (SCN) and *H. schachtii* (SBCN) are common pathogens of soybeans and sugar beets in Michigan, respectively. *Pratylenchus penetrans* (RLN) is another phytopathogenic nematode that parasitizes both soybeans and sugar beets. It co-habits with SCN in *ca* 50% of Michigan soybean fields. SCN resistant cultivars are widely used; however, there are no *SBCN* resistant sugar beet varieties available. Michigan sugar beet growers have adopted oilseed radish (OSR) cvs *Colonel* or *Adagio* as trap crops for control of SBCN. This study evaluated 26 potential trap crops for the control of SCN. Berseem clover, Dackon oilseed radish and Oriental mustard have potential as trap crops for SCN. Another analysis evaluated RLN on SCN resistant soybeans. RLN has the ability to reproduce on all seven plant introduction (PI) SCN resistant soybean lines. The nematode also has the ability to break down resistance of a PI 88788 cultivar. Lastly, field and laboratory evaluation of over 30 sugar beet lines for resistance to SBCN was also conducted. All three USDA lines tested showed resistance. Two commercial lines (Beta 5534N and Beta 5374) showed the most stability for resistance to SBCN in both the field and greenhouse studies.

Dedication

I would like to dedicate this thesis to Philip Bates,
my husband and my best friend. He supported me through this process
and bore all my craziness with love and tenderness.

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Wow, where to even start, so many have helped me during this process. It is tough to decide who should be listed first. So I have decided to thank my furry feline friend, Yoda, first. She was and is always there to support me during the good and bad. Next I would like to thank John Davenport. His guidance and friendship through my time here has been immeasurable. And thanks for never leaving me in a soybean field. Fred Warner is also another who I would like to thank. He is the ultimate nematode guru.

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Family always has to be on this page. So I thank them. Without their prayers and support I probably would have never had made it. My husband is an awesome, Godly man who has been my rock of support throughout this whole

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Introduction

Heterodera glycines (soybean cyst nematode, SCN) and *H. schachtii* (sugar beet cyst nematode, SBCN) are considerable economic pests throughout the world, causing significant damage to their respective hosts.

In the State of Michigan, *H. glycines* and *H. schachtii* are major pests of soybeans and sugar beets, respectively. To date, there is no *H. schachtii* resistant sugar beet in commercial production, but there are dozens of *H. glycines* resistant soybean varieties available to Michigan soybean growers. The genetic variability of this resistance, however, is limited (verbal correspondence with Warner, 2006). *Pratylenchus penetrans* (root-lesion nematode, RLN) is another phytopathogenic nematode that is known to parasitize both soybeans and sugar beets. Economic loss due to *P. penetrans* on these crops is still unknown. It is speculated that *P. penetrans* and *H. glycines* interact to cause a decrease in soybean production, but alone, *P. penetrans*, has limited effect upon soybean production (Melakeberhan, 1998; Lawn and Noel, 1986).

The impact of current Michigan soybean-sugar beet management systems in the presence of these three phytopathogenic nematodes is illustrated in Figure 1. Soybean production in Michigan is significantly impacted by *H. glycines* which also increases the risk to Sudden Death Syndrome of Soybean (SDS), which is caused by the fungal pathogen, *Fusarium solani*, (Xing and Westphal, 2006). Nematicides are costly and therefore not a viable option for control of *H. glycines*. For that reason, an integrated management approach is vital, primarily focusing on trap crop systems. By the author's definition, a trap crop for nematodes is a

crop that allows the nematode to penetrate and enter the root; however, the nematode is not able to complete its life cycle and therefore no reproduction takes place. This in turn potentially lowers the field population without the use of other control procedures.

Michigan sugar beet yield is significantly impacted by *H. schachtii*. The result is fewer profits for Michigan sugar beet growers due to a decrease in sugar content and a reduction in the number of viable stands. Control for this nematode usually includes crop rotation and some pre-plant, and in the past, post-plant nematicides (Caswell *et al.*, 1986). These methods are highly labor intensive, thus the focus has turned to discovering resistant germplasm lines with the hope of a commercial sugar beet seed available to Michigan growers in the near future.

The goal of this research was to develop *H. glycines* and *H. schachtii* control tactics designed to increase the nematode management options for Michigan growers, as proposed in Figure 2.

Literature Review

Nematode Taxonomy

Heterodera glycines and *H. schachtii* are classified in the phylum Nematoda and the genus *Heterodera*. Their current classification (according to DeLay, 2002) is as follows:

Genus *Heterodera*

Taxonomy

Classification: Nematoda = Phylum

Chromadorea = Class

Chromadoria = Subclass

Tylenchida = Order

Tylenchomorpha = Infraorder

Tylenchina = Suborder

Tylenchoidea = Superfamily

Heteroderidae = Family

Heteroderinae = Subfamily

Heterodera = Genus

Heterodera Morphology - Sexual dimorphism is present in the genus *Heterodera*. The males are vermiform while the females are swollen, lemon shaped, and white to cream colored. Cysts are various shades of brown. The metacorpus of the females is enlarged and fills the neck region. The vulva is subterminal and the anus is terminal. Males have a rounded cephalic region, curved spicules and no bursa. The second-stage juveniles have a heavily

sclerotized offset cephalic framework. An infective juvenile's stylet is also very prominent with anterior-directed knobs. The juvenile's tail is very pointed and reminiscent of a "rattle snake tail." There is a ventro-lateral overlap of the esophageal glands over the intestines in all vermiform stages (Ferris, 2006 and Tylka, 1994).

Life Cycle – Following embryogenesis, a first-stage juvenile is enclosed in each egg. It molts into a second stage-juvenile and then hatches inside the cyst. With the right chemical signals from its environment (usually food and hormonal cues) the second-stage juvenile (J2) emerges from either the head or vulval regions of the cyst.

Once a plant root is found, the J2 penetrates into the root and then moves intracellularly until it reaches the vascular cylinder. Cellulases may aide the nematode in intercellular migration through the root cortex to the vascular cylinder (Urwin *et al.,* 1997). Cellulases aid in the break down of cell wall material, allowing the nematode to move freely throughout the cell walls.

Once the juvenile reaches its final feeding location and becomes sedentary, it initiates specialized feeding sites called syncytia (Burrows, 1992). A syncytium consists of a multinucleate mass of protoplasm resulting from the fusion of cells. This may happen due to the nematode injecting material secreted by the pharyngeal glands (Smant *et al.*, 1998). Little is known, however, as to the exact make up of the secretions. The purpose of this feeding site is to transfer nutrients from the plant's vascular tissue to the feeding nematode (Burrows, 1992). Developing syncytia often consist of dense granular cytoplasm, usually

surrounded by highly vacuolated, normal cells (Burrows, 1992). The syncytia often appear thick and opaque due to a proliferation of mitochondria, endoplasmic reticulum, and ribosomes (Burrows, 1992). In order for the increase of subcellular organelles, the genes for their productions must be turned on, or up-regulated. This is explained by the "apparent metabolic stimulation within the syncytial feeding sites" (Burrows, 1992). In addition to an increase of subcellular organelles the "cell wall adjoining the xylem increases its thickness by forming finger-like wall invaginations lined with plasma membrane" (Gheysen *et al.*, 2002). This allows water to be transported from the xylem to the feeding sites (Gheysen *et al.,* 2002).

Cytokinins play a role in the feeding site formation, as activators in the cell cycle (Smant *et al.*, 1998). Cytokinins are responsible for inducing the expression of cyclin D3 at the start of S-phase and the cyclin-dependent kinase 2 prior to the S phase (Smant *et al.* 1998). Cytokinins also influence the G_2-to-M phase (Smant *et al.*, 1998). SBCN produces a total cytokinin amount of $\approx 1.8 \times 10^{-18}$ mol/J2 per hour; whereas, the average root produces $\approx 10^{-18}$ mol/nL (Smant *et al.*, 1998). "The lower levels produced by *H. schachtii*, combined with the pharyngeal secretions, may be sufficient for initiation of the syncytia" (Smant *et al.*, 1998). It is estimated that juveniles "withdraw from the syncytia an amount equivalent to fourfold the total syncytia volume" (Gheysen *et al.*, 2002).

As the nematode pushes its stylet into the root, a feeding tube is formed. The feeding tube is located within the plant cell cytoplasm (Kosack-Hammond *et al.,* 2000). Every time the nematode feeds an entirely new feeding tube is

produced; by the completion of nematode infection, hundreds of feeding tubes are present in the syncytial cell (Kosack-Hammond *et al.*, 2000). There is, however, a size exclusion limit of 20 to 40 kDa to the feeding tube (Kosack-Hammond *et al.*, 2000). This exclusion allows only small soluble proteins, sugars, or other organic compounds to pass through the nematode feeding tube. Once the juvenile is sedentary it then molts into a third stage sausage - shape which is non-feeding. If the juvenile is predetermined to be a female, its body expands and pushes out of the root and molts a final time to the classic lemon - shaped white female.

If the juvenile is predetermined to be a male, it molts a final time from the sausage-shape into a vermiform. The male then leaves the root in search of a female. Males and females mate and the fertilized females begin to die, turning a brown color. Her body becomes hardened and houses up to 250 eggs. The cyst is tolerant of freezing temperatures, so the developing nematodes over-winter (endure freezing temperatures) within the protection of the cyst. It is also possible that the eggs over-winter in the soil (Ferris, 2006 and Tylka, 1994).

Symptomology - The stylet of the infective juvenile (J2) is persistently thrusted into different sites of the epidermal cells until the cell wall is weakened enough to cause a hole. The J2 then enters the root through the hole, moves intracellularly until it reaches the vascular cylinder. Once there the J2 begins a more subtle exploration of cells to find the initial syncytial cell (ISC). Cytoplasmic streaming is seen as the ISC's nucleus increases in size. Damage also occurs

when the male exits the root in search of a female, as well as when the female pushes her body out of the root leaving her neck still in the root.

Heterodera glycines

History

H. glycines is suspected to have originated in China along with the soybean (Riggs, 2004). Book 26 of *The Annuls of Lü Buwei*, (China, 239 B.C. as reviewed by Riggs, 2004) mentions *H. glycines* as one of the "three robbers" of crops and gives rules of tillage for the management of the "three robbers." One of the robbers "the land stealing the crops" is suspected to be soil-borne pathogens, soybean cyst nematode being one of them (Riggs, 2004). A report from China in 1899 confirms damage done to soybean seedlings was caused by *H. glycines*; however, soybean farmers called the disease "fire-burned seedlings" long before the report was released (Riggs, 2004). In Japan," Moon-night Disease" of soybeans was described in 1881 and confirmed as caused by *H. glycines* in 1916 (Riggs, 2004). In 1951, soybean cyst nematode was classified as *Heterodera gottingiana*. One year later was renamed as a new species by Ichinohe, *Heterodera glycines* (Riggs, 2004).

In 1954, *H. glycines Ichinohe* was reported in the United States in North Carolina. It is suspected to have been introduced through infected soil from flower bulbs from Japan (Riggs, 2004). It is also speculated that *H. glycines* was in the United States long before the 1954 detection. In 1893 W. P. Brooks in Massachusetts demonstrated the benefit of dusting soybean roots with soil from three soybeans originally from Japan. It was later proven that *Bradyrhizobium*, a nitrogen-fixing bacterium, was the benefit (Riggs, 2004). In the late 1950's and early 1960's, soybean cyst nematodes were found in Missouri, Tennessee, Arkansas, Kentucky, Mississippi, and Virginia (Riggs, 2004). *H. glycines* was first

detected in Michigan in Gratiot County in the spring of 1987 (Warner and Bird, 2000).

Distribution

H. glycines is widely distributed. It can withstand a wide range of temperatures and is found world wide in Japan, China, Korea, Indonesia, South America, Soviet Union, Canada and the United States (Riggs, 2004). In the United States it is present in 26 states including Michigan, North Carolina, Texas, Oklahoma, and Florida. In South Carolina alone, approximately one-third of all soybean fields are infested. It is found in all soybean-producing states of the Midwest (Riggs, 2004).

Host Range

H. glycines has a broad host range. A majority of crop plants, however, do not support reproduction of *H. glycines*. It parasitizes most leguminous plants such as soybeans (*Glycines max*), adzuki bean (*Vigna angularis*) and snapbean (*Phaseolus vulgaris*) (Riggs, 1992). A greenhouse study conducted in 2005, *H. glycines* reproduced on all dry edible beans grown in Michigan (Bird *et al.* unpublished data, 2005). It has also been reported to reproduce on sugar beets and some cultivars of tomatoes (Riggs, 2004 and Riggs, 1992).

Symptomology

In the field, plants may appear yellowed and stunted; this is often referred to as "yellow dwarf" disease in soybeans. These symptoms are most apparent on sandy soil where moisture is low. However, in soils that are heavy and moisture is optimum, there may be no symptoms. Infested plants have a lack of nitrogen-fixing nodules and the roots may appear stunted.

Ecology

H. glycines can be found wherever soybeans are produced. Due to its ability to reproduce on leguminous plants, it may also be found in regions where dry-beans are raised as well as clovers and alfalfas. It co-exists with many other plant-parasitic nematodes in many fields. This co-habitation is speculated to cause an increase in nematode damage on certain plants.

Disease Complexes

H. glycines commonly occur in sites colonized by *Pratylenchus penetrans (*root - lesion nematode, RLN*)*. In Michigan, *P. penetrans* co-exists with *H. glycines* in about 50% of all soybean fields (personal communication with F. Warner, 2006). In one field in Cass County, Michigan there is a small correlation between the number of *P. penetrans* and *H. glycines* on an *H. glycines*-resistant soybean cultivar (Appendix 1). With the increase of *P. penetrans* there was also an increase in success of *H. glycines* upon a resistant soybean (Bird, unpublished data, 2005). Melakeberhan (1998) found that *P. penetrans* had no effect upon plant growth of *H. glycines*-resistant soybean variety 'Bryan.' In 1986, Lawn and Noel provided evidence that when polyphagous communities of nematodes were present in the field they presented a problem for control. When *P. penetrans* and *Meloidogyne incognita* (non-target nematodes) are present along with *H. glycines* a problem arises with the use of species-specific resistant cultivars to control *H. glycines*.

Fusarium solani is the causal agent for Sudden Death Syndrome (SDS) of soybeans (Rupe *et al.*, 1997). Rupe *et al.* suggested that rotation to any non-soybean crop greatly reduced *H. glycines* populations as well as lowered the *F.*

solani populations. Recently, Xing (2006) and colleagues found that as *H. glycines* populations increased in the field there was a higher probability of SDS infestation (Xing *et al.*, 2006).

Economic Loss

Heterodera glycines may reduce soybean yields between 5 to 90 percent (Riggs and Wrather, 1992). In the U.S. alone, over 1.1 billion dollars are lost annually due to this nematode. In 2003 and 2005, *H. glycines* caused 102,705 and 105,981 tons loss to soybean yield, respectively (Wrather and Koenning, 2006). In Michigan, *H. glycines* is detected in 36 soybean growing counties (personal communication with G.W. Bird, 2006). Michigan soybean growers experience an average of 5% annual yield loss due to *H. glycines* (personal communication with F. Warner, 2006).

Current Control Strategies for *Heterodera glycines*

Crop Rotation - Current Michigan *H. glycines* crop rotation strategies are based upon a SCN risk index. This is determined by viable units (eggs and second-stage juveniles) per 100 cm^3 of soil (MSU Diagnostic Services, 2006). The risk system is on a scale from 0-3, where 0 (no SCN detected) is no risk and 3 (>10,000 [eggs+J2s]) is high risk. For example, if the SCN risk index for a field is a 3 (high risk), the recommendation would be two years without growing soybeans. The third year the grower could plant a *H. glycines* - resistant soybean variety. During the years out of soybean, a non-host should be grown (i.e. corn, sugar beets, or wheat). In Michigan, the usual rotation is corn-corn-soybean (or corn-wheat-soybean). If the risk index was 0, no *H. glycines* were detected and

the grower could grow a *H. glycines* susceptible soybean variety (MSU Diagnostics Services, 2006).

Genetic Resistance - There are over 100 Plant Introduction Lines (PI) of *Glycines max* (soybeans) with known resistance to *H. glycines*. Only seven have been used in commercial variety development (Niblack *et al.*, 2002). In Michigan, only three are available in commercial varieties (personal communication with G.W. Bird, 2006). These include PI 54840 (Peking), PI 88788, and PI 437654. PI 88788 is the current dominate source of resistance used in commercially available soybean varieties.

H. glycines is a parasite that has evolved diversity among and within field populations. This enables some populations to reproduce upon certain *H. glycines* resistant soybean varieties. In 1970, a bioassay was developed to detect and quantify this diversity of field populations of *H. glycines* (Niblack *et al.*, 2002). The assay allowed for a more detailed protocol for development of resistant cultivars (races). It also provided growers with specific information about which race of *H. glycines* they had in their field and which resistant PI line(s) to plant. The race concept, however, had several significant faults. In 2002, Niblack *et al.* published an alternative, the HG Type Test. The HG Type Test determines if the field population reproduces on any of the seven PI lines used in commercially available seed. The most common HG Type in Michigan is 2.5.7 which indicates that the average Michigan field population of *H. glycines* is able to reproduce upon PI 88788, PI 209332 and PI 548316; the growers should refrain from planting soybean varieties that contain these resistance lines.

Soybean Cropping System

In Michigan, soybeans are the second largest commodity grown with 2.13 million acres planted to soybeans every year (Andersen, 2005). Soybeans are typically planted as early as the end of April and as late as early June. The most common rotation is corn-soybean-corn. Some producers grow dry edible beans or green beans as well. There over 10,000 acres of organic edible soybeans in Michigan which are usually shipped to Asia for consumption (personal communication with J. Davenport, 2006). Additional pests found in soybean fields include grubs, spider mites, and occasionally soybean aphids (DiFonzo *et al.*, 2006).

Heterodera schachtii

History

The sugar beet cyst nematode disease of sugar beets was first described in Halle, Germany in 1859 by Schacht (Gray and Kerr, 1992). Schacht described the symptoms as *rubenmudigkeit* (beet weariness). The causal agent was described by Schmidt in 1871 and classified as *Tylenchus schactii*. It was not until 1930 that *T. schachtii* was placed in the genus *Heterodera*. *H. schachtii* is believed to have been detected in the United States between 1895 and 1918 (Gray and Kerr, 1992). By 1992, *H. schachtii* was present in 17 states in the U.S (Gray and Kerr, 1992).

The first field survey for *H. schachtii* in Michigan was conducted in 1920 by Gerald Thorne. *H. schachtii* was not detected, however, until 1948 (Knobloch and Bird, 1981; Bockstahler, 1950). In 1998, Miller conducted an industry-wide survey in Michigan and found that 54% of the 214 sugar beet fields sampled were positive for *H. schachtii* (Miller, 1999).

Distribution

H. schachtii is present in 40 sugar beet growing countries as well as 17 states in the United States. In Europe, it ranges from Spain to Bulgaria. *H. shachtii* is also found in regions of Russia, Turkey, Israel, South Africa, and Australia. In the United States it is found in both eastern and western states and it has been detected in parts of Canada (Ferris, 1999).

Host Range

H. schachtii parasitizes at least 200 plant species, most in the families *Chenopodiaceae* and *Cruciferae* (Gray and Kerr, 1992). *H. schachtii* cysts have

been recovered from cabbage, Brussels sprouts, tomatoes, cauliflower, broccoli, kale, radishes, turnips, spinach, and table beets (Gray and Kerr, 1992). *H. schachtii* also parasitizes weed species. For example, mustard, pigweed, lambs-quarter, shepardspurse, and purslane are all hosts for the nematode. *H. schachtii* is categorized as the major pest on sugar beets in Michigan (Knobloch and Bird, 1981).

Symptomology

H. schachtii is responsible for approximately 90% of all nematode-related sugar beet damage (Steele, 1984). Fields affected by *H. schachtii* often appear wilted and underdeveloped. Leaves of affected plants may remain green but can develop a distinct yellowing (Steele, 1984). Beneath the soil the plant roots have excessive fibrous root formation and the storage roots often appear sprangled or have severe branching (Steele, 1984). When the infective juvenile enters into the tap root it destroys the zone of elongation causing excessive branching. This produces the occasional forked beet. This in turn severely decreases sugar content of the beet and ultimately reduced economic return.

Ecology

H. schachtii co-exists with multiple different phytopathogenic nematode species in fields. One in particular that causes increase economic loss in Michigan is *H. glycines*. To date, Michigan is the only state in which these two nematodes co-inhabit the same field. These two nematodes are very closely related taxonomically, in that they are sibling species and are in the same grouping on a parsimonious tree. Nematodes collected from a particular field in Michigan have been collected and successfully mated under laboratory

conditions. The field nematodes have been confirmed to have similar molecular patterns as that of the laboratory mated hybrids, confirming a possible hybrid between *H. gycines* and *H. schachtii* (personal communications with G.W. Bird, 2006).

Disease Complexes

H. schachtii occurs not only with other nematodes but with fungal pathogens in the field. In 1970, Jorgenson reported that in greenhouse trials, damage to sugar beet plants was far greater when both *Fusarium oxysporum* (wilt disease agent) and *H. schachtii* were present than when only the nematode was present (reviewed by Powell, 1971). Root-rot fungal pathogens, such as *Rhizoctonia solani*, are also known to occur with *H. schachtii*. Once the nematode penetrates the root, it facilitates ensuing penetration by the fungus, (Powell, 1971). Polychronopoulos and his colleagues looked at this interaction and found that the syncytia cells induced by the nematode are very suitable substrates for the fungal growth (reviewed by Powell, 1971).

Economic Loss

Heterodera schactii is responsible for approximately 90% of all nematode related sugar beet damage (Steele, 1984). In Michigan, *H. schachtii* is a key pest that lowers sugar beet yield potential significantly. In surveys conducted in 1999, *H. schachtii* was present in approximately 50% of Michigan sugar beet acreage surveyed (Miller, 1999). In fields with high nematode populations and visible foliar symptoms yield loss was as great as 10 tons per acre. In fields with relatively low population of *H. schachtii* and no visible foliar symptoms, yield loss range from 2 to 4 tons per acre (Miller, 1999).

Current Control Strategies for *Heterodera schachtii*

Crop Rotation - Similar to the crop rotation used for *H. glycines* control, *H. schachtii* crop rotation strategies are based upon SBCN risk indices. This is determined by viable units ([egg+J2]) per 100 cm^3 of soil (MSU Diagnostics Services, 2006). The SBCN risk system is on a scale from 0-5, where 0 (no *H. schachtii* detected) is no risk and 5 (>5,000 [eggs+J2s]) is high risk. Unfortunately, there is still not a clear understanding of the level of nematode pressure that causes yield or economic loss on a sugar beet plant (personal communication with F. Warner, 2006). Current rotation recommendations are the responsibility of the Michigan Sugar Company (a grower cooperative, formally Michigan Sugar and Monitor Sugar). The company makes the final recommendation as to how long a field will be out of sugar beet production. Currently, fields are planted to sugar beets one year out of three. The two years out of sugar beets are planted to a *H. schachtii* non-host, such as corn, wheat, potato, soybean, or dry beans.

Sugar beet growers have adopted oilseed radish (OSR) cultivars *Colonel* or *Adagio* as trap crops for control of *H. schachtii*. Field trials indicate that a spring crop of OSR before sugar beets is a more reliable practice for lowering *H. schachtii* population densities and increasing subsequent sugar beet yields than a late summer planting of OSR following wheat (Bird *et al.* unpublished data). *H. schachtii* populations have been estimated to decrease as much as 50% the first year in oil seed radish (Bird *et al.* unpublished data). Greenhouse trials indicate that trap crop efficacy is cultivar specific. While OSR cvs *Colonel* and *Adagio* are

appropriate trap crops for *H. shachtii*, other cultivars of OSR are not (see Appendix B).

Genetic Resistance - Currently there are no *H. schachtii*-resistant sugar beet varieties available to Michigan sugar beet growers. In 2005, a *H. schachtii* resistant variety (Beta 5534N) was field tested in 11 grower trials sponsored by Michigan Sugar Company. In fields where *H. schachtii* populations were low, there was a 6.9 ton per acre increase in yield. In fields where *H. schachtii* populations were high, the yield increase was as high has 10.4 tons per acre (personal communication with G.W. Bird, 2006).

Extensive research has been done in laboratories to identify *H. schachtii* resistance genes in a variety of plant species. One such gene, Hsl $^{pro-1}$, is on chromosome 1 of *Beta. procumbens* (Cai *et al.,* 1997). Plants that carry this gene display an incompatible reaction between host and pathogen (Cai *et al.*, 1997). The nematode invades the roots, but most die in the late J2 stage. In this process the syncytial cells degrades and leaving the nematode without adequate nutrition by then it is incapable of moving to a new location (Cai *et al.*, 1997). In some cases females develop, although abnormally. The females are transparent due to lack of eggs. This process prevents the nematode from completing its lifecycle (Cai *et al.*, 1997).

Also there has been some work in modifying sugar beets as well as other plants to express resistance to *H. schachtii*. Some transgenic plants have been developed with the incorporation of proteinase inhibitors as nematode anti-feedants (Urwin *et al.,* 1997). A modified rice cystatin protein Oc-IΔD86,

expressed as a transgene in *Arabidopsis thaliana* has a profound effect on the size and fecundity of females (Urwin *et al.*, 1997). Ingestion of this cystatin by the nematode caused a loss of cysteine proteinase activity in the intestine and ultimately decreased normal nematode growth (Urwin *et al.*, 1997).

Biological Control - A proportion of the fungi associated with the soil surrounding the plant roots can be nematophagous. Trapping fungi like *Hirsutella rhossiliensis,* aid in the plant defense methods of the nematode (Jaffee *et al.*, 1998). Trapping can occur with initial binding of the nematode then a lectin binding, followed by a penetration peg and hyphal development inside the nematode. Other fungi have the advantage of attacking eggs inside cysts (Pyrowolakis *et al.*, 1999).

Sugar beet cropping system

In Michigan 160,000 acres of sugar beets are grown each year (Andersen, 2005). Sugar beets are grown in a three year rotation on a heavier soil, usually on clay-loam or dry lake beds. Sugar beets are usually planted as soon as the ground thaws from winter. This could be as early as the end of March. The majority of planting takes place during the first two weeks of April. A corn crop generally precedes sugar beets to decrease the chance of disease the following year in sugar beets. Harvest begins in early October and continues into November. Most rotations and variety choices are determined by the sugar company under which all Michigan sugar beet growers are contracted (personal communication with J. Davenport, 2006). Additional pests in sugar beet fields include aphids (foliar and root), springtails, white grubs, and Spinach leaf miner (DiFonzo *et al.,* 2006).

Objectives and Hypotheses

The goals of this research were to develop *Heterodera glycines* and *H. schachtii* control tactics designed to increase the nematode management options for Michigan growers.

Heterodera glycines trap crop development

The objective of this component of the research was to evaluate a large number of potential trap crops and develop a technique that Michigan soybean growers could incorporate into their current crop system to lower *H. glycines* field populations.

Primary screening

Hypothesis 1: All plant cultivars tested will allow for successful *H. glycines* reproduction.

Host Status

Hypothesis 2: Plant cultivars tested will allow for successful *H. glycines* development.

Demonstration Plot

Hypothesis 3: Michigan growers will not accept an additional method for the control of *H. glycines.*

Crop Management

Hypothesis 4: The trap crop system is difficult to incorporate into Michigan soybean grower's current soybean planting regime.

Yield Impact

Hypothesis 5: The trap crop has no impact upon soybean yields.

Heterodera glycines resistant varieties in the presence of *P. penetrans*

The objective of this component of the research was to evaluate *P. penetrans* reproductive potential on all seven PI Lines. Evaluate the development of *H. glycines* in the presence of *P. penetrans* on a PI 88788 sourced *H. glycines* resistant soybean.

H. glycines resistant PI Lines host status for *P. penetrans*

Hypothesis 6: *P. penetrans* will not reproduce on any of the PI Lines.

Impact of *P. penetrans* on *H. glycines* development on a *H. glycines* resistant soybean variety

Hypothesis 7: *P. penetrans* has no effect upon *H. glycines* development on a *H. glycines* resistant soybean variety.

Heterodera schachtii resistant variety development

The objective of this component of this research was to evaluate USDA sugar beet germplasm and commercially available sugar beet varieties for resistance to *H. schachtii.*

Breeding Line germplasm characterization

Hypothesis 8: USDA sugar beet germplasm does not express resistance to *H. schachtii.*

Commercial germplasm characterization

Hypothesis 9: Commercial varieties of sugar beets do not express resistance to *H. schachtii.*

Field Evaluation

Hypothesis 10: All varieties and germplasm tested under field conditions do not express resistance to *H. schachtii*.

Beta 5534N Characterization

Hypothesis 11: There is no significant difference between Beta 5534N and Crystal 963 in relation to *H. schachtii* development and reproduction.

Materials and Methods

Heterodera glycines trap crop development

Primary screening

H. glycines extraction and development - Cysts were collected from soil from Cass County, Michigan. Soil (100 cm^3) was processed using a centrifugal-floatation method (Jenkins, 1964). The cysts were then crushed over a 60 mesh sieve and rinsed into a 15 x 85 ml test tube. The supernanent contained second-stage juveniles (J2s) and eggs only. The [eggs + J2] were counted using a dissecting microscope and calibrated to obtain a ratio of 2000 [J2s + eggs]/ml.

Plant development - Thirty seeds of each variety tested (26 varieties tested, Table 1) were planted in moist (90% sand) soil in an aluminum container (24.13 cm x 29.21 cm x 5.08 cm). The plant containers were placed on a greenhouse (East Lansing, Michigan) bench under a 16 hour light period in a 23C day and 21C night atmosphere for 14 days. The plants were watered once a day and fertilized with Peters 20-20-20 fertilizer solution (25 grams/3.78 L).

Plant inoculation - 14 days after planting, (150 cm^3, 20.95 cm x 4.12 cm) Conetainers were filled half way with steam pasteurized soil (90% sand). A hole was formed, using a pencil, in the soil and a single seedling was placed inside. One ml of J2 and egg mixture (1 ml. at 2000 [egg +J2] per ml) and 1 ml of water was placed directly onto each plant's roots using a syringe. Soil was then placed on top of the roots and each plant was watered. A total of seven seedlings were planted in seven Conetainers for each variety and replicated seven times. The Conetainers were placed in a rack and arranged randomly, then placed into the

greenhouse under 16 hour light period at a temperature of 23C day and 21C night for 35 days.

Nematode extraction - At the end of 35 days the soil was processed using a centrifugal-floatation technique described by Jenkins (1964). Each sample was inspected for the presence or absence of cysts and females.

Data analysis - An ANOVA and Tukey-Kramer Multiple Comparison Test was conducted using SAS® (Version 9.1 SAS Institute INC. Cary, NC.) program using a P-value 0.05 as alpha. A non-parametric test was also conducted to rank the potential trap crops.

Host Status

Microtile preparation - Soil infected with *H. glycines* from Michigan Cass County, Michigan was used to fill 4 liter microtiles. Soil (100 cm^3) was separated and processed for nematodes according to the above protocol. Cass County field soil (3.5 L) was used to fill each microtile. Four trenches were dug in a field plot located in East Lansing, Michigan. Each microtile was placed into the trench and field soil was used to fill around the microtile. The cultivars used in this experiment were the following: *H. glycines* susceptible soybean, *H. glycines* resistant soybean, corn, Berseem clover, Oriental mustard, Dackon common oilseed radish, and Common lespedeza. Five seeds of the soybeans were planted in the middle of a microtile and replicated 12 times in 12 different microtiles. Four seeds of the corn, replicated 12 times, and 10 seeds of the remaining cultivars were planted in the middle of a microtile and replicated 12 times.

Sampling of microtile - At 14 days after planting a single microtile was dug from the ground and placed into a plastic bag. The microtile was then destructively sampled. The soil was knocked out of the tile using the side of a table. The plant inside was then saved and the roots were washed. The soil was placed into a plastic bag and put into a cooler that was kept at 40C. This was done to each treatment once a week for six weeks. The roots were subjected to acid fusion staining to detect the stages of nematodes.

Root Staining - All procedures were followed as outlined by Byrd *et al.* in 1983.

Demonstration Plot

A 9.14 m by 9.14 m plot was cleared in a recently planted soybean field (Monroe County, Michigan) using garden hoes. The treatments were *H. glycines* susceptible soybean, corn, Berseem clover, *H. glycines* resistant soybean, Common Lespedeza, Oriental mustard, and Dackon common oilseed radish.The treatments were replicated seven times; each plot measured 0.91 m by 0.91 m with a 0.61 m alley in between replicates (Figure 3). Plots were arranged in a complete random block design. The treatments were hand planted (Table 2) plots were sampled at planting (mid-June) and at the harvest of the soybean field (early October). Seeding rates can be seen in Table 2. Approximately 1 liter of soil was sampled from each treatment at planting as well as at harvest.

Nematode extraction- Soil (100 cm^3) was processed using a centrifugal-floatation technique (Jenkins, 1964). Each sample was inspected for the

presence or absence of cyst and white females. Cysts were crushed by hand using a tissue homogenizer and [eggs + J2] per each cyst were counted.

Data Analysis - An ANOVA and Tukey-Kramer Multiple Comparison Test was conducted using SAS® (Version 9.1 SAS Institute INC. Cary, NC.) program using a P-value 0.05 as alpha.

Crop Management

Greenhouse study

H. glycines susceptible soybean, Berseem clover, Oriental mustard, Dackon common oilseed radish and a fallow were all used to test the practicality of a trap crop. *H. glycines* infested field soil from Cass County, Michigan, (800 ml) was used to fill plastic square pots. A sample of soil (100 cm^3) was set aside to get an initial nematode count. Two seeds of clover, radish, mustard and soybean were planted individually in the middle of a pot. The fallow pot was left undisturbed. The plant containers were placed in a greenhouse (East Lansing, Michigan) under a 16 hour light period in a 23C day and 21C night atmosphere for two weeks. The plants were watered once a day and fertilized with Peters 20-20-20 fertilizer solution (25 grams/3.78 L). The plants were allowed to grow for 35 days and then were spray killed using Roundup WeatherMax at 33 oz/A +AMS at 7.7 kg/378.54 L of H_2O using a Allen Spray Machine (Laboratory spray chamber that delivers 187 L/ha (20 GPA) at 173 KPa (25 psi) with a TeeJet 8001E nozzle). Fourteen days post spray, two *H. glycines* resistant soybeans were planted in each pot. After 43 days, the soybeans were destructively sampled.

Nematode extraction - The soil and roots were scrubbed and then processed using a centrifugal-floatation technique (Jenkins, 1964). Each sample was inspected for the presence or absence of cysts and white females. Cysts were crushed by hand using a tissue homogenizer and the number of [eggs + J2] per cyst was counted.

Data analysis - An ANOVA and Tukey-Kramer Multiple Comparison Test was conducted using SAS® (Version 9.1 SAS Institute INC. Cary, NC.) program using a P-value of P = 0.05 as alpha. A Fisher's LSD was also conducted.

Yield Impact

A 91.44 m x 15.24 m plot was used and each treatment was planted using a Great Plains® Drill in Saginaw County, Michigan. Soil samples were taken before each treatment was planted to asses the initial *H. glycines* population density. Approximately 1 liter of soil was sampled and processed using a centrifugation-floatation method (Jenkins, 1964). Each plot was 9.14 m wide with four rows in a randomized block design with seven replicates. Seeding rates of each treatment are shown in Table 3. WatchDog® data loggers were placed in three of the replicates in different areas of the field. One sensor was placed 15.24 cm below the soil and another was placed 15.24 cm above the soil. After five weeks, the trap crops were spray killed using 24 oz/A of Roundup WeatherMax + AMS at 7.7 kg/378.54 L of H_2O using a flat-fan herbicide sprayer. Fourteen days post-spray of the trap crops, an *H. glycines* susceptible soybean was planted with a White Seed Boss 5100 using the same population as the *H. glycines* resistant soybean. Soil samples were again taken before the soybeans

were planted. A pre-emergence was also sprayed, Pursuit Plus at 2pt/A + 24 oz Roundup Weathermax using a flat-fan herbicide sprayer. At the end of the trial, mid-October, another soil sample was taken from each plot. Soil (100 cm^3) was processed for nematodes using the centrifugation-floatation method as described by Jenkins in 1964.

Harvest - At the end of the growing season the center two rows were harvested using a small-plot combine (mid-October). The beans were run through a fanning mill to remove soil pellets as well as debris. Beans were then weighed and a sub-sample was tested for seed moisture and test weight. Percent moisture and a test weight were recorded. Yield was then calculated from the above information using a soybean yield conversion equation.

Nematode extraction - Soil (100 cm^3) was processed using a centrifugal-floatation technique (Jenkins, 1964). Each sample was inspected for the presence or absence of cysts and white females. Cysts were crushed by hand using a tissue homogenizer and eggs and J2s per each cyst were counted.

Data analysis - An ANOVA and Tukey-Kramer Multiple Comparison Test was conducted using SAS® program (Version 9.1 SAS Institute INC. Cary, NC.) using a P-value of 0.05 as alpha.

Heterodera glycines resistant variety in the presence of *P. penetrans*

H. glycines resistant PI Lines Host Status for *Pratylenchus penetrans*

Pratylechus penetrans extraction and development - *Pratylechus penetrans* was obtained from greenhouse cultures of *P. penetrans* (RLN) infested corn plants. The roots were processed using Bird's method (1971) with the substitution of Ethyl Mercuric Chloride (EMC) solution for a 0.01% NaOCl solution. *P. penetrans* were counted using a dissecting microscope and diluted to obtain 2000 *P. penetrans*/ml

Plant development - Seeds of each indicator line tested were planted in moist (90% sand) sandy-loam in an aluminum container (24.13 cm x 29.21 cm x 5.08 cm). The containers were placed upon a greenhouse bench under a 16 hour light period in a 23C day and 21C night atmosphere for two weeks. The plants were watered once a day and fertilized with Peters 20-20-20 fertilizer solution (25 grams/3.78 L).

Plant inoculation - At the end of two weeks (150 cm^3, 20.95 cm x 4.12 cm) Conetainers were filled half way with steam pasteurized loam (90% sand). A hole was formed in the soil, using a pencil, and a single plant was placed inside. *P. penetrans* (1 ml at 2000 *P. penetrans* /ml) at different stages and 1 ml of water was placed directly onto each plant's roots using a syringe. Soil was then placed on top of the roots and each plant was watered. A total of seven seedlings were planted for each variety and replicated seven times and the Conetainers were placed in racks, arranged randomly, then placed in a greenhouse (East Lansing,

Michigan) under 16 hour light period at a temperature of 23C day and 21C night for 35 days.

Nematode extraction - The roots were processed using Bird's method (1971) with the substitution of Ethyl mercuric chloride (EMC) solution for a 0.01% NaOCl solution. Total amount of *P. penetrans* was counted from a 1 ml aliquot.

Data analysis - An ANOVA and Tukey-Kramer Multiple Comparison test was conducted using SAS® (Version 9.1 SAS Institute INC. Cary, NC.) program using a P-value of 0.05 as alpha.

Development of *Heterodera glycines* on an SCN Resistant Soybean Cultivar in the Presence of *Pratylenchus penetrans*

Pratylechus penetrans extraction and development - *Pratylechus penetrans* was obtained from greenhouse cultures of *P. penetrans* (RLN) infested corn plants. The roots were processed using Bird's method (1971) with the substitution of Ethyl Mercuric Chloride (EMC) solution for a 0.01% NaOCl solution. *P. penetrans* were counted using a dissecting microscope

H. glycines (SCN) extraction and development - Cysts were collected from soil from Cass County, Michigan. Soil (100 cm^3) was processed using a centrifugal-floatation method (Jenkins, 1964). The cysts were then crushed over a 60 mesh sieve and rinsed into a 15 x 85 ml test tube. The supernanent contained second-stage juveniles (J2s) and eggs only. The [eggs + J2] were counted using a dissecting microscope

Plant development - Seeds of a PI 88788 resistance source and an *H. glycines* (SCN) susceptible soybean were planted in moist (90% sand) soil. The

plant containers were placed in a greenhouse (East Lansing, Michigan) under a 16 hour light period in a 23C day and 21C night atmosphere for 14 days. The plants were watered once a day and fertilized with Peters 20-20-20 fertilizer solution (25 grams/3.78 L).

Plant inoculation - At the end of two weeks (150 cm^3, 20.95 cm x 4.12 cm) Conetainers were filled half way with steam pasteurized sandy soil (90% sand). A hole was formed, using a pencil, in the soil and a single plant was placed inside. Either 2000 SCN [J2s + eggs]/ml; 2000 RLN/ml; 1600 SCN [J2s+eggs]/ml: 400 RLN/ml; 1000 SCN [J2s+eggs]/ml: 1000 RLN/ml or 400 SCN [J2s+eggs]/ml: 1600 RLN/ml was placed directly onto each plant's roots using a syringe. Soil was then placed on top of the roots and each plant was watered. A total of seven plants were planted for each variety and replicated seven times and the Conetainers were placed into a rack and arranged at random. The plants were then placed in the greenhouse under 16 hour light period at a temperature of 23C day and 21C night for 35 days.

Nematode extraction - At the end 35 days the soil was processed using a centrifugal-floatation technique (Jenkins, 1964). The roots were processed using Bird's method (1971) with the substitution of EMC solution for a 0.01% NaOCl solution. Total *P. penetrans* was counted from a 1 ml aliquot. All stages of *H. glycines* and each cyst was crushed using a tissue homogenizer and each J2 and egg were counted per a 1 ml aliquot.

Data analysis - An ANOVA and Tukey-Kramer Multiple Comparison Test was conducted using SAS® (Version 9.1 SAS Institute INC. Cary, NC.)

program using a P-value of 0.05 as alpha.

Heterodera schachtii resistant variety development

Breeding line germplasm characterization

H. schachtii extraction and development - Cysts were collected from soil from Michigan's Saginaw Valley. Soil (100 cm^3) was processed using a centrifugal-floatation technique (Jenkins, 1964). The cysts were then crushed over a 60 mesh sieve and rinsed into a 15 x 85 ml test tube. The solution contained J2s and eggs only. The solution was then counted using a dissecting microscope to obtain a ratio of 2000 [J2s + eggs]/ml

Plant development - Seeds of four germplasm (Hil-2, HM E-17, N 224, N 172) lines tested were planted in moist (fine) grain vermiculite in an aluminum container (24.13 cm x 29.21 cm x 5.08 cm). The plant containers were placed on temperature tanks set at 24C under a 16 hour light period for 14 days. The plants were watered once a day and fertilized with Peters 20-20-20 fertilizer solution (25 grams/3.78 L).

Plant inoculation - At the end of 14 days, (150 cm^3, 20.95 cm x 4.12 cm) Conetainers were filled half way with steam pasteurized sandy soil (90% sand). A hole was formed, using a pencil, in the soil and a single plant was placed inside. J2s and eggs (1 ml at 2000 [eggs+J2] per ml) were placed directly onto each plant's roots using a syringe. Soil was then placed on top of the roots and each plant was watered. A total of seven seedlings were planted for each line and replicated seven times. Conetainers were then placed into a rack and arranged at random. Another Conetainer rack was filled with the same number of

Conetainers with the four different lines. The plants were then placed in the greenhouse under 16 hour light period at a temperature of 23C day and 21C night for 42 and 56 days.

Nematode extraction - At the end of both 42 and 56 days the plants were taken out of the green house and the nematodes were extracted. Soil was then processed using a centrifugal-floatation technique (Jenkins, 1964). Each sample was inspected for the presence or absence of cysts and white females to give a percent mature female.

Data analysis - *An* ANOVA and a Tukey-Kramer Multiple Comparison test were conducted using SAS® (Version 9.1 SAS Institute INC. Cary, NC.) program using a P-value of 0.05 as alpha. Mature females were determined by adding cyst and white females together. This sum was divided by cysts counts to give the percentage of [egg + J2s] that matured to cysts over each given time frame.

Commercial germplasm characterization

H. schachtii extraction and development - Cysts were collected from soil from Michigan's Saginaw Valley. Soil (100 cm^3) was processed using a centrifugal-floatation technique (Jenkins, 1964). The cysts were then crushed over a 60 mesh sieve and rinsed into a 15 x 85 ml test tube. The solution left over contained J2's and eggs only. The solution was then counted using a dissecting microscope to obtain a ratio of 2000 [egg +J2s] /ml

Plant development - *Seeds* of 20 commercial sugar beet lines (obtained from Michigan Sugar Company) were planted in moist (fine) grain vermiculite in an aluminum container (24.13 cm x 29.21 cm x 5.08 cm). The plant containers

were placed on temperature tanks set at 24C under a 16 hour light period for 14 days. The plants were watered once a day and fertilized with Peters 20-20-20 fertilizer solution (25 grams/3.78 L).

Plant inoculation - At the end of two weeks, (150 cm^3, 20.95 cm x 4.12 cm) Conetainers were filled half way with steam pasteurized sandy soil (90% sand). A hole was formed in the soil and a single plant was placed inside. J2s and eggs 1 ml. at 2000 [egg+J2s] per ml) were placed directly onto each plant's roots using a syringe. Soil was then placed on top of the roots and each plant was watered. A total of seven plants were planted for each variety and the Conetainers were placed into a rack and arranged at random. The plants were then placed into a green house under 16 hour light period at a temperature of 23C day and 21C night for 42 and 56 days.

Nematode extraction - At the end of both 42 and 56 days the plants were taken out of the greenhouse and the nematodes were extracted. Soil was then processed using a centrifugal-floatation technique (Jenkins, 1964). Each sample was inspected for the presence or absence of cysts and white females to give a percent mature female.

Data analysis - *An* ANOVA, Tukey-Kramer Multiple Comparison and Fisher's Least Significance Difference tests were conducted using SAS® (Version 9.1 SAS Institute INC. Cary, NC.) program using a P-value of 0.05 as alpha. Mature females were determined by adding cyst and females together. The sum was divided by cyst counts to give the percentage of [egg + J2s] that matured to cysts over each given time frame. On the 56 day trial a Fisher's Least Square

Difference was performed to allow for greater separation. Classification of resistance was determined by a higher susceptibility index the greater the probability of susceptibility (the percentage of mature females upon the variety)

Field Evaluation

Pre-plant sampling – A field in Saginaw County, Michigan was divided into four sections and each section was sampled four times using a cone-soil probe, 15.24 cm below the soil surface. Approximately 1 liter of total soil was taken during sampling.

Planting - Each of the four sections that the field was divided into became a replicate. A wooden stake was pushed 5.08 cm into the soil and a single sugar beet seed was placed into the hole by hand. This was done 10 times for each of the 22 varieties and replicated 4 times.

Harvest - All sugar beets were hand dug.

Harvest sampling - Once the sugar beets were harvested one soil sample was taken from each variety/line tested.

Nematode extraction - Soil (100 cm^3) was processed using a centrifugal-floatation technique (Jenkins, 1964). Each sample was inspected for the presence or absence of cysts and females. The stages of development were recorded. Also each cyst was crushed by hand using a tissue homogenizer and the number of eggs and juveniles were also enumerated.

Data analysis - Initial sample field population density mean of *H. schachtii* was calculated. If treatment sample mean was less than five times the initial sample mean there was a good probability of resistances. If the treatment mean

was greater than 5 times the initial sample mean then it was a good probability of susceptibility. If the treatment mean equaled 5 times the initial sample mean then susceptibility or resistance was indeterminate.

Beta 5534N characterization

H. schachtii extraction and development - Cysts were collected from soil from Michigan's Saginaw Valley. Soil (100 cm^3) was processed using a centrifugal-floatation technique (Jenkins, 1964). The cysts were then crushed over a 60 mesh sieve and rinsed into a 15 x 85 ml test tube. The supernanent left over contained J2s and eggs only. The supernanent was then counted using a dissecting microscope to obtain a ratio of 2000 [J2s + eggs]/ml.

Plant development - Seeds of two sugar beet varieties tested were planted in moist (fine) grain vermiculite in an aluminum container (24.13 cm x 29.21 cm x 5.08 cm). The plant containers were placed on temperature tanks set at 24C under a 16 hour light period for 14 days. The plants were watered once a day and fertilized with Peters 20-20-20 fertilizer solution (25 grams/3.78 L).

Plant inoculation - At the end of two weeks, (150cm^3, 20.95 cm x 4.12 cm) Conetainers were filled half way with steam pasteurized sandy soil (90% sand). A hole was formed, using a pencil, in the soil and a single plant was placed inside. J2s and eggs (1 ml at 2000 [eggs+J2s] per ml) were placed directly onto each plant's roots using a syringe. Soil was then placed on top of the roots and each plant was watered. A total of seven plants were planted for each variety the Conetainers were then placed in a rack and arranged at random. The plants

were then placed in the greenhouse under 16 hour light period at a temperature of 23C day and 21C night for 35 days.

Nematode extraction - At the end of 35 days the plants were taken out of the greenhouse and the nematodes were extracted. Soil was processed using a centrifugal-floatation technique (Jenkins, 1964). Each sample was inspected for the presence or absence of cysts and females and cysts were crushed by hand using a tissue homogenizer and eggs and J2s were counted.

Data analysis - An ANOVA and Tukey-Kramer Multiple Comparison test were conducted using SAS® (Version 9.1 SAS Institute INC. Cary, NC.) program using a P-value of 0.05 as alpha.

Deposition of Voucher Specimens

Cysts of *Heterodera glycines* were collected from Cass, Monroe, and Saginaw Counties, Michigan. Cysts of *Heterodera schachtii* were collected from Bay County, Michigan. Cysts of an unknown *Heterodera* spp. were collected from Bay County, Michigan. The cysts were placed in glass vials of alcohol, labeled and were deposited in the A.J. Cooke Arthropod Collection at Michigan State University Entomology Museum under voucher number 2006-06 (Appendix 4 and 4.2).

Results and Discussion

Heterodera glycines trap crop development

Primary Screening

Results

Eighteen of the twenty-six crops tested had no detectable *H. glycines* cysts or females (Table 4). There was a highly significant, ($P<0.0001$) impact of host crops upon nematode populations. When crops with no *H. glycines* development were excluded from the analysis, the remaining crops significantly supported development for *H. glycines* ($P=0.0018$). Resistant soybean and sugar daddy peas had a significant positive impact upon the nematode population ($P=0.0007$ and $P<0.0001$, respectively). Since the resistant soybean and the pea are considered hosts of *H. glycines*, they were also then removed from analysis. The remaining crops were: Berseem clover, Sudax, Oriental Mustard, Dackon Oilseed Radish and Generic Oilseed Radish. There was no significant difference among treatments, ($P = 0.4610$). A non-parametiric test was conducted and Oriental mustard, Dackon oilseed radish, and Berseem clover were found to have a strong trend of positive influence on development for *H. glycines*.

Discussion

Much work has been done to determine a number of plant species that are poor hosts for *H. glycines* (Miller and Ahrenes, 1969; Riga *et al.*, 2001; Rao-Arelli *et al.*, 1991). Those poor hosts were the foundation for the development of an efficient trap crop system. Twenty-six different crops were initially screened to determine their nematode-trapping ability (Table 1). Riggs determined many of

those crops to be poor hosts for the *H. glycines* (reproductive potential was very low). Taking that into account, only reproductive potential was measured in the initial screening trial. This allowed for creating a new list of those where no cyst or females were detected, which could mean the plant trapped the nematode and it was not allowed to continue on in its life cycle. The evaluation identified three plant cultivars with potential for use as a trap crop for the control of *H. glycines*: Berseem clover, Dackon Oilseed Radish, and Oriental Mustard. This decision was based upon the low developmental success of *H. glycines*.

Host Status

Results

Based on visual observation of stained roots, *H. glycines* were not detected in the corn roots. Common Lespedeza was included in this study due to its suspected susceptibility for *H. glycines*. The lowest number of vermiform nematodes were seen at the first sampling (Table 5), but towards the end of sampling Lespedeza had a greater number of vermiform and sausage nematodes than the *H. glycines* susceptible soybean (Table 5 and 6). Only vermiform nematodes were detected throughout the sampling period on Berseem clover (Tables 5 and 6 and Figure 4). Nematode numbers declined on Berseem clover, Dackon oilseed radish and Oriental mustard. The vermiform nematodes increased as sampling continued on the *H. glycines* resistant soybean. The sausage nematodes increased as the sampling continued of that treatment.

Discussion

As expected the *H. glycines* susceptible soybean supported all stages of nematodes throughout the sampling period. There appeared to be a difference in mechanism of trapping occurring in Berseem clover, Dackon oilseed radish, and Oriental mustard. Only vermiform nematodes were found throughout the sampling of the Berseem clover, however; both vermiform and sausage nematodes were observed but the number drastically decreased as sampling continued in the oilseed radish and mustard. This could possibly be explained by Berseem clover classically trapping the nematodes (allowing for entrance into the root but failing to develop a feeding site) while oilseed radish and mustard may not allow the nematode to develop a sufficient feeding site, so development is incomplete. Further studies, however, are required to test this hypothesis.

Demonstration Plot

Field Trial:

The difference between harvest and at planting egg: J2 ratios were calculated and an ANOVA was run. The *H. glycines* susceptible soybean had the most significant effect on increasing reproduction and development of *H. glycines* over one growing season approximately 4 months, (P=0.0039). Using an alpha of 0.1 both Oriental Mustard and Berseem clover had the most significant effect on lowering *H. glycines* reproduction and development over one growing season, (P=0.068 and P = 0.086, respectively, Table 7)

Overall, based on this field trial, growing a susceptible soybean as a trap crop increased the *H. glycines* population. Growing Oriental mustard lowered the

nematode population substantially. Much more extensive field trials are needed before the assessment of the feasibility of the effectiveness of the trap crop system.

Farmer Education:

In late August a farmer field day was held. It was sponsored by Michigan State University Monroe County extension. There were approximately 80 soybean growers in attendance. A handout was created to give to those in attendance (Appendix C). Growers were able to walk by the field trial and see what each of the possible trap crops look like as well as ask questions. A short talk was given to explain the objectives of the study and to define what a trap crop is in relation to nematodes, in particular *H. glycines*. Most growers responded very positively to the idea of a trap crop for *H. glycines*. Others were concerned with the possibility of the trap crop becoming a nuisance or a problem "weed" in the coming growing season.

Crop Management

Results

A difference between initial [egg + J2] count and final [egg + J2] counts were calculated by subtracting harvest counts from initial counts. All treatments were significant ($P < 0.0001$) at impacting *H. glycines* population densities. When a Fisher's LSD was conducted, the *H. glycines* susceptible soybean had the most significant positive effect on [egg+J2] counts (mean difference of 22624, $P < 0.0001$). Oriental mustard was separated out as having the most negative

effect on *H. glycines* [egg+J2] counts (mean difference of –1628, Table 8), but with no significant difference between treatments.

Discussion

Overall based on the greenhouse trial it could be concluded that growing a susceptible soybean as a trap crop followed by a *H. glycines* resistant soybean, increases the *H. glycines* population. However, Oriental mustard does appear to have the most significant effect on the nematode population based on comparison of the mean difference calculation to the other treatments. It could be concluded that growing Oriental mustard or possible any of the other treatments (there was no significant difference between the treatments) except *H. glycines* susceptible soybean prior to planting a *H. glycines* resistant soybean, the overall *H. glycines* population were reduced at the end of the growing season.

Yield Impact

Results

Berseem clover had the best overall stand compared to the other trap crops (fallow treatment excluded) over the course of one month. The ambient air temperature during the time the trap crops were in the field was on average 13.7C and the soil was on average 14.2C. Temperature per day can be seen in Figure 5 (temperature in degrees F). The four treatments had no effect on the yield of a *H. glycines* susceptible soybean over one growing season (Table 9). The fallow control had an average yield of 41.74 bu/A. The Oriental mustard had the highest yield compared to the other treatments. It also had 1.14bu/A more than the fallow control (Table 9). Mean *H. glycines* development over the course

of the growing season can be seen in Figure 6. Trap crop effect on *H. glycines* population densities at both samplings of planting of the trap crop and planting of the *H. glycines* susceptible soybean were not significant. Whereas, the harvest samples were highly significant for *H. glycines* cyst and [eggs + J2s] ($P = 0.0004$ and $P < 0.001$, respectively). Berseem clover had the most positive impact upon *H. glycines* population at the harvest of the soybeans. The other trap crops (Fallow, Oriental mustard, *H. glycines* resistant soybean, and Dackon oilseed radish) had no difference among themselves. The *H. glycines* resistant soybean showed the most reduction of the *H. glycines* population densities over the three sampling periods (Figure 6).

Discussion

H. glycines is physically active in the soil at temperatures as low as 10C (verbal correspondence with Bird, 2006). The optimum temperature for the nematode is 23C (Caswell *et al.,* 1986). Based on that knowledge, *H. glycines* would have been active during the period in which the trap crops were in the field. The nematode would have been able to penetrate the plant roots and potentially do damage. The trap crop treatments had no effect on the overall soybean yield which could be accounted for by the low stands of each trap crop. This could have led to unclear conclusions and less reduction in nematode population densities. Berseem clover was the only treatment that has the ability to be frost seeded and would succeed if planted one month before soybeans. Also, keeping the field fallow prior to soybeans also has the potential to decrease nematode densities. Oriental mustard was the only treatment that had a positive

impact on yield. The mustard's impact on yield could either be that the plant is trapping the nematode or that it has nematicidal properties. Additional field trials are required to further understand the yield impact of the *H. glycines* trap crops.

Heterodera glycines resistant cultivars in the presence of *P. penetrans*

H. glycines Resistant PI Lines Host Status for Pratylenchus penetrans

Results

Pratylenchus penetrans reproduced on all seven *H. glycines* resistant PI lines and the susceptible variety, Lee 74 (Figure 7). Final population densities were highest on Lee 74 (~900/g root) and lowest on PI 89722 (~300/g root) (Figure 6). The ANOVA was highly significant (P= 0.0060). The highest amount of variability was in PI 88788 and the susceptible control, Lee 74.

Discussion

There are currently seven sources of *H. glycines* resistance used in commercial soybean varieties (Niblack *et al.* 2002). The HG type test is used to determine the diversity among *H. glycines* populations and their ability to develop on resistant soybean varieties (Niblack *et al.* 2002). It is believed that *P. penetrans* co-exists in *ca* 50% of Michigan soybean fields with *H. glycines*.

Data shows that each of the seven PI Lines are successful as hosts for *P. penetrans*. Lee 74, which is susceptible to *H. glycines*, is also susceptible to *P. penetrans* (success of host ability). This could lead to the conclusion that if a cultivar is susceptible to *H. glycines* it may very well likely be susceptible to *P. penetrans*. If a cultivar is resistant to *H. glycines* it may not be resistant to *P.*

penetrans. This could lead to many other problems. Having a soybean cultivar resistant to only one type of nematode it may leave the plant susceptible to other nematodes that may infect in a different mode, like *P. penetrans*.

Impact of *P. penetrans* on *H. glycines* development on a *H. glycines* resistant soybean variety

Results

Where *H. glycines* was alone (2000 [eggs+J2s]) upon the susceptible bean there was an average of 265 times more *H. glycines* than compared to the resistant bean. When *P. penetrans* was present at a low number (400 RLN/ml) there were only 37 times more *H. glycines* on the susceptible than on the resistant bean. Whereas, when *P. penetrans* was present at an equal number (1000 nematodes/ml) to *H. glycines* there were 23 times more *H. glycines* upon the susceptible than on the resistant (Figure 8 and 9). An ANOVA of the *H. glycines* developmental potential upon the *H. glycines* susceptible soybean showed that the treatments were all significant with a P-value of 0.0002. Each treatment when compared to one another showed no significance for *H. glycines* development (P-value 0.1349). The PI 88788 bean it was shown that the ANOVA was significant for *H. glycines* development with a P-value of 0.0010. When looking at the treatments where the nematodes were present separate from the treatments where *H. glycines* (SCN) and *P. penetrans* (RLN) were alone it was seen that together they were not significant (P-value 0.2351). Tukey-Kramer test reviled that the treatment where the nematode ratio was 1000 SCN: 1000 RLN it more significant than the other treatments (P-value 0.0003).

Discussion

Only three of the seven sources of resistance to *H. glycines* that have been commercialized are available for use in Michigan (PI 54840, PI 88788, and PI 437654). PI 88788 is the dominate source of resistance used in many of the commercially available soybean seeds. It was noted that when *P. penetrans* was not present or in very small numbers, either due to experimental error or failure to develop, the total *H. glycines* numbers were similar to that of the control counts. However, when *P. penetrans* was present there was a significant increase in cyst counts. The nematode numbers upon the susceptible were what were to be expected. When large numbers of *H. glycines* were added, large numbers were observed in the final count. The same was true when small quantities of *H. glycines* were added; small quantities were observed in the final count. Based on the above results it can be concluded that in the presence of *P.penetrans*, *H. glycines* has a higher rate of successfully developing upon a PI 88788 *H. glycines* resistant source soybean (Figure 6 and 7). PI 88788 is the primary source of resistance to *H. glycines* in production and that *P. penetrans* is shown to possibly break down that PI 88788 resistance a recommendation would be to determine at what rate is *P. penetrans* present in the soil and not just determining which resistance would be the best for the field. This would be due to that an equal number of RLN: SCN ratio there was a higher developmental potential of *H. glycines* upon the PI88788 line as opposed to just inoculating with *H. glycines* only.

Heterodera schachtii resistant variety development

Breeding line germplasm characterization

Results

Clear differences were observed between individuals and germplasm lines in response to *H. schachtii* infection (Table 10), particularly by 56 days after inoculation under the conditions used here. One germplasm, Hil-2, had uniform resistance to *H. schachtii*. Apparent segregation was evident for N224 and N172, also. Susceptible line E17 was variable, but counts of cysts and females were higher than with other germplasm. By 42 days, it was evident but not statistically significant, as to which germplasm was going to be more or less susceptible, suggesting that this is not long enough for the nematode to complete its lifecycle.

Discussion

This experiment was an initial test into the development of a *H. schachtii* assay in the greenhouse whereby selections for further breeding could be performed. A small number (perhaps as few as 16) of breeding lines appear to be resistant to *H. schachtii*, but no commercial cultivars are marketed at this time, although the positive control used here (a breeding line from Syngenta, Hil-2) may be the most advanced germplasm available. Resistance in Hil-2 is derived from *Beta procumbens*.. Differences were clearer on average at week eight, however another time interval would have to be done to determine if the white females seem would develop into viable cysts. The cysts that were seen on Hil-2 were small and, when crushed, not may eggs or J2s were seen, leading to believe that the plant variety may have slowed the nematode's development. More time intervals may be needed to get a clearer picture of resistance of the

varieties. At six weeks may not be long enough to develop significant discrimination. The plant's response to the nematode could be monitored using the method described here to find when the resistance takes place and how it affects the plant and the nematode.

Commercial germplasm greenhouse screening

Results

At the end of the 42 day time interval, each of the 20 varieties could be separated into 5 categories: resistant, somewhat resistant, susceptible, and highly susceptible, based on *H. schachtii* development. This separation can be seen in table 11 and figure 11. Six of the twenty varieties were shown to be mostly resistant at the end of the 42 day period. Those varieties were: Beta 5471, Beta 5374, 5X Spartan, Holly 02HX272, Crystal 963, and HM E-38. All of those varieties had a female percent maturity of fewer than 33%. At the end of the 56 day time period, only three varieties could be classified as most resistant. Those varieties were: Beta BK 1383R, Beta 5374, and Holly 02HX272. These varieties had less than 25% female maturity. The 56 day values can be seen in Table 12 and Figure 12.

Discussion

Two time periods were used in this experiment to determine if resistance broke down as time increased. It was shown that in certain varieties that resistance does indeed break down in some varieties. The variety 5X Spartan by the end of the eight weeks had dropped from most resistant to highly susceptible. However, four of the varieties showed improvement at the end of the eight week

period. Crystal 271 and Beta BK 1381R both were classified as somewhat resistant at the end of the eight weeks, moving up from susceptible. Beta 5374 was only variety that was classified as most resistant at both 42 and 56 days and remained at the same position. This would lead to the conclusion that Beta 5374 has the most stable resistance to *H. schachtii* as compared to the other commercial varieties.

Field Evaluation

Results

Out of the twenty two different sugar beet varieties that were planted only two showed probability of resistance. The initial sampling means can be seen in Table 11. It was seen that Hil-2 and 2927-4 were the only varieties that showed a probability of resistance based on the analysis done. Hil-2 had a mean cyst count of 13.3 with a [J2s + egg] mean count of 1120 (Figures 12 and 13). The 5*mean initial count was 17.5 for cyst and 1137.5 for [J2s + egg] counts. Hil-2 treatment means were less than the initial means. 2927-4 was the other variety that showed a probability of resistance. The variety 2927-4 had a mean cyst count of 13 and a [J2s + egg] count of 880. Both varieties showed numbers less than 5*initial mean.

Discussion

In the field it was seen that only two varieties had the probability of being a resistant to *H. schachtii*, Hil-2 and 2927-4. Unfortunately, no definite conclusions can be drawn due to many of the varieties had poor yield, thus leading to a

decrease of replications. As well as, the initial sampling only obtained an average nematode count for the entire field, rather than for each treatment.

Beta 5534N Characterization

Results

When each variety of sugar beet was inoculated with the same numbers of *H. schachtii* J2's and eggs, (2000), there was 40% less females (cysts or white) upon Beta 5534N than the commercial susceptible variety. Further, the number of eggs and juveniles per cyst significantly declined for the Beta host (P-value <0.001). When the cysts were crushed an average of 32.8 eggs and juveniles were accounted for as opposed to the average 91 found in the commercial variety cysts (Figure 14).

Discussion

Recently, a sugar beet variety, Beta 5534N, was developed by a commercial sugar beet seed company that is potentially resistant to *H. schachtii*. Field tests conducted by Michigan Sugar Company found that beet yield was increased 6-10 tons/A (personal communication with G.W. Bird, 2006). A greenhouse experiment was conducted to determine the tolerance of Beta 5534N to *H. schachtii* as compared to a popular commercial *H. schachtii* susceptible variety.

Overall, Beta 5534N decreased overall *H. schachtii* population as well as decreased the fecundity of females that were successful enough to complete development under greenhouse conditions. From this study, Beta 5534N is a sugar beet variety that shows promise to being resistant to *H. schachtii*.

New Management Practices for the Soybean-Sugar beet Management System in the Presence of Three Phytopathogenic Nematodes

Heterodera glycines trap crop development

Out of 26 cultivars tested, three were carried into further experiments. Those were Berseem clover, Dackon oilseed radish, and Oriental mustard. The recommended planting of a trap crop is one month prior to the planting of the cash crop (soybeans). Economically, the three potential trap crops are currently not feasible. The oilseed radish is priced at $1.80/lbs and was planting it at 22lbs/A this equates to $39.80/A. The clover is priced at $1.50/lbs and was planted it at 12lbs/A this equates to $18.00/A. The mustard is priced at $1.50/lbs and was planted it at 10lbs/A this equates to $15.00/A. (Verbal correspondence with Davenport, 2006) However, if a grower is able to afford one of the above crops it is recommended that the field be in the southern part of the state (warmer climate) and that the trap crop be planted one month prior to planting of soybeans. Then again, further field studies are required to illuminate the impact of the trap crop under Michigan growing conditions. Also, additional laboratory analyses are needed to better understand the mechanism of the three cultivars in their reduction of *H. glycines* population densities.

Impact of *Pratylenchus penetrans* on *Heterodera glycines* resistant cultivars

P. penetrans is able to reproduce on all seven PI Lines used in commercial varieties of soybeans. Since *P. penetrans* and *H. glycines* co-exsist in *ca* 50% of all Michigan soybean fields and only PI 54840, PI 88788, and PI 437654 are grown, most of which is PI 88788, and it is likely that *P. penetrans* has the ability to successfully parasitize those varieties. Having this ability to reproduce on all seven *H. glycines* resistant lines could enable the nematode to cause soybean yield losses. Having a majority of all *H. glycines* resistant varieties carry PI 88788 resistances and knowing that *P. penetrans* has the ability to successfully reproduce on this line, seeing how *P. penetrans* affected the variety's resistance to *H. glycines* was the most logical step. It was found that *P. penetrans* does in fact affect the PI 88788 resistance source variety to *H. glycines*. It was seen that *P. penetrans* enables *H. glycines* to successful reproduce on a PI 88788 resistant source variety, which without *P. pentrans*; *H. glycines* would not be able to reproduce as well. When *H. glycines* control recommendations are made they are based upon the *H. glycines* population of a given field. Based on the above research the field population of *P. penetrans* should also be considered when developing a *H. glycines* control strategy for a grower. PI 88788 may in fact not be the best *H. glycines* resistant source for a grower to plant if the *P. penetrans* field population is relatively high.

Heterodera schachtii resistant variety development

All experiments that were conducted were preliminary in the development of a *H. schachtii* resistant sugar beet variety for Michigan. In both USDA greenhouse and field trials Hil-2 showed to be the most promising for a resistant sugar beet variety. Unfortunately, this variety was a private sector developed variety and could not continue into future USDA screening trials. The variety 2927-4 was another variety that showed probability of being a resistant variety in the field trial. This variety was developed in Salinas, CA. and has the potential of entering future *H. schachtii* resistant screening trials. The current commercial varieties available for Michigan sugar beet growers all proved to be susceptible to *H. schachtii.* In spite of this, at the end of both 6 and 8 week greenhouse trials Beta 5374 proved to have the most resistance characteristics as compared to all the other commercially available varieties tested. Recently, a private sector *H. schachtii* resistant variety has been developed and field trials were set up all around the state of Michigan. The greenhouse trial reveled that compared to a commercially available *H. schachtii* susceptible variety that there were 40% less cysts overall on the resistant variety. There was also 36% less eggs+J2s as compared to the susceptible variety. Overall, *H. schachtii* resistance development is a challenging endeavor. Based on the aforementioned research USDA available germplasm for resistance to *H. schachtii* is small. The commercially varieties available for Michigan sugar beet growers that were tested only one proved to have resistance characteristics, majority are very susceptible. For Michigan sugar beet growers Beta 5534N is a *H. schachtii*

tolerant variety that will be available in the very near future that will aide in the control of *H. schachtii*.

Table 1. Cultivars screened in preliminary trap crop evaluation greenhouse trial for the control of *Heterodera glycines*.

Common Name	Variety	*H. glycines* host status (from Riggs, 2004)
Alfalfa	Vernal	Poor Host
Alfalfa	wL252HQ	Poor Host
Clover	Berseem	Poor Host
Clover	Crimson	Poor Host
Clover	Kura	Poor Host
Clover	White	Poor Host
Clover	White Blossom, Sweet	Poor Host
Clover	Yellow Blossom, Sweet	Poor Host
Corn	DynaGrow SSK27 RR	Non-Host
Soybean	Kenwood 94	Host, Control
Soybean	INA	Resistant Host
Sorghum-Sudan Grass Hybrid	Sudax	Unknown Host
Sugar beet	Crystal 963	Unknown Host
Vetch	Hairy	Poor Host
Cowpea	Red Ripper	Unknown Host
Cabbage	Early Jersey Wakefield	Unknown Host
Rye Grass	Annual	Unknown Host
Oil seed Radish	Adagio	Unknown Host
Oil Seed Radish	Colonal	Unknown Host
Oil Seed Radish	Rimbo	Unknown Host
Oil Seed Radish	Generic	Unknown Host
Oil Seed Radish	Dackon	Unknown Host
Oil Seed Radish	Arena	Unknown Host
Mustard	Oriental	Unknown Host
Oat	IDA	Unknown Host
Pea	Sugardaddy	Host

Table 2. Seeding rate of *Heterodera glycines* trap crops in the Monroe County field trial/demonstration plot.

Treatment	Per Plot	Per Acre
SCN Susceptible Soybean	33 Seeds	160,000 Seeds
Corn	7 Seeds	31,000 Seeds
Clover, Berseem	1.12 g	5.44 kg Seed
SCN Resistant Soybean	33 Seeds	160,000 Seeds
Lespedeza, Common	1.88 g	9.07 kg Seed
Mustard, Oriental	0.94 g	4.53 kg Seed
Oilseed Radish, Dackon	2.1 g	9.97 kg Seed

Table 3. Seeding rate of treatments for Saginaw County trap crop yield determination field trial.

Treatment	Per Acre
SCN Resistant Soybean	142,000 seeds
Clover, Berseem	5.44 kg
Mustard, Oriental	4.53 kg
Oilseed Radish, Dackon	8.16 kg

Table 4. Mean number of cyst and females of *Heterodera glycines* obtained from 100cm^3 soil in the preliminary trap crop greenhouse trial.

Treatment	Females[1]	Cyst[1]
Vernal Alfalfa	0 b	0 b
wL252HQ Alfalfa	0 b	0 b
Berseem Clover	0.2 a	0 b
Crimson Clover	0 b	0 b
Kura Clover	0 b	0 b
White Clover	0 b	0 b
White Blossom, Sweet Clover	0 b	0 b
Yellow Blossom, Sweet Clover	0 b	0 b
DynaGrow Corn	0 b	0 b
Kenwood 94 Soybean	3.2 N/A[2]	334.71N/A[2]
INA Soybean	0.3 N/A[2]	6.5 N/A[2]
Sudax	0.14 a	0 b
Crystal 963 Sugarbeet	0 b	0 b
Hairy Vetch	0 b	0 b
Red Ripper Cowpea	0 b	0 b
Early Jersey Wakefield, Cabbage	0 b	0 b
Annual Rye Grass	0 b	0 b
Adagio Oilseed Radish	0 b	0 b
Colonel Oilseed Radish	0 b	0 b
Rimbo Oilseed Radish	0 b	0 b
Generic Oilseed Radish	0 b	0.2 b
Oriental Mustard	0 b	0.4 a
IDA Oats	0 b	0 b
Dackon Oilseed Radish	0 b	0.42 a
Sugardaddy Pea	0.6 N/A[2]	8.5 N/A[2]
Arena Oilseed Radish	0 b	0 b

[1] Column means followed by the same letter are not significantly different (P =0.05) according Fisher's LSD.(N=7)
[2] Not included in statistical analysis

Table 5. Mean early second-stage juveniles of *Heterodera glycines* in roots of seven plants at weekly intervals during host status trial.

Treatment[1]	20-June	27-June	4-July	11-July	18-July
SCN Susceptible Soybean	45	28	186	17	8
Corn	0	0	0	N/S[2]	0
Clover, Berseem	32	47	0	5	N/S[2]
SCN Resistant Soybean	82	23	192	15	20
Lespedeza, Common	0	4	8	46	54
Mustard, Oriental	9	24	0	N/S[2]	N/S[2]
Oilseed Radish, Dackon	38	57	12	9	12

[1] N=7
[2] No plants were found to be up on that sampling day

Table 6. Mean late fourth-stage (sausage-stage) juveniles of *Heterodera glycines* in roots of seven plants at weekly intervals during host status trial.

Treatment[1]	20-June	27-June	4-July	11-July	18-July
SCN Susceptible Soybean	30	59	46	28	8
Corn	0	0	0	N/S[2]	0
Clover, Berseem	0	0	0	0	N/S[2]
SCN Resistant Soybean	45	34	18	5	2
Lespedeza, Common	35	4	9	35	59
Mustard, Oriental	13	5	0	N/S[2]	N/S[2]
Oilseed Radish, Dackon	3	2	0	0	6

[1] N=7
[2] No plants were found to be up on that sampling day

Table 7. Mean difference (final-initial) of *Heterodera glycines* [egg +J2] population densities in Monroe County Farmer Education field trial.

Treatment	Mean difference (initial-final) [1]
H. glycines susceptible soybean	3528.6 b
Corn	- 135.9 a
Clover, Berseem	- 46.3 a
H. glycines resistant soybean	- 76.6 a
Lespedeza, Common	- 290.9 a
Mustard, Oriental	- 486.7 a
Oilseed Radish, Dackon	- 169.7 a

[1] Column means followed by the same letter are not significantly different (P =0.05) according to Fisher's LSD. (N = 7)

Table 8. Mean difference (final – initial) of *Heterodera glycines* [egg + J2] population densities in greenhouse trap crop validation trial.

Treatment	Egg + J2 mean difference (final –initial) [1]
SCN Susceptible Soybean	22624 a
Clover, Berseem	-1439 b
Fallow	-1468 b
Oilseed Radish, Dackon	-1605 b
Mustard, Oriental	-1628 b

[1] Column means followed by the same letter are not significantly different (P =0.05) according to Fisher's LSD. (N=7)

Table 9. Soybean yield in bu. /A from Saginaw County trap crop field trial, all trap crops were spray killed and plots were planted to an SCN susceptible soybean and then harvested in October, 2006.

Treatment	Mean[1] (bu/A)	Standard Deviation	Range
Fallow	41.74	± 4.75	36.99 - 46.49
Mustard, Oriental	42.88	± 4.29	38.59 - 47.17
SCN Resistant Soybean	40.07	± 2.31	37.70 - 42.38
Oilseed radish, Dackon	41.52	± 2.45	39.07 - 43.97
Clover, Berseem	41.40	± 2.37	39.03 - 43.77

[1] No significant difference in treatments

Table 10. Summary means of *Heterodera schachtii* cyst and female counts at day 42 and 56 on four sugar beet breeding line germplasm lines greenhouse trial.

Treatment	42 Day		56 Day	
	Females[1]	Cyst	Females[1]	Cyst[1]
Hil-2	7.0 a	8.0	0.0 a	3.2 a
HM E-17	48.3 ab	8.3	110.0 b	46.3 b
N224	49.4 ab	13.1	41.7 a	18.9 a
N 172	59.4 b	15.4	20.9 a	26.0 ab

[1] Column means followed by the same letter are not significantly different (P =0.05) according Fisher's LSD. (N = 7)

Table 11. Percentage of eggs or juveniles of *Heterodera schachtii* that were able to successfully mature into cysts/ females on 20 commercial sugar beet varieties at the end of 42 days in the greenhouse.

Treatment	Female-Cyst (%)[1]	Clasification of Resistance[2]
Beta 5471	1 a	Resistant
Beta 5374	1.2 a	Resistant
5X Spartan	1.7 a	Resistant
Holly 02HX272	1.8 a	Resistant
Crystal 1353	1.9 a	Resistant
HM E-38	4.2 a	Resistant
HM E-33	14.9 ab	Somewhat Resistant
Beta BK 1383R	16.4 ab	Somewhat Resistant
Crystal 271	18.7 abc	Susceptible
Beta BK 1381R	20.6 abc	Susceptible
Crystal R353	21.0 abc	Susceptible
HM 7172RZ	23.5 abc	Susceptible
Beta 5736	26.0 abc	Susceptible
HM RH-5	27.9 abc	Susceptible
HM 2761RZ	28.6 abc	Susceptible
HM E-17	36.9 bcd	Highly Susceptible
5X Prompt	39.4 bcd	Highly Susceptible
Crystal 913	44.3 bcd	Highly Susceptible
Beta 5310	46.4 bcd	Highly Susceptible
Crystal 963	65.9 bcd	Highly Susceptible

[1] Column means following by the same letter are not significantly different (P =0.05) according to Fisher's LSD. (N=7)
[2] Classification of resistance was determined by if there was a higher susceptibility index, the greater the susceptibility (the percentage of mature females upon the variety)

Table 12. Percentage of eggs of juveniles of *Heterodera schachtii* that were able to successfully mature into cysts/ females on 20 commercial sugar beet varieties at the end of 56 days in the greenhouse.

Treatment	Female-Cyst (%)[1]	Classification of Resistance	Developmental Dynamics[2]
Beta BK 1383R	18.9 a	Resistant	+
Beta 5374	21.5ab	Resistant	0
Holly 02HX272	24.1 abc	Resistant	0
HM E-33	30.5 abc	Somewhat Resistant	–
HM RH-5	30.7 abc	Somewhat Resistant	+
Crystal 1353	32.4 abc	Somewhat Resistant	–
HM E-38	32.6 abc	Somewhat Resistant	–
Crystal 271	35.6 abc	Somewhat Resistant	+
Beta BK 1381R	36.4 abc	Somewhat Resistant	+
Beta 5471	37.7 bc	Somewhat Resistant	–
HM E-17	41.6 cd	Susceptible	+
HM 2761RZ	41.7 cd	Susceptible	0
Crystal R353	43.3 cd	Susceptible	0
Beta 5736	43.4 cd	Susceptible	0
5X Prompt	44.3 cd	Susceptible	0
5X Spartan	47.4 cde	Susceptible	0
HM 7172RZ	58.9 de	Highly Susceptible	–
Beta 5310	61.2 e	Highly Susceptible	+
Crystal 913	61.4 e	Highly Susceptible	+
Crystal 963	80.9 f	Most Susceptible	0

[1] Column means following by the same letter are not significantly different (P =0.05) according to Fisher's LSD. (N = 7)
[2] Comparative changes (42 vs. 56 days) in female-cyst development as a percent of original population
+ = Increase
– = Decrease
0 = No change

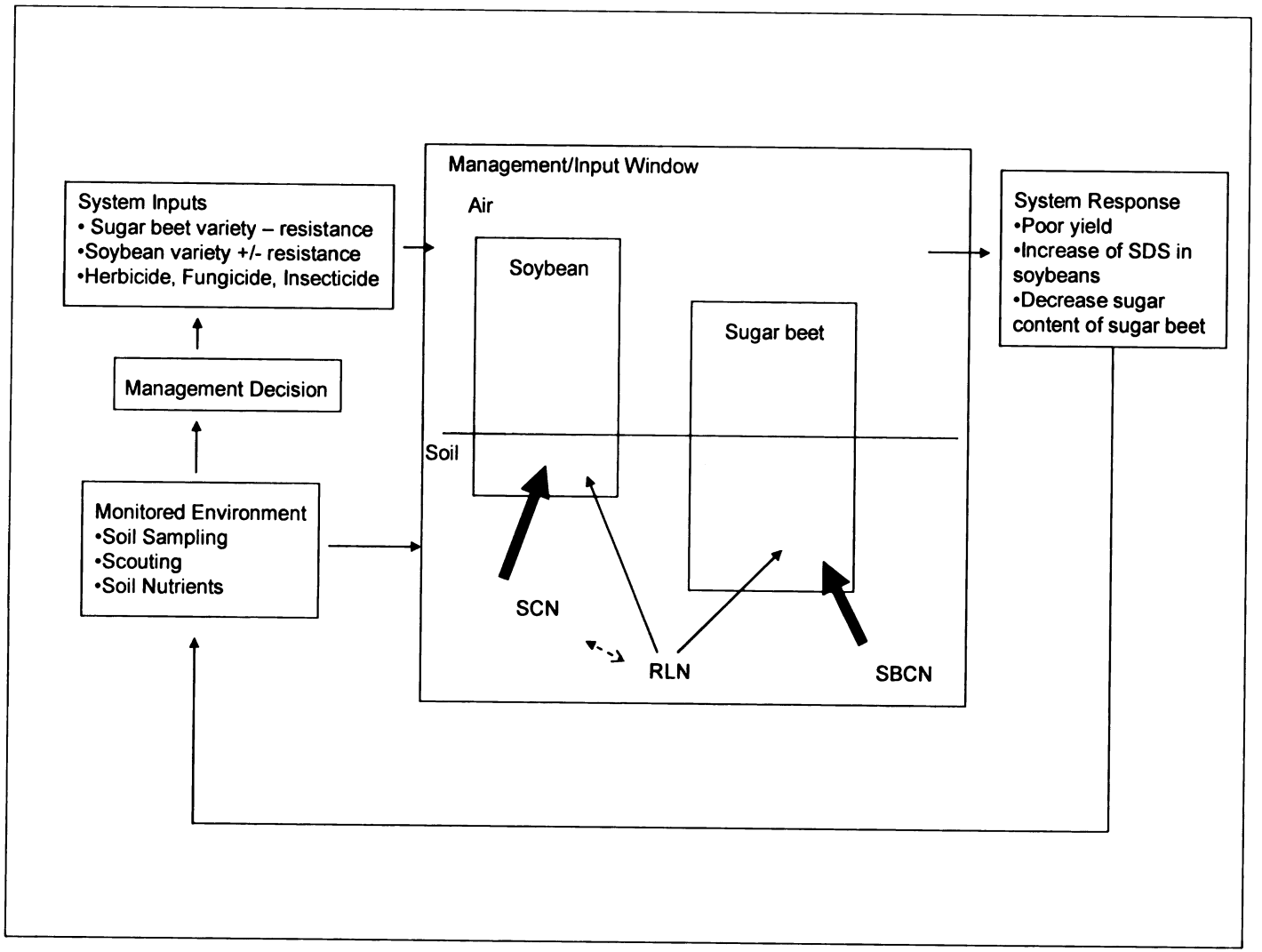


Figure 1. Conceptual model of the current Michigan soybean-sugar beet management system in the presence of three phytopathogenic nematodes, *Heterodera glycines*, *H. schachtii*, and *Pratylenchus penetrans*.

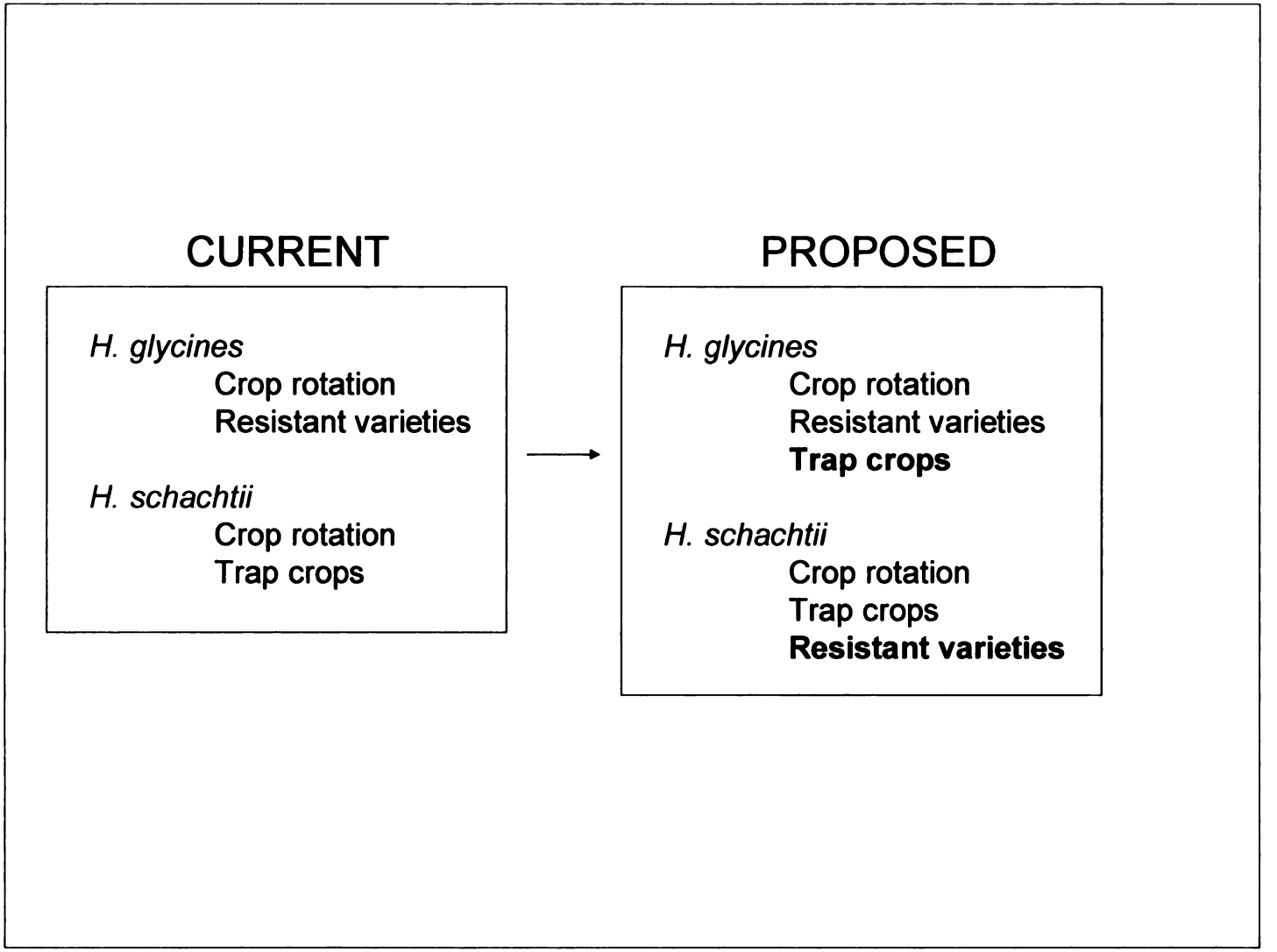


Figure 2. *Heterodera glycines* and *H. schachtii* current and proposed management practices for Michigan. (Management practices in bold are primary focus of research)

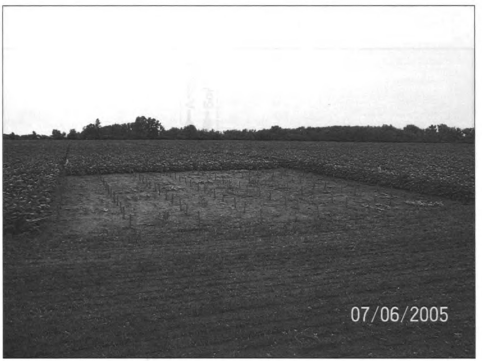


Figure 3. 2005 Monroe County grower education field plot.

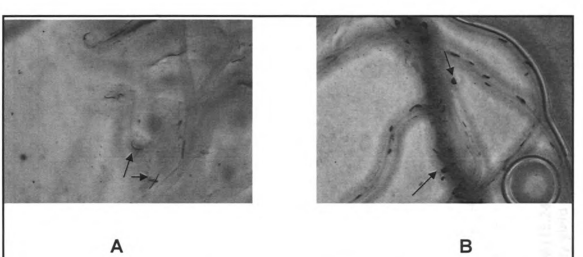


Figure 4. Photographs of *Heterodera glycines* development in Berseem clover (A) and *H. glycines* susceptible soybean (B) after 14 days. (Arrows indicate *H. glycines* stages)

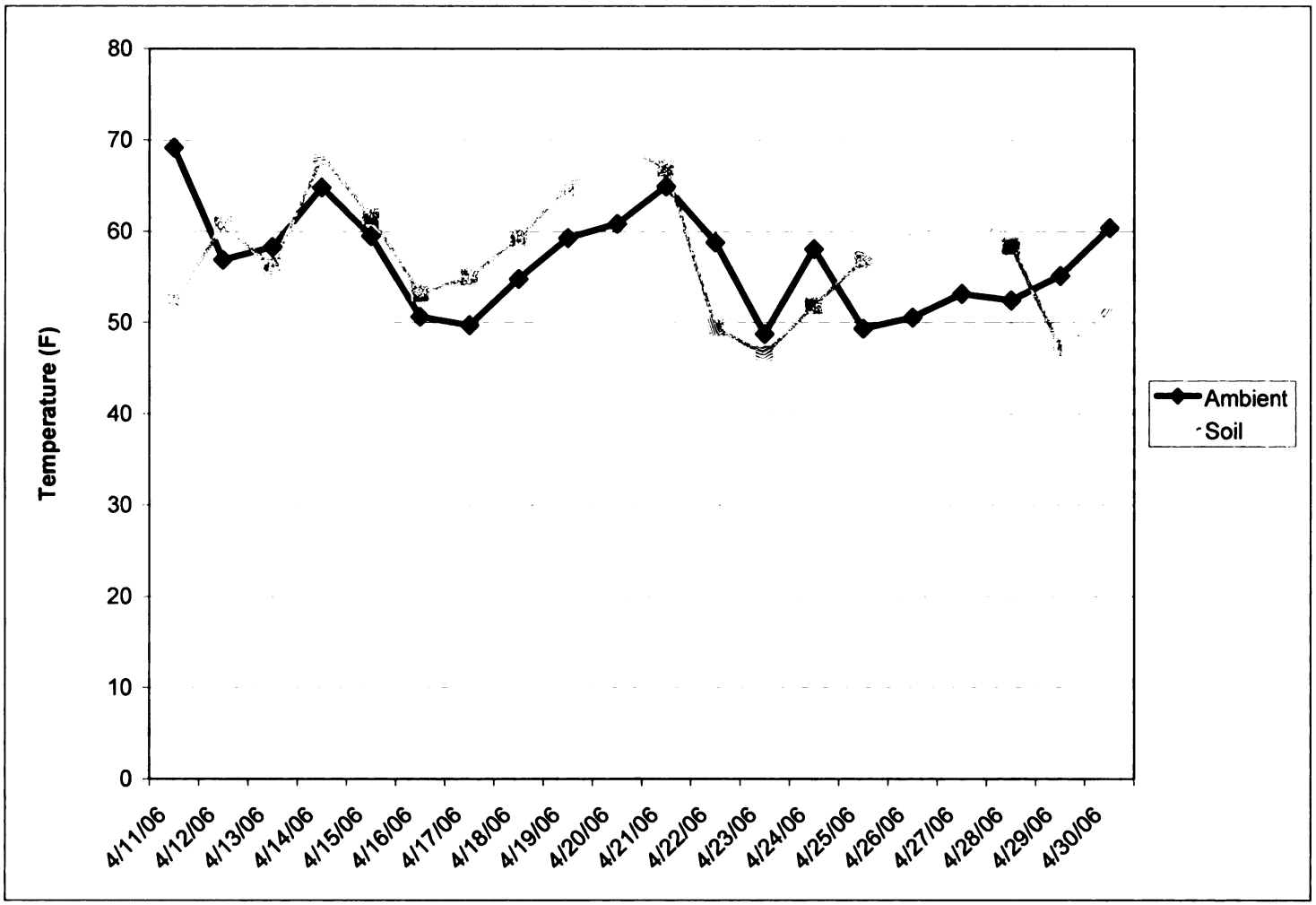


Figure 5. Mean soil (15.24 cm below ground) and ambient (15.24 cm above ground) over one month temperature monitoring for Saginaw county yield impact trap crop trial.

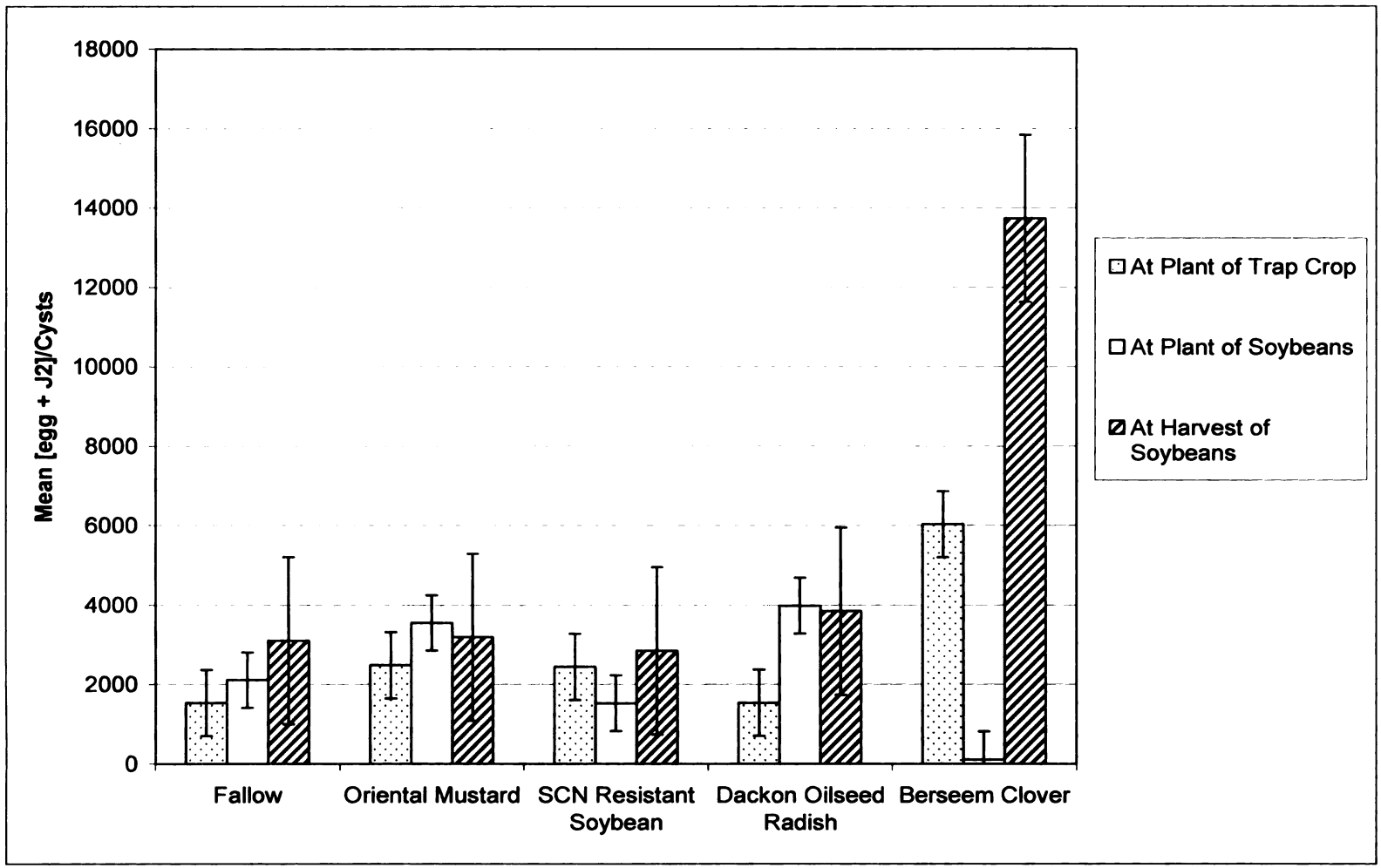


Figure 6. Mean [eggs+J2s] per cyst in 100cm^3 of soil from three sampling periods during the Saginaw County trap crop yield impact trial.

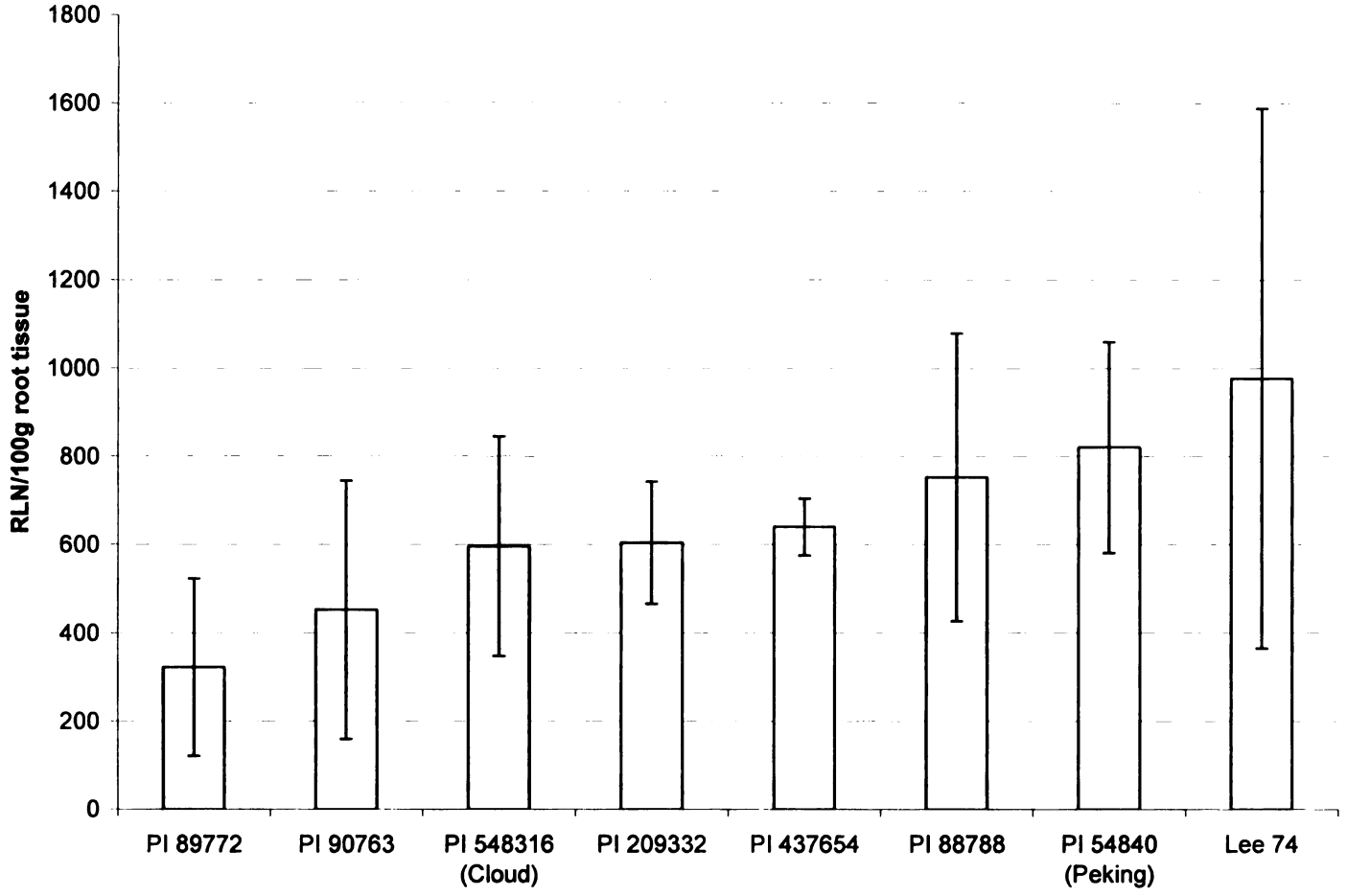


Figure 7. Mean reproductive potential of *P. penetrans* upon *H. glycines* resistant PI Lines. (Lee 74 is a *H. glycines* susceptible control for doing the HG Type Test)

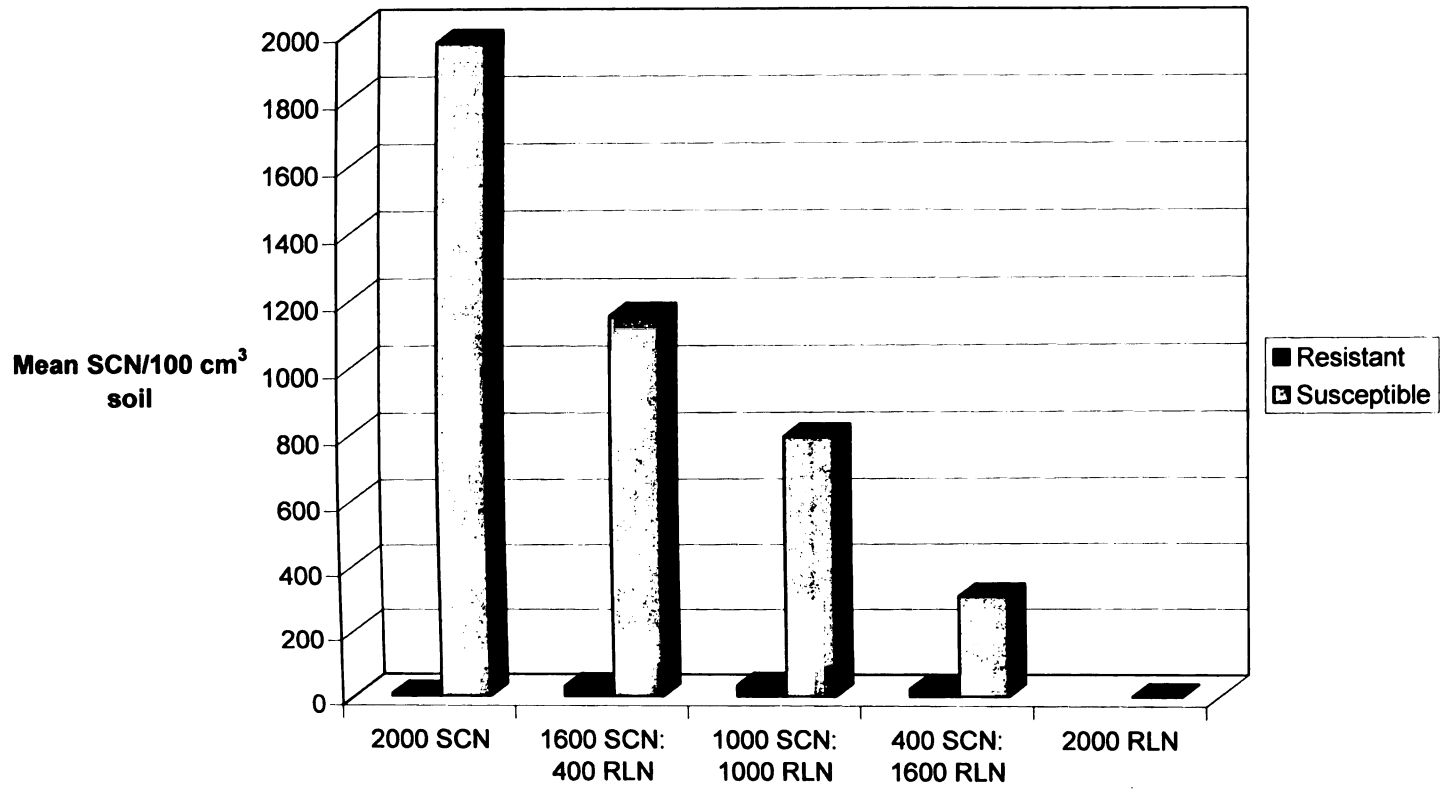


Figure 8. Influence of *Pratylenchus penetrans* on *Heterodera glycines* reproduction on *H. glycines* resistant *Glycine max* (PI 88788)

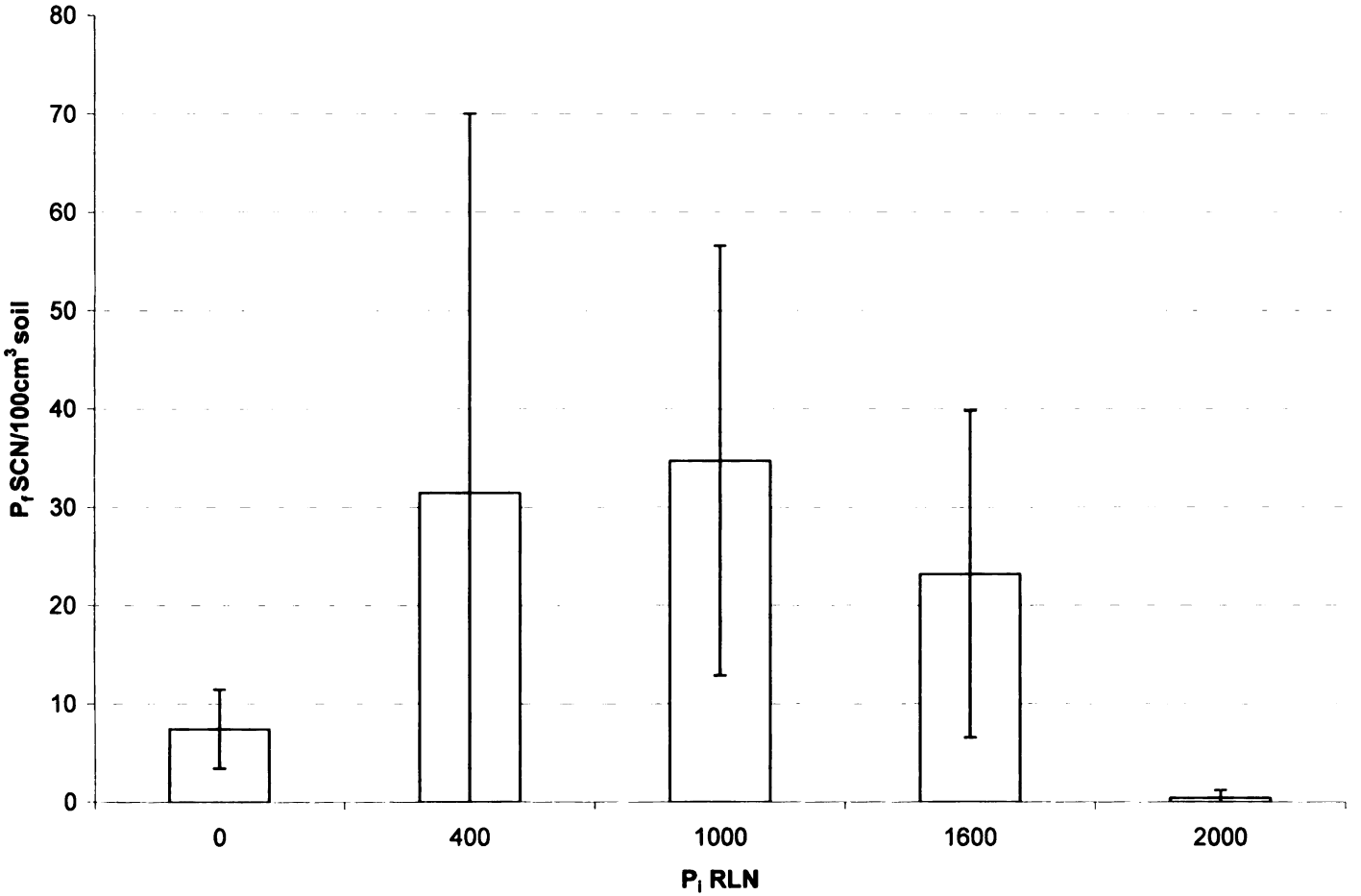


Figure 9. Relationship among initial (P_i) *Pratylenchus penetrans* and final (P_f) population densities of *Heterodera glycines* on *H. glycines* resistant (PI 88788) soybean.

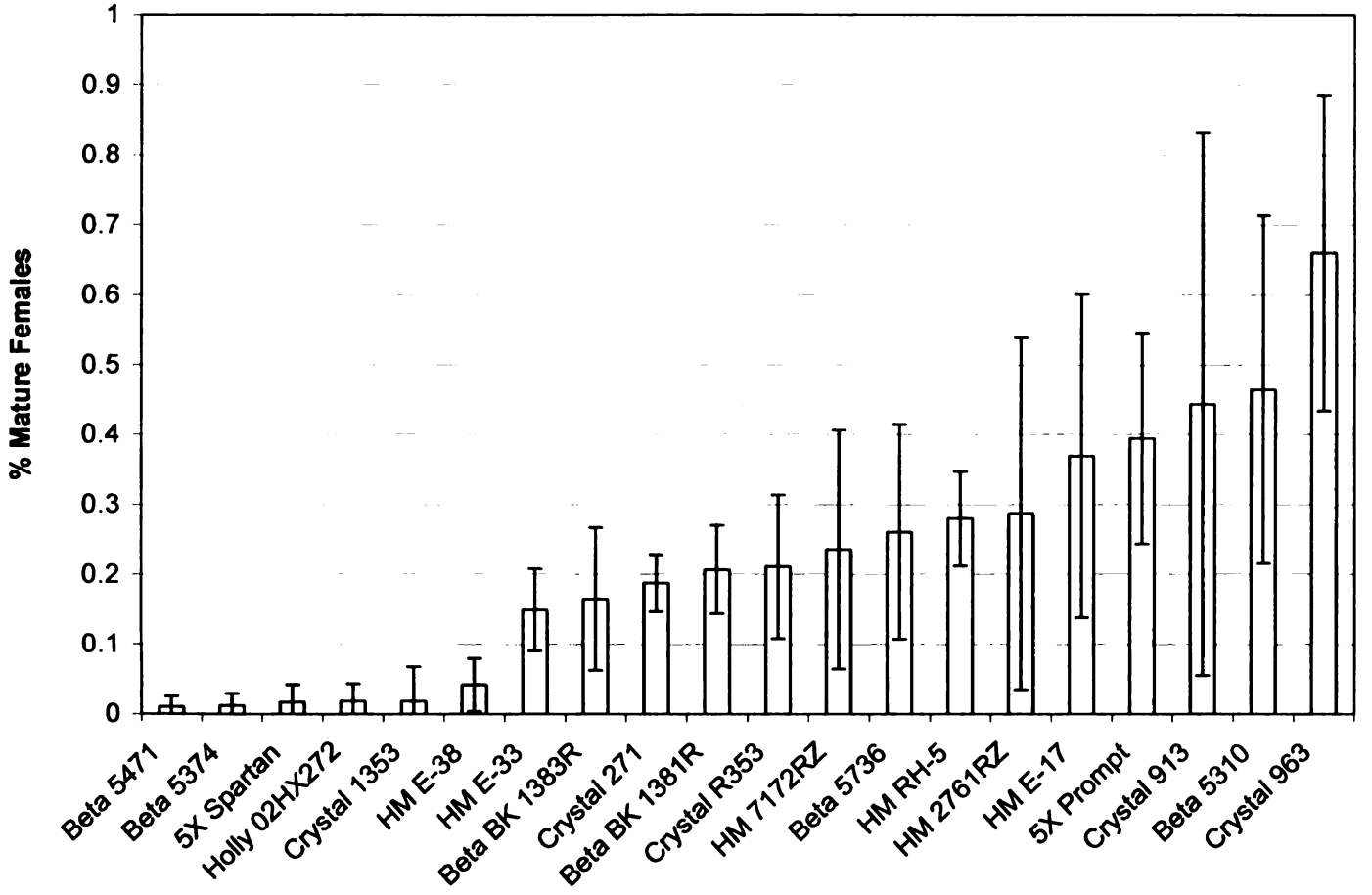


Figure 10. *Heterodera schachtii* female development on 20 commercial sugar beet varieties after 42 days under greenhouse conditions.

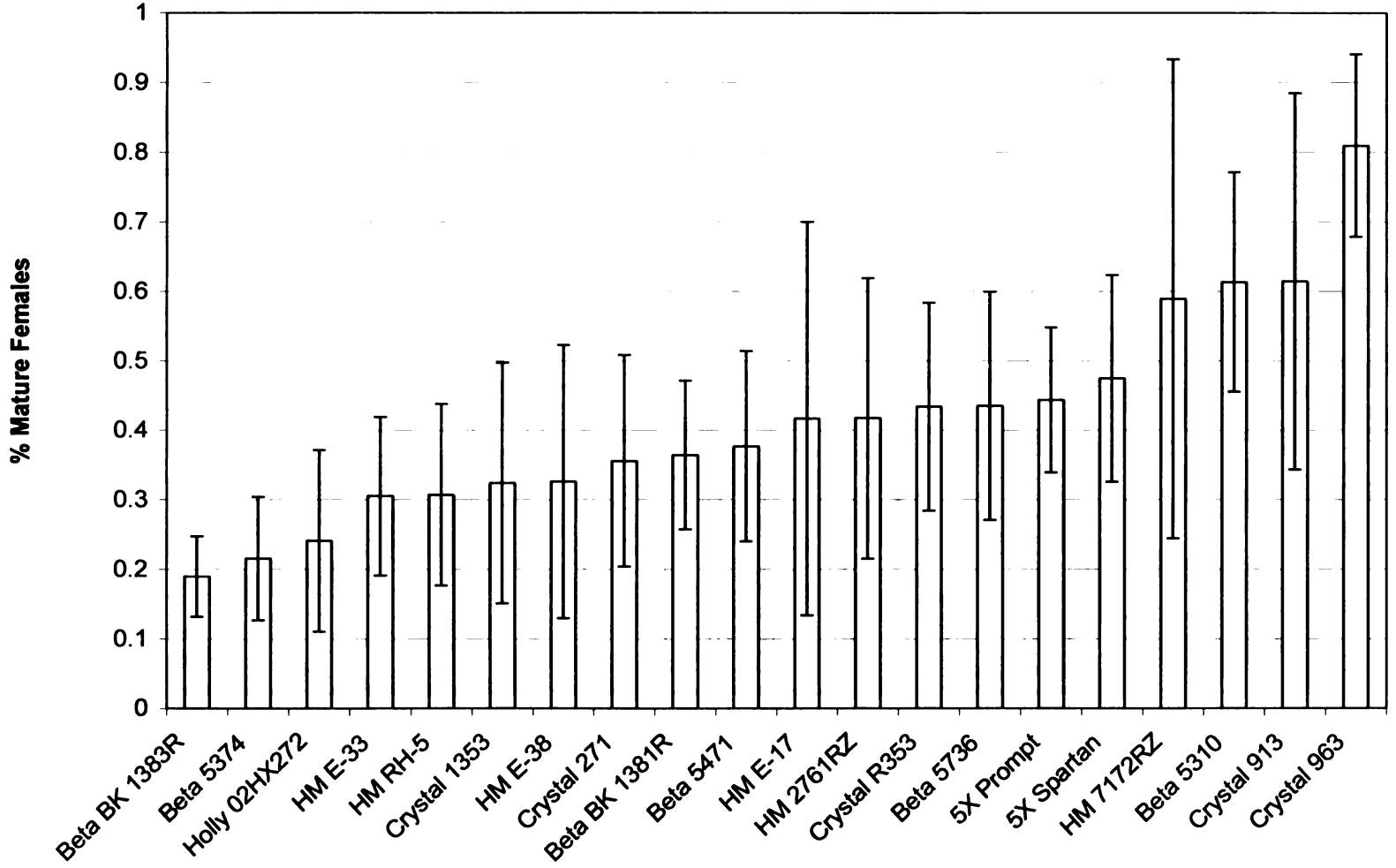


Figure 11. *Heterodera schachtii* female development on 20 commercial sugar beet varieties after 56 days under greenhouse conditions.

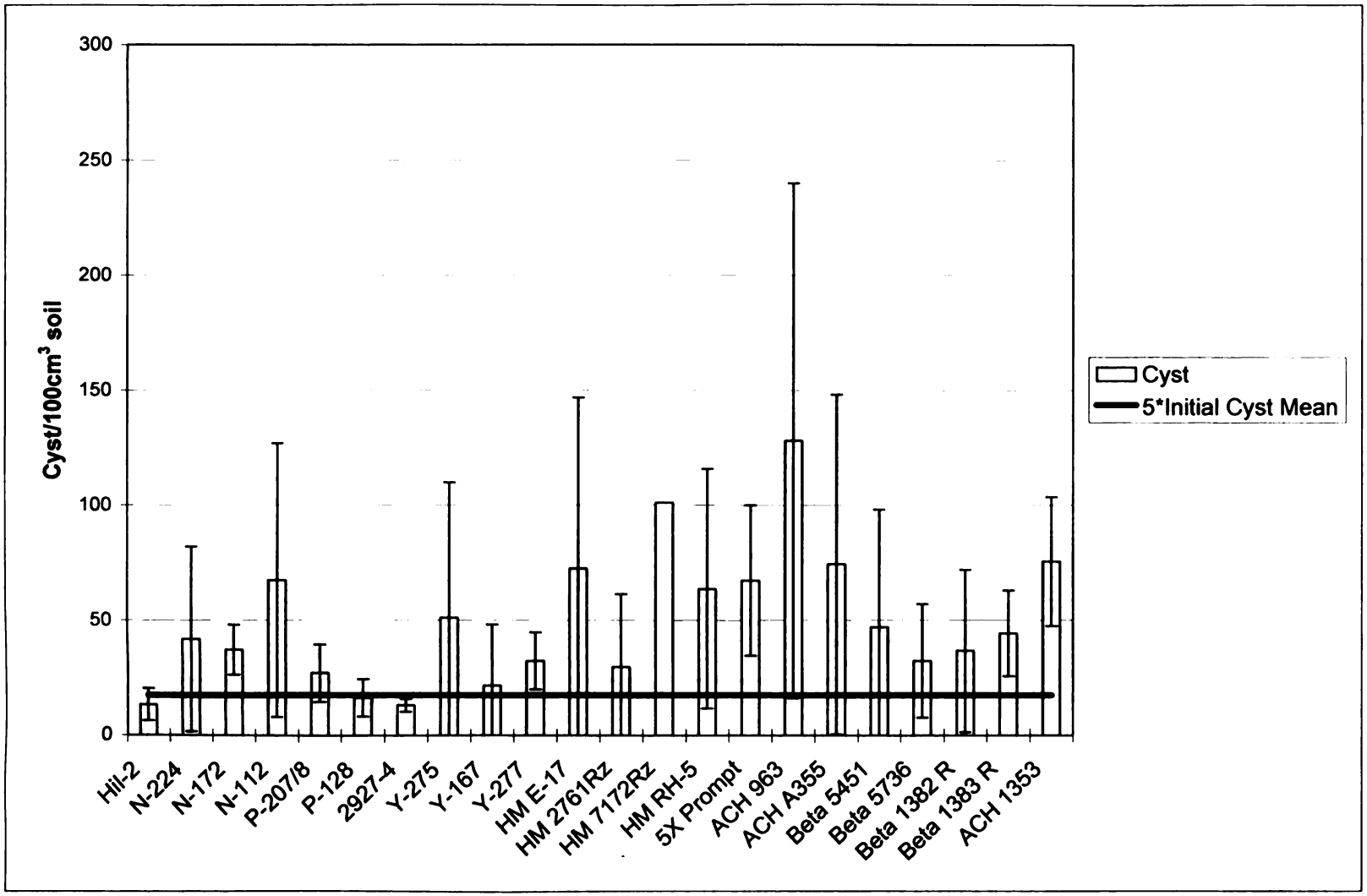


Figure 12. ***Heterodera schachtii*** **cyst development on 22 sugar beet lines under Michigan field growing conditions in Bay County, Michigan (P_i = 17.5 cyst/ 100cm^3 soil)**

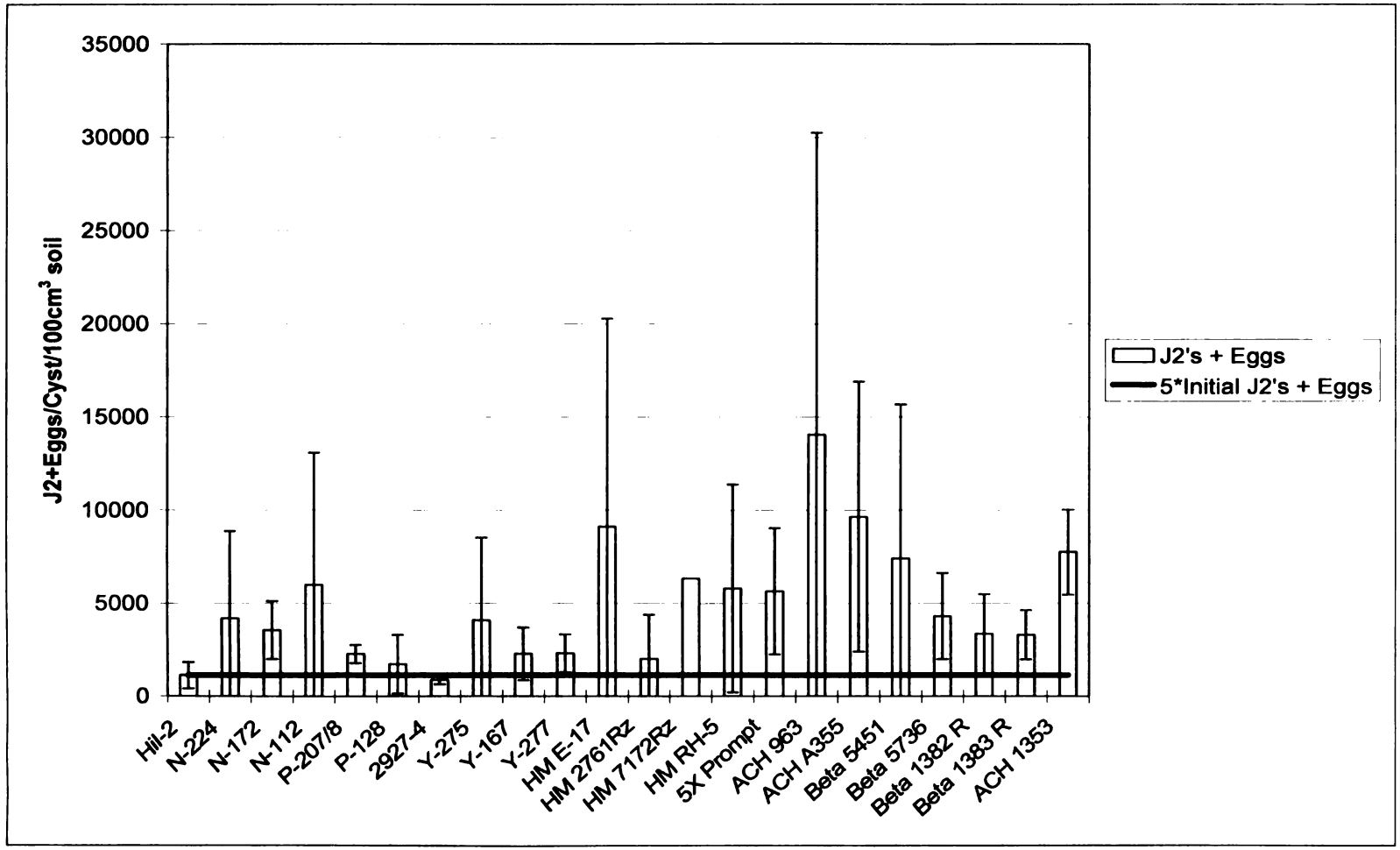


Figure 13. Reproductive potential ([eggs+J2s/cyst]) of *Heterodera schachtii* on 22 sugar beet lines grown under Michigan field growing conditions in Bay County, Michigan (P_i = 1387.5 [eggs+J2s]/100cm3 soil)

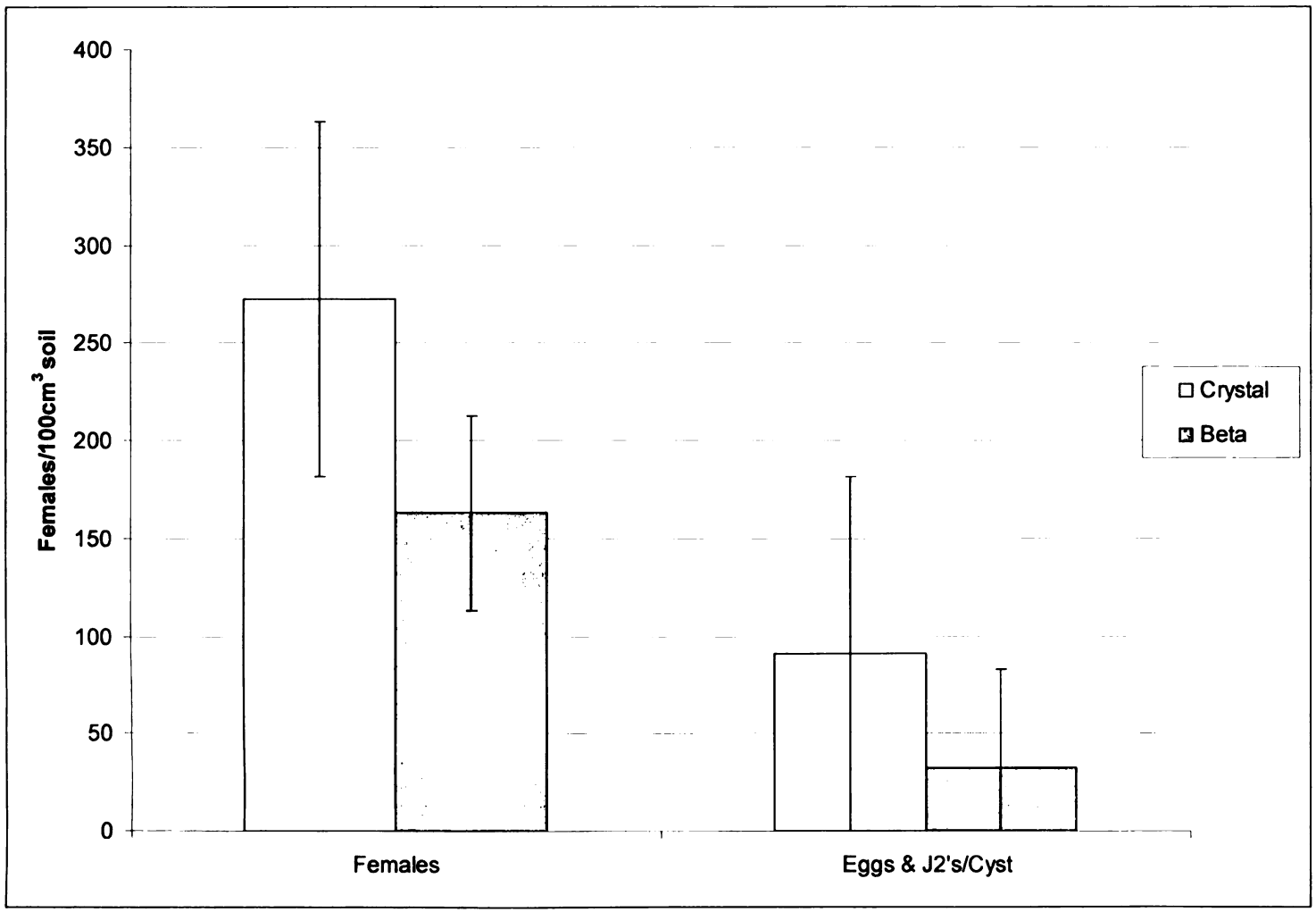
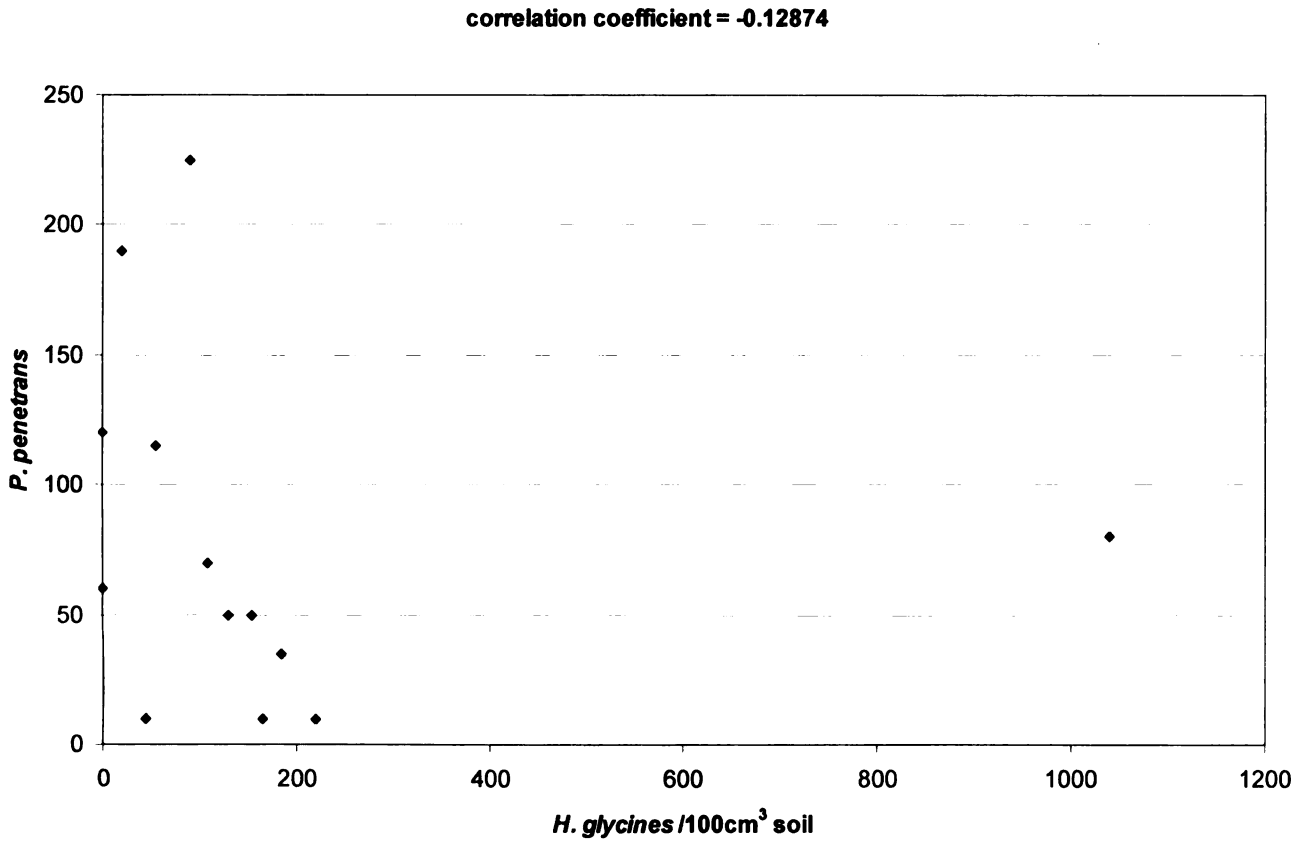
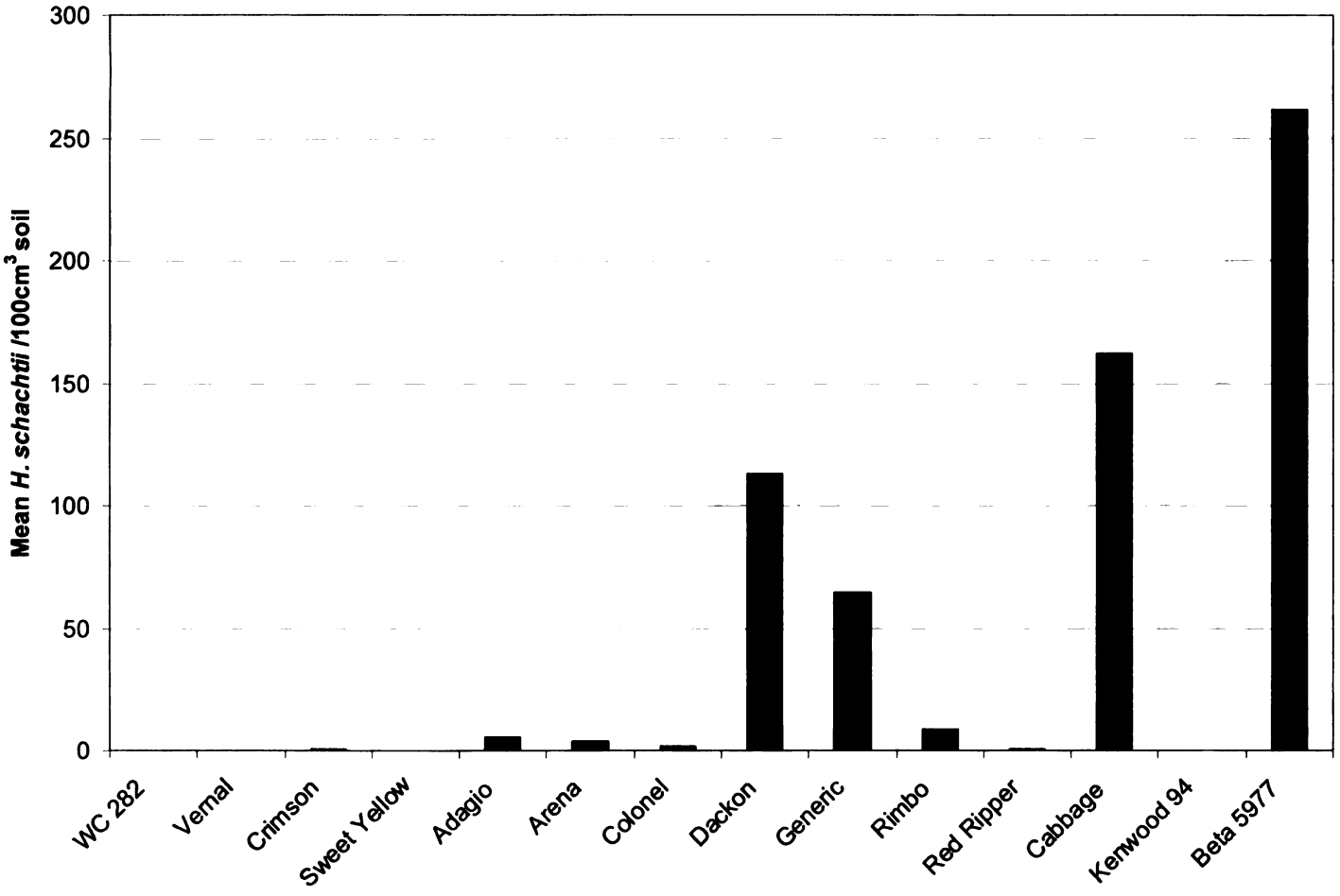


Figure 14. *Heterodera schachtii* female and [egg + J2] population densities on *H. schachtii* susceptible Crystal 963 and *H. schachtii* tolerant Beta 5534N.

Appendix



Appendix 1. Relationship between *Heterodera glycines* to *Pratylenchus penetrans* on a *H. glycines* resistant soybean variety under field conditions at Kendle Farm, Cass County, MI.



Appendix 2. 2002 Greenhouse Trial to determine the influence of cover crop varieties on the reproduction of *Heterodera schachtii*.

Appendix 3. Handout for Monroe County Farmer Field Day

Monroe County Soybean Cyst Trap Crop Trial

Treatments and Objective for Planting

1: SCN Susceptible Soybean (33 seeds or 160,000 seeds/A)
2: Corn (7 seeds or 31,000 seeds/A) (SCN non-host)
3: Clover, *Berseem* (1.12g or 12 lbs seed/A) (Possible "trap crop"
4: INA SCN resistant soybean variety PI 437654 + PI 88788 (33 seeds or 160,000 seeds/A)
5: Lespedeza, *Common* (1.88g or 20 lbs/A) (Possible "trap crop")
6: Mustard, *Oriental* (0.94 g or 10 lbs/A) (Possible "trap crop"
7: Oil Seed Radish, *Dackon Common* (2.1g. or 22 lbs/A) (Possible "trap crop)

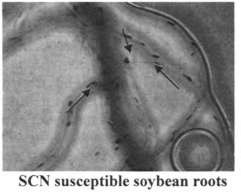
Note: Planted on 6/16/05
There is a 2' alley between each replicate
Each treatment is 3'x3'

VII	**2**	**3**	**4**	**5**	**6**	**7**	**1**
VI	**3**	**4**	**5**	**6**	**7**	**1**	**2**
V	**4**	**5**	**6**	**7**	**1**	**2**	**3**
IV	**5**	**6**	**7**	**1**	**2**	**3**	**4**
III	**6**	**7**	**1**	**2**	**3**	**4**	**5**
II	**7**	**1**	**2**	**3**	**4**	**5**	**6**
I	**1**	**2**	**3**	**4**	**5**	**6**	**7**

Road

N↑

Berseem clover roots stained for SCN at 2 weeks post-plant (photo shows SCN juveniles)



SCN susceptible soybean roots stained for SCN at 2 weeks post-plant (photo shows multiple stages of SCN)

(Page two of handout)

Introduction:

In Michigan, soybeans are a highly economical crop. Unfortunately for soybean grower's soybean cyst nematode (SCN) was first detected in Michigan in 1987 and causes yield loss in 31 soybean growing counties (Warner and Bird 2000). SCN can greatly reduce soybean yields ranging from 5 percent to more than 90 percent (Riggs and Wrather, 1992). Regrettably, nematicides are extremely costly and therefore not a viable option for control of SCN. Therefore, an integrated management approach is vital, primarily focusing on trap crop systems. By the author's definition a trap crop for nematodes is a crop that allows the nematode to penetrate and enter the root; however, the nematode is not able to complete a life cycle and therefore no reproduction will take place. Sugar beet growers here in Michigan have adopted the use of oilseed radish (OSR) varieties for control of *Heterodera schachtii*, sugar beet cyst nematode (SBCN). Field studies, unpublished by the Bird laboratory indicate that a spring crop of OSR before soybeans is a more reliable practice for lowering *H. schachtii* population densities and increasing subsequent sugar beet yields than a late summer planting of OSR following wheat. In addition, Warner, unpublished, in 2003, found in a greenhouse study that the role of cover crops in the control of nematode populations is cultivar specific. He and his colleagues found that while OSR cvs *Colonel* and *Adagio* are appropriate trap crops for *H. schachtii*, other cultivars of OSR are not. In addition, OSR cv *Colonel* was found to be a good host for reproduction of both *Pratylenchus penetrans* (Root-lesion nematode) and *Melodogyne hapla* (Root-knot nematode). Confounding the problem more is the fact that many fields in Michigan have all four nematodes, (SCN, SBCN, Root-lesion, and Root-knot nematode). Additionally to the discovery of a trap crop for soybean cyst nematode, further understanding of the interaction and contribution to biotic potential of these four nematodes. Also, their interactions and how they affect resistant cash crops need to also be expounded upon.

Objectives:

The primary focus of this project will be to develop a trap crop system to control soybean cyst nematode due to its economical importance to the soybean industry. This is complicated by the fact that most soybean fields in Michigan do not only have soybean cyst nematode but three other semi-economical important nematodes. These would be Root-lesion nematode, Sugar beet cyst nematode, and Root-Knot nematode. These nematodes are found in fields that grow both sugar beets and soybeans. Fortunately, there are soybean cyst nematode resistant soybeans and there is a sugar beet cyst nematode resistant sugar beet variety in development. However, the author found in an unpublished greenhouse study that having equal populations of Root-Lesion nematode and soybean cyst nematode upon a SCN resistant variety soybean, it therefore becomes susceptible to soybean cyst nematode. Further experiments into these interactions upon resistant varieties will be looked at using greenhouse studies. In addition to the greenhouse studies of interaction, two field trials for trap crops will be set up around the state.

Appendix 4

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: **2006-06**

Title of thesis or dissertation (or other research projects):

Bionomics and Control of Two *Heterodera* spp. in Michigan

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Cassandra Lee Bates

Date November 28, 2006

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 4 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 4 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Appendix 4.2

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Heterodera glycines	Collected from: Azalia, MI.					14			A.J Cooke Arthropod Collection
	Edwardsberg,MI.					8			
	St. Charles, MI.					15			
Heterodera schachtii	Bay City, MI.					15			
Heterodera spp.	Bay City, MI.					10			

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Cassandra Lee Bates

Date November 28, 2006

Voucher No. 2006-06

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator 29 NOV 2006 Date

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