Evaluations of novel, synthetic compounds for control of the soybean cyst nematode (*Heterodera glycines* Ichinohe)

by

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A thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Plant Pathology

Major Professor: Gregory L. Tylka

Iowa State University

Ames, Iowa

2001
Graduate College
Iowa State University

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Signatures have been redacted for privacy
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ACKNOWLEDGEMENTS

I would like to recognize the following people:

Plant Pathology Graduate Students: Alka, Walber, Leonor, Ryan, Emily, Cassie, Dean, Wei, Paul, Hari, Christine, and Dirk - for their support and friendship.

Plant Pathology Faculty members - for sharing their passion for science and research.

Mary Jo Vivian and Dave Volkers - for their energy invested and their ability to answer all questions.

Program of Study committee members: Dr. Joel Coats, Dr. Greg Miller, Dr. Thomas Baum, and Dr. Gregory Tylka - for their guidance and thought-provoking advice. Also, Dr. Tylka's role as my major professor and for his example as a public educator.

The Nematology lab - a special thanks to Susan Souhrada, Chris Marett, and David Soh, who introduced me to the world of 'tode busting.

I am grateful for the cooperative efforts of those who supplied the experimental compounds, and also for their interest and involvement with this study.

♦ Methyl pelargonate was provided by Dr. Jerry Feitelson and Chuck Dullum, previously of Mycogen Corporation.
♦ CHP was provided by the laboratory of Dr. Joel Coats of the Department of Entomology at Iowa State University.
♦ The AA and Va compounds were provided by the laboratory of Dr. George Kraus of the Chemistry Department at Iowa State University.

Lee Brady, the greatest finding of this research - for his ideas and inspiration.

My colleagues: Dirk Charlson, Dean Reynolds, and Walber Gavassoni - for their camaraderie, and for being my best teachers.

Dr. Braun, thank you for your encouragement.

This thesis is dedicated to my parents, Jim and Kathy Knips, for their enthusiastic and never-ending support, and for their sudden interest in nematodes.
GENERAL INTRODUCTION

*Glycine max* (soybean)

**Production and uses**

The soybean (*Glycine max* (L.) Merill) is a leguminous crop, believed to have originated in China (Norman, 1978). Major production was not established in the United States until 1940, and at that time, soybeans mainly were used as a forage crop (Norman, 1978). Between 1945 and 1975, production increased over eight-fold, due in part to the development of twelve maturity groups better suited to the growing regions. Soybeans are now the second major cash crop in the United States.

The corn belt states, which include Iowa, Illinois, Minnesota, Indiana, Ohio, and Missouri, account for 60% of the U.S. soybean production. In 1998, Iowa led the nation in production with 501,600,000 bushels worth more than $2.5 billion (Iowa Agricultural Statistics, 1999).

The versatility of the soybean is evident when considering the range of nonedible and edible soy products. The major force that influences the market for nonedible soy products today is environmental concerns over the use of synthetic chemicals (D. J. Myers, Associate Professor of Food Science and Health Nutrition, Iowa State University, personal communication). Potentially popular nonedible soy products include wood adhesives, plastics, and fibers. Efforts to improve these products focus on increasing water resistance and reducing microbial degradation (D. J. Myers, personal communication).
Biodiesel, introduced into the United States in 1992, is another valuable product of soybeans. Biodiesel is a biodegradable and nontoxic fuel option produced by renewable biological sources such as vegetable oils. Reduction of harmful emissions and utilization of surplus soybean oil are two important advantages of biodiesel over alternative fuel sources (Caruana, 1992).

There also is a growing demand for edible soy products. Soy protein as a food additive repeatedly has been shown to reduce the risk of heart disease, breast cancer, and osteoporosis (Messina, 1995). The continual pursuit of healthy living, whether aimed at reduction of pollution or improved eating habits, suggests that the popularity of soy products is more than a passing phase.

*Heterodera glycines* (soybean cyst nematode)

**Past and present**

While the popularity of soy products has been increasing, yields are being threatened by a soil pathogen in all major soybean-producing areas in the United States. (Doupnik, 1993; Pratt and Wrather, 1998; Wrather et al., 1995). This pathogen, *Heterodera glycines* Ichinohe, the soybean cyst nematode, recently has gained increased attention by the global agricultural community. Soybean cyst nematode has become the most damaging pathogen among the top soybean-producing countries of the world (Wrather et al., 1997). The ability of this pathogen to reduce yield, combined with incomplete management strategies, warrant intense research efforts.
The first documentation of *H. glycines* was in Japan in 1915 (Hori, 1915). The first report of *H. glycines* in the United States was in North Carolina in 1954 (Winstead et al., 1955). Evidence suggests that the pathogen was transported to the United States through infested soil imported for *Bradyrhizobium japonicum* (Kirchner) Buchanan cultures from China and Japan (Noel, 1992). With the dramatic increase in soybean production, *H. glycines* has become the major cause of yield loss among U.S. soybean producers in the last 20 to 25 years (Doupnik, 1993; Pratt and Wrather, 1998; Wrather et al., 1995).

Currently, *H. glycines* is present in 90 of the 99 counties in Iowa. Up to 74% of the fields in Iowa are infested with this pathogen (Workneh et al., 1999). A range of 0-50% yield loss has been attributed to the presence of *H. glycines* in infested fields (Niblack et al., 1992). The yield reduction varies depending on growing conditions.

**Biology**

*Heterodera glycines* is a microscopic, plant-parasitic roundworm. It is an obligate parasite and, therefore, cannot reproduce or survive in the absence of a living host. The host range for this pathogen includes mostly legumes (Riggs, 1992). Among these hosts, the most economically important is the soybean.

*H. glycines* juveniles develop within free eggs in the soil and also within eggs retained inside the nematode body wall. Molting from the first-stage juvenile to the second-stage juvenile occurs inside the egg. It is the vermiform, second-stage juvenile (J2) that hatches from the egg and penetrates a soybean root. Nematodes develop into male and female third- and then fourth-stage juveniles while inside the roots. Upon reaching maturity,
the vermiform male migrates back into the soil whereas the swollen female ruptures through the surface of the root. The male then locates a female on the root surface and fertilization occurs. After fertilization, the female begins producing eggs. Eggs are deposited in an egg mass outside of the body and within the female body cavity. Upon death, the body of the female hardens and becomes a cyst. The cyst contains, on average, 200-300 eggs. These eggs have been shown to remain viable but dormant in the cyst for up to 11 years (Inagaki and Tsutsumi, 1971). Chemical components of the cyst wall are believed to have a direct effect on this period of dormancy (Okada, 1972). This long-term survival makes control efforts difficult.

**Symptoms**

Aboveground symptoms of *H. glycines* can include chlorosis, stunting, slow closing of the rows, and mid-day wilting. When present, these symptoms often are seen in circular or oval areas in the field. Symptoms below ground can include stunting and discoloration of roots, wounds created at feeding sites of juveniles and protrusion points of adult females on roots, and reduced numbers of nitrogen-fixing nodules. It is possible that symptoms above ground may not be present or may be mistaken for symptoms caused by other diseases or environmental stress, even when damaging levels of *H. glycines* occur (Tylka, 1995). The most diagnostic sign of *H. glycines* is the presence of adult females and cysts on infected soybean roots.

The fact that *H. glycines* can cause considerable damage in fields with the presence of nondescript symptoms or even no symptoms is a major contributor to the severity of the
problem today. Growers may not assume nematodes to be causing the yield loss. By the time symptoms first become visible, *H. glycines* populations may be devastatingly high.

**Management options**

There are several management strategies for the producer to choose from, each having both strengths and weaknesses. The most effective control at this time is achieved through an integration of two approaches: growing resistant soybean varieties and nonhost crops.

The first of these management strategies is planting soybean cultivars that have genetic resistance to *H. glycines*. A problem experienced with this approach is termed “directional selection” (Strickberger, 1996). Some proportion of the nematode population in a field still will be able to feed and reproduce on a resistant variety of soybean. Reproduction by this portion of the nematode population will increase the numbers of nematodes that are able to thrive on the new variety of soybean. Populations of resistant nematodes contribute to what is referred to as a “race shift” (Luedders and Dropkin, 1983; Niblack, 1992). Reportedly, a new race of *H. glycines* can evolve as quickly as in a single growing season when exposed to a resistant variety. But, it also is possible for resistant varieties to maintain effectiveness for many years.

The selection pressure for this “race shift” phenomenon can be decreased by varying the source of resistance (Young, 1994). However, nearly all resistant soybean cultivars on the market are derived from the same source, PI88788 (Uphoff et al., 2000). Because of the risk of a race shift, development of new varieties of resistant soybeans is continually needed.
The second management strategy, growing nonhost crops, is an inexpensive and effective way to lower nematode numbers (Agrios, 1988). There are multiple factors to consider in choosing this management strategy. The host range of the nematode affects the selection of suitable nonhost crops. In much of the upper Midwest, the existence of a strict corn/soybean rotation makes it difficult to find nonhost crops in addition to corn that are suitable for the area that also have economic value for the producer. The number of seasons to grow the nonhost crop is another variable. Because nonhost crop choices are limited and the nematode has long-term survival potential, growing a nonhost crop successively for the number of successive seasons required to eradicate \textit{H. glycines} from a field is not practical. In addition, a monoculture of a nonhost crop can increase problems with insects, weeds, and other pathogen populations. As a result of these limitations, the intent of crop rotation is to reduce the nematode population, not eliminate it.

The current recommendation by Iowa State University is a six-year rotation that involves planting a nonhost crop every other year, resistant soybean varieties of different sources on the second and fourth years, and a susceptible soybean variety the sixth year (Tylka, 1995). If only one source of resistance is available to the grower, two different soybean varieties with the same source of resistance are recommended for year two and year four. Growing the same soybean variety with the same source of resistance for these two years is recommended only when multiple sources of resistance or different resistant varieties are not available.

A third management option is the application of nematicides. In the years prior to the 1980s, nematicide use was an important and useful method of controlling \textit{H. glycines} and
other plant-parasitic nematodes (Johnson and Feldmesser, 1987). In the last 15 years, use of nematicides has declined sharply. It became evident that suppression of nematode populations on a variety of crops with compounds such as 1,2-dibromo-3-chloropropane (DBCP), 1,2-dichloropropene (DD), ethylene dibromide (EDB), and methyl bromide, the associated environmental contamination and mammalian toxicity outweighed their benefits (Johnson and Feldmesser, 1987).

Compounds with active ingredients that are registered for nematicidal use often have limitations for practical use. Some chemicals are registered only for specific uses or restricted times of application (Farm chemicals handbook, 2000). The chemicals not restricted or banned have become impractical to use as crop values declined.

In order to overcome past obstacles to nematicide use, recent research on *H. glycines* management has focused on the development of nontoxic, biodegradable, inexpensive control compounds that either stimulate or inhibit egg hatch.

**Nematicides**

**Classification**

Available nematicides can be separated into two classes based on the mode of action: cholinesterase inhibitors and metabolic inhibitors. Cholinesterase inhibitors include organophosphates and carbamates (Hassall, 1990). Metabolic inhibitors include chlorinated or brominated hydrocarbons.

Organophosphates are a large group of chemicals that inhibit cholinesterase, an enzyme involved in neurotransmission (Matsumura, 1985). The half-life of
organophosphates is relatively short, often only one to five days under most conditions. Organophosphates mimic the shape of acetylcholine, which is the natural substrate of cholinesterase (Hassall, 1990; Matsumura, 1985). An example of a registered organophosphate that is used as a nematicide is phemamiphos (Nemacur™) (Farm chemicals handbook, 2000). This is a product similar to terbufos (Counter™), a nematicide as well as a chemical used against corn rootworm. Other organophosphates registered for use as nematicides are disulfoton (Di-syston™), ethoprop (Mocap™), and phorate (Thimet™) (Farm chemicals handbook, 2000).

Carbamates, the newest additions to the group of cholinesterase inhibitors, generally are synthetic derivatives of plant alkaloids. Their mode of action is considered to be similar to organophosphates, but the action appears to be reversible and a more rapid dissociation of the enzyme-inhibitor complex characteristically occurs (Hassall, 1990). The relatively high water solubility of these compounds is a limitation due to rapid degradation and reduced time of pathogen exposure. Most carbamate compounds are more expensive than organophosphates, causing another limitation to their use (Hassall, 1990). There are several carbamates registered for use against nematodes including carbofuran (Furadan™), aldicarb (Temik™), and oxamyl (Vydate™) (Farm chemicals handbook, 2000). Both organophosphates and carbamates are often insecticidal. Doses intended for nematocidal effects generally are two- to four-times greater than for insecticidal use (Johnson and Feldmesser, 1987).

Inhibitors of basal metabolism, the second class of nematicides, are stable compounds with long-lasting residual effects (Matsumura, 1985). As a result of the metabolic inhibition,
there are neurotoxic effects. For example, if acting as nerve poisons, these compounds would inhibit enzymes in the Krebs cycle, specifically succinate dehydrogenase (J. R. Coats, Professor of Entomology, Iowa State University, personal communication). One well-studied example of a chlorinated hydrocarbon is 4, 4'-dichlorodiphenyltrichloroethane (DDT) (Matsumura, 1985).

**General environmental concerns**

In the past, nematicides were determined to have a detrimental effect on the environment. Levels of nematicide accumulated in the soil and groundwater due to poor natural and microbial degradation (Johnson and Feldmesser, 1987; Thomason, 1987). Some chemicals also were removed from the market after causing unacceptable levels of toxicity to mammals (Johnson and Feldmesser, 1987; Thomason, 1987).

Residual activity impacts both compound effectiveness as well as the potential for environmental hazards. A nematode population has a greater likelihood of being affected by a compound with extended residual activity than by a compound that quickly degrades. However, there also is an increased risk for environmental contamination with extended residual activity. While the chemical is still active, its effects might extend further than was intended. If the chemical is not in the targeted segment of the soil profile, a major concern focuses on its displacement. Where is the nematicide and what effects occur? Are the chemical components prone to leaching or evaporation, and how much contamination occurs in ground water supplies?
Movement of pesticide residue is affected by water and air (Matsumura, 1985). The role of water in transportation of residues suggests obvious problems. Contamination of groundwater and pollution of waterways is continually monitored. Lakes are a frequent body of water in the Midwest, and their slow exchange rate of water extends the impact of pollution effects (Matsumura, 1985). Another concern regarding pesticide movement is long-range dispersion, either dust-mediated or through true pesticide vapors (Matsumura, 1985).

Compounds used in this study

The four compounds investigated in this research project are AA, Va, CHP, and methyl pelargonate. AA, Va and CHP are hatch-inhibitors that are synthetic analogs of natural compounds, whereas methyl pelargonate is an emulsion of a natural compound with nematicidal properties.

• AA and Va

Glycinoeclepin A is a natural compound extracted from kidney bean roots that has been found to stimulate hatch of *H. glycines* eggs (Masamuni et al., 1982). Several synthetic analogs of this compound have been created, and in vitro hatch studies have shown significant inhibitory effects with several of the compounds (Kraus et al., 1994; Kraus et al., 1996). Two of these compounds that have shown consistent inhibition of *H. glycines* hatch were used in this project. The first is 2-hydroxymethylenecyclopentanone, hereafter referred
to as compound “AA”. The second is 2-(1-ethoxycarbonyl-1-hydroxymethylene)cyclo-pentanone, hereafter referred to as compound “Va”.

Incubation of H. glycines eggs in AA and Va at concentrations between 10 and 100 µg a.i./ml inhibits hatch (Kraus et al., 1994; Kraus et al., 1996). Stimulation of hatch also has been determined to occur during incubation in AA at concentrations of 1, 2.5, and 5 µg a.i./ml (Thompson, 1997).

Currently, the mode of action of these compounds is not known. One hypothesis is that AA and Va may inhibit hatch by fitting into a receptor that would normally be occupied by a hatch-inducing compound such as glycinoeclepin A (G. L. Tylka, Professor of Plant Pathology, Iowa State University, personal communication).

• CHP

The third compound in this project is 1-cyano-1-hydroxy-2-propene, hereafter referred to as CHP. CHP is a synthetic analog of a natural glucosinolate compound. Glucosinolates are categorized as alkaloids, a group of secondary metabolites produced by plants that may function in defense from insects, pathogens, or competing plants (Schmeltz, 1971). There is a strong similarity between CHP and natural cyanohydrin alkaloids produced by flax (J. R. Coats, personal communication).

CHP has been shown to be an effective and irreversible inhibitor of H. glycines egg hatch (Tylka et al., 1997). CHP also was tested on the house fly (Musca domestica L.), the lesser grain borer (Rhizopertha dominica (F.)), brine shrimp (Artemia franciscana Kellog), and larvae of the yellow fever mosquito (Aedes aegypti (L.)) in a study conducted by
Peterson et al. (2000). Results showed the toxicity of this compound to be similar to the commercial fumigant chloropicrin as well as to dichlorvos, a restricted-use pesticide (Peterson et al., 2000).

Volatility (Tylka et al., 1997) is the characteristic that sets CHP apart from the previously described compounds AA and Va. This characteristic may make compounds more effective in treating soil and also allow for better permeation through the cyst wall. Compounds applied as soil fumigants tend to be respiratory inhibitors. It is understood that these compounds are catalyzed to ultimately form hydrocyanic acid, but the mode of action of CHP is still under study. Understanding how hatch is inhibited by CHP may be useful in timing of applications and improvements in formulation.

• **Methyl pelargonate**

The final compound in this research project is methyl pelargonate. Research has shown fatty acids and their derivatives to be toxic to nematodes (Sayre et al., 1965). The benefits of pelargonic acid esters compared to traditional nematicides are that they are relatively inexpensive to produce, toxicity to mammals is low, and minimal hazards are posed to the environment due to the high degree of degradation by soil microbes (Davis et al., 1997).

Soil treatments of methyl pelargonate effectively reduced the number of adult females and cysts of *H. glycines* per gram of root and galls of *Meloidogyne incognita* per gram of root at 3.2 µg a.i./liter (Davis et al., 1997). Phytotoxic effects occurred with higher concentrations.
The mode of action for methyl pelargonate is unknown as of yet, but it is believed to affect the permeability of the nematode cuticle or hypodermis by interfering with the waxy cuticular coat through a detergent effect (Davis et al., 1997).

Objectives

The motivation behind this study is to minimize yield reductions caused by *H. glycines* for soybean growers. Specific objectives are to evaluate four biorational compounds as potential *H. glycines* management tools by determining:

1) effects of AA, Va, CHP, and methyl pelargonate on *H. glycines* population densities in growth chamber, field soil, and laboratory environments,

2) mobility responses of second-stage juveniles of *H. glycines* to AA, Va, CHP, and methyl pelargonate.

3) contact and volatile influences of AA, Va, CHP, and methyl pelargonate on *H. glycines* egg hatch.

Implications

The results of these experiments will help to better understand the activity of the chosen compounds as they correspond to both the life cycle of the nematode and the growth and development of the soybean. By examining these interactions, timing and application methods for the compounds may be identified. Due to the rising costs resulting from *H. glycines* yield loss, steps are needed to find economical and practical methods of control for fields already infested with this damaging pathogen. The ultimate goal of this project is to
investigate the use of control compound application in order to decrease population densities of *H. glycines* and to raise attainable soybean yields in the Midwest.

**Thesis organization**

The preceding pages have been a general introduction to the soybean crop, *H. glycines*, and nematicide use. What follows is organized into two chapters and a general discussion. The two chapters are prepared as journal articles for submission to the Journal of Nematology.

**Literature Cited**


CHAPTER 1
CONTROL OF SOYBEAN CYST NEMATODE WITH SYNTHETIC COMPOUNDS

A paper to be submitted to the Journal of Nematology

A. M. Knips, G. L. Tylka, J. R. Coats, & G. A. Kraus

Abstract: Effects of 2-hydroxymethylenecyclopentanone, 2-(1-ethoxycarbonyl-1-hydroxymethylene)cyclopentanone, 1-cyano-1-hydroxy-2-propene, and methyl pelargonate on *Heterodera glycines* population densities were examined in growth chamber, microplot field, and laboratory environments. In a growth chamber study, the above-mentioned compounds were incorporated manually into *H. glycines*-infested soil, and *H. glycines*-susceptible soybeans were planted as seeds or seedlings in pots of the infested, treated soil. Five weeks after planting, soybean growth and chlorosis as well as *H. glycines* egg and second-stage juvenile (J2) population densities in the soil and on the roots were determined. In one run of the growth chamber study, egg population densities were lower in soil treated with each of the compounds relative to those in the water control pots. However, these differences did not occur in the other run of the experiment. In two consecutive field seasons, the compounds were applied to *H. glycines*-infested microplots and manually incorporated into the soil. Half of the microplots were planted with *H. glycines*-susceptible soybeans and half remained unplanted. Egg and J2 soil population densities were determined at planting, full bloom, and harvest, and final soybean seed yield was recorded. There were no differences in *H. glycines* population densities within planted or unplanted treatments of the microplot field study for either season. In the first season, the yield of plants in
microplots treated with 1-cyano-1-hydroxy-2-propene was less than in all other treatments, most likely due to a phytotoxic effect on a majority of plants. In laboratory experiments, *H. glycines* eggs were incubated in solutions of one of the above-described compounds and transferred to fresh solution every two days for 20 days. Hatched J2 were counted after each transfer. All four compounds reduced hatching relative to hatch in water beginning on the fourth day and continuing for the duration of the experiment. Although the chosen compounds inhibited *H. glycines* egg hatch in the laboratory, they did not have consistent, adverse effects on *H. glycines* in growth chamber and microplot field experiments.

**Key Words:** fatty acid, glucosinolates, glycinoeclepin A, *Heterodera glycines*, management, microplot, soybean cyst nematode, ZnSO₄

**Introduction**

*Heterodera glycines*, the soybean cyst nematode, was first reported in the United States in North Carolina in 1954, apparently after introduction from China or Japan (Winstead et al., 1955). With dramatic increases in soybean [*Glycine max* (L.) Merr.] production, *H. glycines* has become the major cause of yield loss among soybean producers in the United States during the last 20 to 25 years (Doupnik, 1993; Wrather et al., 1995). In Iowa, incidence of detected populations of *H. glycines* has increased from 45% to 90% of all counties since 1990 (G. Tylka, unpublished data). It is a realistic assumption that the pest eventually will affect all soybean-growing regions of the United States. (Noel, 1986).

Present options for management of *H. glycines* include growing soybean cultivars with genetic resistance as well as growing nonhost crops. A third, less-used, tactic is application of nematicides to infested soil. The potential exists to integrate chemical control
with other tactics to broaden *H. glycines* management programs. However, despite effective control of other plant-parasitic nematode species on a variety of crops with compounds such as 1,2-dibromo-3chloropropane (DBCP), 1,2-dichloropropane (DD), ethylene dibromide (EDB), and methyl bromide, nematicide use has declined in the past 15 years (Johnson and Feldmesser, 1987). Increased amounts of toxins in the soil and ground water, toxicity to mammals, and decreasing crop values have eliminated the use of nematicides as a management option for agricultural crops with a low per hectare value (Thomason, 1987).

In response to these problems, recent laboratory research has focused on the development of nontoxic, biodegradable control compounds that either stimulate or inhibit *H. glycines* egg hatch. Ideally, stimulation of egg hatch in a field of nonhost crops would result in death of hatched J2 by starvation, and inhibition of hatch in a field of soybeans would prevent the rapid increase of *H. glycines* population densities (Tylka, 1995). The purpose of this study was to evaluate the effect of four novel, potential control compounds on *H. glycines* population densities in growth chamber, field soil, and laboratory environments.

**Materials and Methods**

**Preparation**

**Treatments.** Compounds evaluated in the experiments were 2-hydroxymethylenecyclopentanone (hereafter referred to as compound AA) and 2-(1-ethoxycarbonyl-1-hydroxymethylene)cyclopentanone (hereafter referred to as compound Va), synthetic analogs of glycinoeclepin A (Masamuni et al., 1982); 1-cyano-1-hydroxy-2-propene (hereafter referred to as compound CHP), a synthetic aglycone; and methyl
pelargonate (hereafter referred to as compound MP), a fatty acid derivative. Compounds AA, Va, and CHP inhibit hatching of H. glycines eggs (Kraus et al., 1996; Tylka et al., 1997), and MP is an emulsion of a fatty acid that is toxic to nematodes (Davis et al., 1997). Treatment solutions were made using sterile, deionized water adjusted to pH 7.0 by additions of 1M potassium hydroxide or 1M hydrochloric acid. Compounds were dissolved in 1 ml of ethanol, then added to 1 L of the described water. Compounds AA, Va, and CHP were tested at a concentration of 100 µg a.i./ml, and MP was tested at concentrations of 300 µg a.i./ml and 1,000 µg a.i./ml unless otherwise noted.

Sterile, deionized water (hereafter referred to as ‘H2O’); sterile, deionized water plus ethanol (hereafter referred to as ‘H2O+EtOH’); and a MP formulation blank (methyl pelargonate minus the active ingredient, hereafter referred to as ‘MP formulation blank’), were used as controls. The solution of H2O + EtOH was made by adding 1 ml ethanol to 1L of H2O; the ethanol was added to H2O to create a control solution to account for the ethanol used to dissolve each compound in solution. A 3.14 mM concentration of zinc sulfate (hereafter referred to as ZnSO4) also was included as a positive control for the growth chamber experiment.

GROWTH CHAMBER STUDY

SOIL PREPARATION AND EXPERIMENTAL SET-UP. Soil was obtained from a field infested with H. glycines in Story County, Iowa, and was mixed with sand to reduce the H. glycines population densities. The sand/soil mixture was divided for five replications, then each replication was mixed manually with a shovel for ten minutes. Treatment solutions of 100 µg a.i./ml AA, Va, CHP, and MP and control solutions of H2O, H2O + EtOH, and ZnSO4
were applied at a rate of 90 ml/1.35 L of the sand/soil mixture with a spray bottle while continuing to mix the soil. Treated soil was poured into 15-cm-diam. clay pots. Three seeds of *H. glycines*-susceptible soybean cultivar ‘Kenwood 94’ were planted in half of the pots, and the remaining pots were not planted. Seven days after planting, soybeans were thinned to one plant per pot. Equal volumes of water were added to each pot as needed. The growth chamber was maintained at 12 hours of light and 12 hours of dark, 26 °C, and a minimum of 80% relative humidity.

**DATA COLLECTION.** Five weeks after planting, 100 cm³ soil samples were taken from all pots, then cysts and juveniles were extracted by elutriation (Byrd et al., 1976). For the planted pots, plant height, vegetative growth stage (Herman, 1996), and chlorosis level (rated 1 to 5 with increasing levels of chlorosis) of the five-week-old soybeans were recorded. Roots were carefully freed from the soil, tops of the plants were removed and discarded, and females and cysts were collected from roots on a set of nested sieves using a stream of water. A 20-mesh sieve (850 µm pore) was used to collect debris, and females and cysts were caught on a 60-mesh sieve (250 µm pore). After females and cysts were dislodged, root systems were dried and weighed. For all samples, eggs were extracted from females and cysts by grinding with a motorized, stainless-steel pestle (Niblack et al., 1993). Eggs subsequently were stained with acid fuchsin (Niblack et al., 1993) and counted with a dissecting microscope at a magnification of 24X using a nematode counting slide (Advanced Equine Products, Issaquah, WA). Extracted J2 were separated from soil sediments by centrifugation for one minute at 220g in 454 g/ml sucrose, a modification of the technique
described by Jenkins (1964), then counted with a compound light microscope at a magnification of 40X.

**EXPERIMENTAL DESIGN AND DATA ANALYSIS.** Each treatment was replicated six times, and the experiment was repeated once. Pots were arranged in a complete randomized block design in a 2m x 4.5m growth chamber. Analysis of variance (ANOVA) was performed on all variables using SAS General Linear Model (GLM) procedure, and means were separated using Fisher’s least-significant-difference (LSD) test (SAS Institute, Cary, N.C.).

**MICROPLOT FIELD STUDY**

**PREPARATION AND TREATMENT OF SOIL.** Microplots were constructed in Story County, Iowa, by inserting 75-cm-diam. fiberglass cylinders 90 cm into soil that was artificially infested with *H. glycines*. During the spring of 1998 and 1999, weeds were removed manually from microplots, and soil from the top 20 cm was loosened and turned over. Four liters of experimental compound were applied and manually incorporated into the upper 15 to 20 cm of soil of each microplot before planting. A second application for several treatments was applied six weeks after planting. Compounds, concentrations, and number of applications are listed in Table 1. Following application of the treatment compounds to the microplot soil, three liters of each treatment solution were kept for use in the corresponding laboratory hatch studies. Weeds within microplots were controlled manually throughout both growing seasons.
PLANTING. Seeds (25/microplot) of *H. glycines*-susceptible soybean cv. Kenwood 94 were planted in 40 microplots on 27 May 1998 and 26 May 1999, and 40 microplots were left unplanted for each of the test years. Data from one unplanted microplot were disregarded due to a low initial nematode population density. Fifteen seeds were planted on each side of each of the 40 planted plots in a line with the microplot seed row. Additionally, the area between each row of microplots was planted with six rows of soybeans for weed control. Microplots were thinned to 18 soybean plants/microplot in 1998. In 1999, plots were thinned to 18 soybeans where possible, and plots with fewer than 18 soybeans were noted.

SAMPLING. Five soil cores, 2-cm diam. and 15-to 20-cm deep, were taken from each microplot at the time of planting, at full bloom stage, and at the time of harvest. Cysts and J2 were extracted from the soil samples by elutriation as described above. Eggs were extracted from cysts, juveniles were separated from soil particles, and egg and J2 population densities were determined following methods described above.

HARVEST. Soybeans were harvested on 13 October 1998 and 28 September 1999 by cutting each stem near the soil line and collecting all plants within each microplot. Plants were dried, then threshed. Soybeans were weighed immediately after threshing, stored in a drying chamber for several weeks, and then weighed a second time. Moisture content was calculated gravimetrically from the two weights, and then grams of yield were calculated after adjustment to 13% moisture content.

EXPERIMENTAL DESIGN AND DATA ANALYSIS. Five replicates of 16 treatments (8 planted, 8 unplanted) were randomized with a different method for the two growing seasons.
In 1998, initial egg counts were used to order the plots sequentially. Beginning with the lowest *H. glycines* population density, the first 16 microplots were grouped as a single replication. The remaining microplots were divided into replications in the same manner. In 1999, the replications were blocked geographically.

For each microplot, egg population densities at the time of harvest were divided by initial egg population densities to obtain the reproductive factor (Rf). Rf, egg and J2 population densities, and yield were transformed to log10(X+1). ANOVA was performed on both transformed and nontransformed data using SAS GLM procedure. Nontransformed values are reported. Means were separated using Fisher’s LSD test (SAS Institute, Cary, NC).

**LABORATORY STUDY**

**EXPERIMENTAL SET-UP AND DATA COLLECTION.** Two experiments were conducted to examine laboratory egg hatch of *H. glycines* in response to the solutions used in the first application and second application of the microplot experiments. A 3.14 mM concentration of zinc sulfate was included in both hatching studies as a positive control due to known stimulating effects on *H. glycines* egg hatch (Clark and Shepherd, 1966; Tefft and Bone, 1984). Methods followed the protocol described by Wong et al. (1993), substituting 6-cm-diam. Corning Snap Seal #1730 black plastic cups (Corning, Corning, NY) for the plastic trays. Microsieves of 3 cm diam. were constructed using nylon screen (Tetko, Briarcliff Manor, NY) with 30-µm-pore openings. The pore size retained eggs on the microsieve and allowed hatched juveniles to pass into the treatment solution. These sieves, initially holding
approximately 8,000 *H. glycines* eggs, were transferred to fresh solution in the black plastic cups every 2 days. Numbers of hatched J2 that had passed into the treatment solution were recorded after each transfer for a total of 20 days.

**EXPERIMENTAL DESIGN AND DATA ANALYSIS.** Five replications of each solution were randomized within complete blocks and stored at room temperature (approximately 26 °C). Data were compared by determining the cumulative percentage of eggs that hatched over time. ANOVA was performed on cumulative percentage hatch for each day using SAS GLM procedure, and the means were separated using Fisher’s LSD test (SAS Institute, Cary, NC).

**Results**

**GROWTH CHAMBER STUDY**

In the first of two runs of the growth chamber experiment, there were differences detected in plant growth among treatments. Plants grown in soil treated with CHP were shorter (α = 0.05) and had smaller (α = 0.05) roots than all other treatments (Table 2). Treatments exhibited differences in vegetative growth stage and chlorosis level (Table 2), but these differences were inconclusive. For example, plants treated with AA had a more advanced growth stage than those treated with H2O, CHP, MP, and ZnSO4, but we do not have an explanation at this time for such a growth response.

The mean egg population density in soil at the time of data collection for all treatments in the first run of the experiment was 12,300 eggs/100cm³ of soil for the planted pots and 9,700 eggs/100 cm³ of soil for the unplanted pots. In addition to the differences in
plant growth mentioned above, differences in nematode population densities also were detected among treatments for the first run of the experiment. The egg population density for Va-treated, planted pots was greater ($\alpha = 0.05$) than that of $\text{H}_2\text{O} + \text{EtOH}$, CHP, and MP (Table 3). For the unplanted pots, the $\text{H}_2\text{O} + \text{EtOH}$ treatment had a greater ($\alpha = 0.05$) egg population density than that of any other test solution (Table 3). In addition, J2 population densities in the soil were greater ($\alpha = 0.05$) with the $\text{H}_2\text{O}$ treatment than the $\text{H}_2\text{O} + \text{EtOH}$- and AA-treated soils. There were no other differences among treatments.

The mean egg population density in soil at the time of data collection for all treatments in the second run of the experiment was 27,400 eggs/100 cm$^3$ of soil for the planted pots and 15,800 eggs/100 cm$^3$ of soil for the unplanted pots. Overall, there were fewer differences among treatments in the second run than in the first run of the experiment. Differences that did occur were associated with the CHP treatment and were not consistent with results obtained when the experiment was first conducted. The egg population densities in planted and unplanted pots treated with CHP were greater ($\alpha = 0.05$) than those in all other treatments (Table 4). Similarly, the population densities of J2 in soil treated with CHP were greater ($\alpha = 0.05$) than those in soil treated with $\text{H}_2\text{O} + \text{EtOH}$, Va, and MP. No other differences were observed among treatments for $H.\text{glycines}$ population densities or soybean development during the second run of the experiment (Table 4).

**MICROPLOT FIELD STUDY**

In 1998, $H.\text{glycines}$ population densities fluctuated from mean Pi of 5,765 and 7,191 eggs + J2/100 cm$^3$ soil to mean Pf of 3,884 and 16,775 eggs + J2/100 cm$^3$ soil for unplanted
and planted microplots, respectively (Table 5). In 1999, *H. glycines* population densities decreased from mean Pi of 12,877 and 12,647 eggs + J2/100cm³ soil to mean Pf of 4,791 and 7,544 eggs + J2/100cm³ soil for unplanted and planted microplots, respectively (Table 6).

The overall mean Rf of all treatments during the 1998 growing season was 0.70 for microplots that were unplanted and 2.50 for microplots that were planted with soybeans (Fig. 1). In 1999, the mean Rf was 0.38 for microplots that were unplanted and 0.78 for microplots that were planted with soybeans (Fig. 2). The changes in nematode population densities through each year were not different among treatments.

Overall mean yield of all treatments was 94.8 grams in 1998 and 168.5 grams in 1999. In four of the five microplots treated with CHP in 1998, the second application caused a phytotoxic response on a majority of the soybeans. The soybean yield in microplots treated with CHP was less (*α* = 0.05) than that of all other treatments in 1998 (Table 7). No other differences in emergence or yield existed among treatments for either growing season (Table 7).

**Laboratory study**

Test solutions from both the 1998 and 1999 microplot field studies inhibited the hatch of *H. glycines* eggs in the laboratory. Hatch in each test solution was less (*α* = 0.05) than hatch in water beginning at day four for both experiments (Figs. 3A, 3B). Eggs incubated in CHP and both concentrations of MP had less than 5% cumulative percent hatch for both experiments, compared to 18% and 22% cumulative percent hatch in H₂O for the first and second experiments, respectively (Figs. 3A, 3B). There were no differences in cumulative
percent hatch throughout either experiment among CHP and the two concentrations of MP and also no difference between cumulative percent hatch for H₂O and the MP formulation blank (Figs. 3A, 3B). Incubation of eggs in zinc sulfate resulted in a cumulative percent hatch of 28% and 44% for the first and second experiments, respectively (Figs. 3A, 3B).

Discussion

The results obtained in this research reflect the variety of experimental variables that are involved in growth chamber, field soil, and laboratory conditions.

Differences occurred among treatments in the growth chamber study, but these differences were not consistent between the two runs of the experiment. Fewer differences among treatments were detected in the second run than in the first run of the experiment. The difference between runs may be due to the range of initial pathogen population densities. Average final *H. glycines* egg population densities from soil in the second run of the experiment were more than twice the average final egg population densities in the first run. It is possible that the initial pathogen population densities for the second run were above a threshold of efficacy for the experimental compounds.

In the first run of the growth chamber study, reduced Rf in unplanted pots was not an expected result for any of the hatch-inhibiting compounds because hatch inhibition alone should maintain the number of eggs in the soil for any given sample. If reductions of population densities in unplanted soil occurs in future studies, it might indicate that these hatch-inhibiting compounds also lead to the death of nematode eggs, perhaps due to the
actions of other soil microorganisms. It is not likely that this secondary effect would be observed under the sterile, controlled conditions of a laboratory hatch study.

For the set of planted pots in the first run of the growth chamber study, reduced egg population densities on roots treated with CHP may be due to effects of this compound on the plant, rather than a physiological effect on the nematode. The reduced foliage and root biomass associated with the CHP treatment relative to all other treatments likely affected nematode population densities by reducing available healthy root tissue for feeding. The fact that there was no difference in soybean development and that nematode reproduction was not reduced by CHP applications for the second run of the experiment supports this hypothesis.

There was a possible detrimental effect of the CHP treatment on plant development in the microplot experiment as well. In the 1998 growing season, the majority of soybeans treated with CHP died within a week of the second treatment application. The suspected cause of this phytotoxicity was contact of CHP on foliage. Therefore, care was taken in the 1999 experiment to prevent splash of the compound onto foliage. Soybeans in the 1999 trial did not show any symptoms of a phytotoxic response. The only effect on yield for either growing season occurred in conjunction with the dead plants treated with CHP in 1998.

With controlled conditions of the laboratory hatch study, each of the four experimental test compounds inhibited *H. glycines* egg hatch. The hatch inhibition by AA, Va, and CHP corroborates results from previous laboratory studies with these compounds (Kraus et al., 1996; Thompson and Tylka, 1997; Tylka et al., 1997). Methyl pelargonate, in addition to the hatch inhibition demonstrated in this study, also has reduced galling by *Meloidogyne javanica* on tomatoes and has interfered with development of *H. glycines*
females on soybean roots in laboratory bioassays (Davis et al., 1997). The hatch inhibition by MP did not occur with the MP formulation blank, thereby demonstrating that the active ingredient is responsible for the observed hatch inhibition. The volatile properties of CHP and MP may increase the chemical penetration of eggshell membranes and enhance the inhibition of hatch.

The inconsistent results that occurred in this study with application of compounds to soil also have occurred in experiments testing other potential control compounds for *H. glycines*. Microplot and field applications of a *H. glycines* pheromone and chemical analogs of the pheromone did not increase soybean yield, even with some reductions in cyst population densities (Meyer et al., 1997). In another study, nematicides aldicarb, EDB, and fenamiphos had inconsistent effects on egg and J2 population densities when tested over three growing seasons (Schmitt, 1987). Environmental variables and formulation limitations each may contribute to these inconsistencies. Outside the laboratory, environmental fluctuations include temperatures, moisture levels, and soil conditions. The impact of each variable may be magnified by inappropriate formulation of the experimental compound. In our study, AA, Va, and CHP were tested as raw products; MP was in a microemulsion formulation. It is possible that improved formulations of these compounds will extend persistence of the compounds in soil and increase the total time that nematodes are exposed to the nematicidal compounds.

There are reports of *H. glycines* population densities in soil and their effects on soybean yields have been managed with application of test compounds. For example, soil and root populations of *H. glycines* were reduced with applications of the allelochemical
Thymol in greenhouse studies (Soler-Serratosa et al., 1996), and aldicarb-treated plots yielded higher than control plots in seven of 16 soil types (Smith et al., 1991). Such instances of successful use warrant the continued investigation of potential control compound applications within a soil environment. At this time, our lack of consistent results with precise application and careful soil incorporation of AA, Va, CHP, and MP show that routine field application is not yet a practical option for control of *H. glycines*.

**Literature Cited**


Table 1. Treatments applied to microplots to examine their effects on *Heterodera glycines* population densities through the 1998 and 1999 growing seasons.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µg a.i./ml)</th>
<th>No. of applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled, deionized water (H₂O)</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Distilled, deionized water plus 7-ml ethanol (H₂O + EtOH)</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>2-hydroxymethylenecyclopentanone (AA)</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>2-(1-ethoxycarbonyl-1-hydroxymethylene)cyclopentanone (Va)</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>1 cyano-1-hydroxy-2 propene (CHP)</td>
<td>100/500^b</td>
<td>2</td>
</tr>
<tr>
<td>methyl pelargonate (MP 300)</td>
<td>300</td>
<td>2</td>
</tr>
<tr>
<td>methyl pelargonate (MP 1000)</td>
<td>1,000</td>
<td>2</td>
</tr>
<tr>
<td>methyl pelargonate formulation blank^c (MP FB)</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

^a Each experimental compound first was dissolved in 7 ml of 100% ethanol to obtain a suspension in water.

^b CHP was applied at 100µg a.i./ml for the first application and 500µg a.i./ml for the second application.

^c The methyl pelargonate formulation blank is identical to the methyl pelargonate micro-emulsion formulation minus the active ingredient.

^d Second application was applied 6 weeks after planting.
Table 2. Effects of various compounds on height, vegetative growth stage, chlorosis level, and dry root weight (DRW) of soybeans.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Height (cm)</th>
<th>V-stage</th>
<th>Chlorosis</th>
<th>DRW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>9.7 ab</td>
<td>2.8 b</td>
<td>3.8 a</td>
<td>0.25 ab</td>
</tr>
<tr>
<td>H₂O + EtOH</td>
<td>11.0 ab</td>
<td>3.3 ab</td>
<td>2.7 ab</td>
<td>0.30 ab</td>
</tr>
<tr>
<td>AA</td>
<td>11.7 a</td>
<td>3.7 a</td>
<td>2.0 bc</td>
<td>0.31 ab</td>
</tr>
<tr>
<td>Va</td>
<td>9.8 ab</td>
<td>3.2 ab</td>
<td>2.7 ab</td>
<td>0.31 ab</td>
</tr>
<tr>
<td>CHP</td>
<td>8.5 b</td>
<td>3.0 b</td>
<td>1.0 c</td>
<td>0.25 b</td>
</tr>
<tr>
<td>MP</td>
<td>9.2 ab</td>
<td>3.0 b</td>
<td>3.7 a</td>
<td>0.32 ab</td>
</tr>
<tr>
<td>ZnSO₄</td>
<td>10.0 ab</td>
<td>3.0 b</td>
<td>2.8 ab</td>
<td>0.34 a</td>
</tr>
</tbody>
</table>

Values are the mean of six replications of the first run of a growth chamber study. Values in the same column with the same letter are not different according to Fisher's LSD values (α = 0.05).

*a AA = 2-hydroxymethylenecyclopentanone, Va = 2-(1-ethoxycarbonyl-1-hydroxymethylene)cyclopentanone, CHP = 1-cyano-1-hydroxy-2-propene, and MP = methyl pelargonate.

*b V-stage represents the average vegetative growth stage (Herman 1996).

*C Chlorosis was rated on a scale of 1-5 for sparse to extensive chlorotic tissue.
Table 3. Effects of potential control compounds on *Heterodera glycines* soil and root population densities (100cm³) in pots.

| Compound^a | Planted Pots | | Unplanted Pots | | |
| --- | --- | | --- | --- | | |
| | Soil J2 | Soil eggs (1,000) | Root eggs (1,000) | Soil J2 | Soil eggs (1,000) |
| H₂O | 55 a | 12.2 ab | 185 a | 20 a | 9.6 ab |
| H₂O + EtOH | 36 a | 10.5 b | 225 a | 3 b | 18.1 a |
| AA | 77 a | 13.5 ab | 245 a | 3 b | 6.2 b |
| Va | 108 a | 16.9 a | 210 a | 6 ab | 8.5 b |
| CHP | 42 a | 8.5 b | 171 a | 7 ab | 6.5 b |
| MP | 52 a | 10.9 b | 237 a | 8 ab | 9.4 b |
| ZnSO₄ | 66 a | 13.4 ab | 161 a | 6 ab | 9.5 ab |

Values are the means of six replications of the first run of a growth chamber study. Values in the same column with the same letter are not different according to Fisher's LSD values (α = 0.05).

^a AA = 2-hydroxymethylenecyclopentanone, Va = 2-(1-ethoxy-carbonyl-1-hydroxymethylene)cyclopentanone, CHP = 1-cyano-1-hydroxy-2-propene, and MP = methyl pelargonate.
Table 4. Effects of potential control compounds on Heterodera
glycines soil and root population densities (100 cm$^3$) in pots.

<table>
<thead>
<tr>
<th>Compound$^a$</th>
<th>Planted pots</th>
<th>Unplanted pots</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soil J2</td>
<td>Soil eggs (1,000)</td>
<td>Root eggs (1,000)</td>
</tr>
<tr>
<td>$^b$</td>
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<td>$^c$</td>
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</table>

Values are the means of six replications of the second run of a
growth chamber study. Values in the same column with the same
letter are not different according to Fisher's LSD values ($\alpha = 0.05$).

$^a$AA = 2-hydroxymethylencyclopetanone, Va = 2-(1-ethoxy-
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1-hydroxy-2-propene, and MP = methyl pelargonate.
Table 5. Main effects of planting status and compound on *Heterodera glycines* soil population densities (100cm\(^3\)) in microplots treated in 1998 with various compounds.

<table>
<thead>
<tr>
<th>Main factor</th>
<th>Pi</th>
<th></th>
<th></th>
<th>Pf</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eggs</td>
<td>J2</td>
<td>Total(^a)</td>
<td>Eggs</td>
<td>J2</td>
<td>Total</td>
</tr>
<tr>
<td>Plant status</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Unplanted</td>
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<td>30</td>
<td>5,802</td>
<td>3,884</td>
<td>7</td>
<td>3,891</td>
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<tr>
<td>Planted</td>
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<td>80</td>
<td>7,271</td>
<td>16,775</td>
<td>69</td>
<td>16,840</td>
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<tr>
<td>Compound(^b)</td>
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<tr>
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<td>4,415</td>
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<td>H(_2)O + EtOH</td>
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<td>5,135</td>
<td>9,200</td>
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<td>5,831</td>
<td>10,370</td>
<td>58</td>
<td>11,427</td>
</tr>
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</table>

Data are the means of five replications.

\(^a\)Total = Combined value of eggs + J2.

\(^b\)AA = 2-hydroxymethylenecyclopentanone, Va = 2-(1-ethoxycarbonyl-1 hydroxymethylene)cyclopentanone, CHP = 1-cyano-1-hydroxy-2-propene, and MP = methyl pelargonate.
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<tr>
<th>Main factor</th>
<th>Pi</th>
<th>Pf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eggs</td>
<td>J2</td>
</tr>
<tr>
<td>A. Plant status</td>
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<td></td>
</tr>
<tr>
<td>Unplanted</td>
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<tr>
<td>Planted</td>
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<td>12</td>
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<td>B. Compound$^b$</td>
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<td>H$_2$O</td>
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<td>H$_2$O + EtOH</td>
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</tr>
<tr>
<td>AA</td>
<td>12,780</td>
<td>14</td>
</tr>
<tr>
<td>Va</td>
<td>11,390</td>
<td>12</td>
</tr>
<tr>
<td>CHP</td>
<td>6,750</td>
<td>7</td>
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<tr>
<td>MP300</td>
<td>11,750</td>
<td>11</td>
</tr>
<tr>
<td>MP1000</td>
<td>26,000</td>
<td>24</td>
</tr>
<tr>
<td>Formulation blank</td>
<td>7,350</td>
<td>6</td>
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</tbody>
</table>

Data are the means of five replications.

$^a$Total = Combined value of eggs + J2.

$^b$AA = 2-hydroxymethylenecyclopentanone, Va = 2-(1-ethoxycarbonyl-1-hydroxy-methylene)cyclopentanone, CHP = 1-cyano-1-hydroxy-2-propene, and MP = methyl pelargonate.
Table 7. Emergence and yield response of soybeans to soil applications of synthetic compounds in two microplot experiments.

<table>
<thead>
<tr>
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<td>92 a</td>
<td>20 a</td>
<td>162 a</td>
</tr>
<tr>
<td>H₂O + EtOH</td>
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<td>92 a</td>
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<td>164 a</td>
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<td>94 a</td>
<td>20 a</td>
<td>168 a</td>
</tr>
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</tr>
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<td>96 a</td>
<td>20 a</td>
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Data are the means of six replications. Values in the same column with the same letter are not different according to Fisher's LSD values ($\alpha = 0.05$).

AA = 2-hydroxymethylene cyclopentanone, Va = 2-(1-ethoxycarbonyl-1-hydroxymethylene)cyclopentanone, CHP = 1-cyano-1-hydroxy-2 propene, and MP = methyl pelargonate.

Number of plants that emerged from 25 seeds per plot.

Yields are grams of soybeans adjusted to 13% moisture.
Figure 1. Reproductive factors of 1998 microplot study comparing *H. glycines* population densities in planted and unplanted microplots treated with H$_2$O, H$_2$O + ethanol, 2-hydroxymethylenecyclopentanone (AA), 2-(1-ethoxycarbonyl-1-hydroxymethylene)cyclopentanone (Va), 1-cyano-1-hydroxy-2-propene (CHP), methyl pelargonate at a concentration of 300 µg a.i./ml (MP 300), methyl pelargonate at a concentration of 1,000 µg a.i./ml (MP 1000), and a methyl pelargonate formulation blank. Reproductive factors were calculated by dividing Pf by Pi for egg + J2 data. Values among treatments within the planted and unplanted microplots were not different according to ANOVA (P > 0.05).
Figure 2. Reproductive factors of 1999 microplot study comparing *H. glycines* population densities in planted and unplanted microplots treated with H₂O, H₂O + ethanol, 2-hydroxymethylenecyclopentanone (AA), 2-(1-ethoxycarbonyl-1-hydroxymethylene)cyclopentanone (Va), 1-cyano-1-hydroxy-2-propene (CHP), methyl pelargonate at a concentration of 300 µg a.i./ml (MP 300), methyl pelargonate at a concentration of 1,000 µg a.i./ml (MP 1000), and a methyl pelargonate formulation blank. Reproductive factors were calculated by dividing Pf by Pi for egg + J2 data. Values among treatments within the planted and unplanted microplots were not different according to ANOVA (P > 0.05).
Figure 3. Laboratory hatch of *Heterodera glycines* eggs incubated in compounds from first application (A) and second application (B) of 1998 microplot soil study: 2-hydroxymethylenecyclopentanone (AA), 2-(1-ethoxycarbonyl-1-hydroxymethylene)cyclopentanone (Va), 1-cyano-1-hydroxy-2-propene (CHP), and methyl pelargonate (MP). Error bars represent Fisher's LSD values at each day.
CHAPTER 2
THE EFFECTS OF NOVEL, SYNTHETIC COMPOUNDS ON
HATCH AND MOBILITY OF Heterodera glycines

A paper to be submitted to the Journal of Nematology
A. M. Knips, G. L. Tylka, J. R. Coats, & G. A. Kraus

Abstract: The effects of 2-hydroxymethylenecyclopentanone (compound AA), 2-(1-ethoxycarbonyl-1-hydroxymethylene)cyclopentanone (compound Va), 1-cyano-1-hydroxy-2-propene (compound CHP), and methyl pelargonate (compound MP) on Heterodera glycines hatch and mobility were assessed in laboratory experiments. Eggs were incubated in trays containing aqueous solutions of the compounds and in trays of deionized water adjacent to trays of these compounds inside sealed boxes. Hatch of H. glycines eggs was inhibited when eggs were incubated in each of the four compounds. Additionally, hatch of eggs incubated in water adjacent to trays of CHP and MP was inhibited, suggesting volatile effects of the compounds. Both contact and volatile inhibition of hatch by CHP and MP were at least partially irreversible. To assess effects of these compounds on H. glycines mobility, three concentrations of the selected compounds were dissolved into agar disks that were placed on sterile water agar. Second-stage H. glycines juveniles (J2) were added to the plates, and the degree of avoidance by J2 was determined. J2 were repelled from 100 and 1,000 µg a.i. AA/ml, 100 µg a.i. Va/ml, and 10 and 100 µg a.i. CHP/ml. Finally, the percent of mobile J2 after one or twenty-four hours of incubation in 100 µg a.i./ml of the experimental compounds
was assessed. Nearly 100% of nematodes incubated in CHP were immobile after both incubation periods, whereas mobility of J2 incubated in all other compounds was not affected.

**Key Words:** Behavior, chemotaxis, fatty acid, glucosinolates, glycinoeclepin A

*Heterodera glycines*, management, soybean cyst nematode, ZnSO₄

**INTRODUCTION**

Soybean (*Glycine max* (L.) Merill) yields presently are reduced by the soybean cyst nematode, *Heterodera glycines* Ichinohe. This pathogen now is found in all major soybean-producing areas in the United States (Doupnik, 1993; Pratt and Wrather, 1998; Wrather et al., 1995). Novel, synthetic compounds that inhibit or stimulate *H. glycines* egg hatch are being studied as potential supplements to use with nonhost crops and resistant soybean varieties for the management of *H. glycines* (Kraus et al., 1994; Kraus et al., 1996; Tylka et al., 1997). Stimulation of egg hatch in the presence of nonhost crops would result in death of hatched second-stage juveniles (J2) by starvation, and inhibition of hatch during soybean production would prevent the rapid increase of *H. glycines* population densities (Tylka, 1995).

Research on nematicidal compounds often assesses the effect of the compounds on *H. glycines* egg population densities, but reproduction is only one component of the pathogen response. The effect of compounds on behavior of *H. glycines* juveniles is another factor to consider. Correlating juvenile mobility response with impact on egg hatch may identify the modes of action of these or similar compounds.
Nematode mobility in response to test compounds is associated with the process of chemoreception. The concept of amphidial chemoreception for host location by plant-parasitic nematodes was introduced by Steiner in 1925 (Steiner, 1925). In 1983, Zuckerman proposed that nematodes receive and recognize chemotactic signals largely due to carbohydrates on the cuticle surface (Zuckerman, 1983). Today, investigation of these and other chemotaxis hypotheses has become a topic of interest to many nematologists.

_Heterodera glycines_ attraction assays often focus on host plant location, pheromone detection, and response to nematophagous fungi (Castro et al, 1989; Huettel, 1986; Jansson and Nordbring-Herz, 1979; Papademetriou and Bone, 1983; Rende et al, 1982). However, there is a lack of research examining the effects of nematicidal compounds on _H. glycines_ mobility. Consequently, the objective of this study was to determine the mobility response of _H. glycines_ J2 to selected compounds and combine this information with an examination of contact and volatile influences of these same compounds on egg hatch.

**MATERIALS AND METHODS**

**NEMATODE INOCULUM PREPARATION**

_Heterodera glycines_ cysts and females were dislodged from roots of _H. glycines_-susceptible soybean cv. Kenwood 94 that were grown in a greenhouse. Eggs were manually extracted by grinding cysts with a motorized, stainless steel pestle (Niblack et al., 1993) and separated from debris by sucrose centrifugation for one minute at 220g in
454 g/ml sucrose (Jenkins, 1964). Eggs subsequently were surface disinfested by incubation for 15 minutes in 0.05% chlorohexidine diacetate (Sigma Chemical Company, St. Louis, MO) (Acedo and Dropkin, 1982) and repeatedly rinsed in sterile water.

Eggs were hatched on a 30-µm-pore sieve incubated in sterile, deionized water adjusted with 1 M KOH or 1 M HCl to pH 7.0 (hereafter referred to as sdw7). At two-day intervals, *H. glycines* J2 were collected for immediate use.

**MOBILITY ASSAYS**

**TREATMENT PREPARATION.** Treatments included 2-hydroxymethylenecyclopentanone (hereafter referred to as compound AA) and 2-(1-ethoxycarbonyl-1-hydroxymethylene)cyclopentanone (hereafter referred to as compound Va), synthetic analogs of glycinoeclepin A (Masamuni et al., 1982); 1-cyano-1-hydroxy-2 propene (hereafter referred to as compound CHP), a synthetic aglycone; and methyl pelargonate (hereafter referred to as MP), a fatty acid derivative. Control treatments were 3.14 mM zinc sulfate (hereafter referred to as ZnSO₄) and sdw7.

AA, Va, CHP, and MP were tested at concentrations of 10, 100, and 1,000 µg a.i./ml. Each compound was dissolved in 1 ml of 95% ethanol (EtOH), then added to sterile, liquified 1.5% Noble water agar (Sigma, St. Louis, MO). Agar mixed with compound was poured into 9-cm-diam. petri plates. To serve as a control, water agar plates were prepared with the same procedure, adding 1 ml of 95% EtOH without compound to the liquified agar. Plates were sealed with Parafilm after cooling, inverted to prevent condensation, and stored at 15° C in the dark. After 24 hours, a sterile, 10-mm-diam. cork borer was used to cut and remove treatment disks from the plates.
EXPERIMENTAL SET-UP. Sterile, 1.5 % Noble water agar was poured into petri plates. Four equal quadrants and a 2-cm-diam. central circle were designated on the bottom of the plates. Four positions were designated 3 cm from the center for treatment applications, one in each quadrant. Two disks of agar impregnated with a compound, and two disks of untreated agar were placed in opposite positions on each agar plate. For the control plates, untreated agar disks were placed in all four positions. After the agar disks were applied, plates were stored for 12 hours at approximately 26° C (hereafter referred to as room temperature) inside an incubation chamber in the dark. Then, approximately 100 2-day-old *H. glycines* J2 were added to the central circle of each plate in a 50-µl-drop of sdw7. Plates were arranged in a randomized complete block design with layered replications and stored at room temperature inside an incubation chamber in the dark.

DATA COLLECTION AND ANALYSIS. The number of nematodes that had moved into each quadrant was recorded 2, 4, 8, 12, 24, 36, and 48 hours after initiation of the experiment. Avoidance factors were calculated by dividing the number of J2 that had migrated into the two quadrants of the plate with untreated agar disks by the number of mobile J2, defined as the J2 that moved beyond the central circle. Random movement in the absence of any attraction or repulsion would result in equal numbers of J2 entering both water and treated quadrants for an avoidance factor of 0.5. An avoidance factor greater than 0.5 indicated avoidance by the nematode or repulsion by the test compound. Likewise, an avoidance factor less than 0.5 indicated that the test compound was attractive to the nematode. Avoidance factors were averaged across the seven time
periods after noting the lack of a linear relationship of avoidance factors over time. Data were combined across all runs, and means were calculated for each treatment. The differences between the mean avoidance factors of each treatment and the internal standard of 0.5 were assessed using Fisher’s least-significant-difference (LSD) test ($\alpha = 0.05$) (SAS institute, Cary NC).

CONTACT TOXICITY ASSAY

EXPERIMENTAL SET-UP. Three-cm-diam. microsieves were constructed as described by Wong et al. (1993) using nylon screen (Tetko, Briarcliff Manor, NY) with 25-µm-pore openings, which retained J2 in the sieves. Test solutions were 100 µg a.i./ml solutions of AA, Va, CHP, and MP, and control solutions were 3.14 mM ZnSO$_4$ and sdw7. *Heterodera glycines* J2 were added to each microsieve, and each microsieve was subsequently placed in a tray containing 3 ml of a test solution. Trays were placed inside sealed plastic boxes (Althor, Wilton, CT) and incubated for one or 24 hours at room temperature in the dark. The J2 then were rinsed on the sieves with sdw7, and approximately 300 J2 were transferred in a 50-µl drop of sdw7 to the center of 1.5% Noble water agar plates. Plates were stored at approximately 26° C without light for six hours.

DATA COLLECTION AND ANALYSIS. The J2 that had migrated beyond the circumference of a 4.0-cm-diam. circle were designated as mobile and were counted and divided by the total number of J2 for each plate. The percentage of mobile juveniles of the treatments was compared using ANOVA to examine main effects of incubation
solution and duration of incubation (Cochran and Cox, 1957). Upon detection of
treatment differences, the means were separated with Fisher’s LSD test (α = 0.05) (SAS Institute, Cary, NC).

LABORATORY HATCH STUDIES

EXPERIMENTAL SET-UP. Three-cm-diam. microsieves were constructed as
described previously (Wong et al., 1993) using nylon screen (Tetko, Briarcliff Manor,
NY) with 30-µm-pore openings, which retained eggs but allowed J2 to pass through the sieve. Two polystyrene trays (75mm x 32 mm x 13 mm) (Althor, Wilton, CT) were
placed inside a polystyrene box (100mm x 100 mm x 50mm) (Althor, Wilton, CT). One tray was filled with 6 ml of sdw7, and the adjacent tray was filled with 6 ml of test solution. Test solutions were 100 µg a.i./ml of AA, Va, and CHP, 300 and 1,000 µg a.i./ml of MP, 3.14 mM ZnSO4, and sdw7. Approximately 4,000 H. glycines eggs were added to each microsieve. Subsequently, one microsieve was placed in each tray, tight-fitting covers were placed on the boxes, and the boxes were stored at room temperature without light. Sieves were transferred to fresh sdw7 or test solution every two days. When hatch of J2 subsided, all sieves were transferred to trays of ZnSO4 and were transferred every two days for the remainder of the experiment to determine if remaining unhatched eggs were viable. The numbers of hatched J2 that passed through the microsieves were recorded after each transfer.

EXPERIMENTAL DESIGN AND DATA ANALYSIS. Three replications of each treatment were arranged in a randomized complete block design, and the hatch study was
performed twice. There were inconsistent levels of hatch inhibition by the CHP treatment during the second run due to impurities of the compound (J. R. Coats, Professor of Entomology, Iowa State University, personal communication), and for this reason, the results of the second run of CHP were not included in the analysis. Cumulative percent hatch by day was computed separately for the contact and volatile data and subjected to ANOVA to determine treatment solution main effects (Cochran and Cox, 1957). When differences were detected among treatments, the means were separated using Fisher’s LSD values for each day ($\alpha = 0.05$) (SAS Institute, Cary, NC).

**RESULTS**

**MOBILITY ASSAY**

Nematode avoidance factors of J2 for all treatments ranged from 0.37 for ZnSO$_4$ to 0.63 for 1,000 µg a.i. AA/ml (Fig. 1). Movement of *H. glycines* J2 in the water control plates was not different from the internal standard of 0.50. The avoidance factors on ZnSO$_4$-treated plates were less ($\alpha = 0.05$) than the internal standard, indicating attraction of the worms to the compound. Nematodes also were attracted to quadrants treated with 1,000 µg a.i. CHP/ml. Conversely, avoidance factors were greater ($\alpha = 0.05$) than the standard for 100 and 1,000 µg a.i. AA/ml, 100 µg a.i. Va/ml, and 10 and 100 µg a.i. CHP/ml. Effects of compound concentrations were varied. Nematode avoidance factors increased with increasing concentrations of AA, but decreased with increasing concentrations of CHP (Fig. 1). There were no differences from the internal standard at any concentration of MP.
CONTACT TOXICITY ASSAY

Incubation in CHP reduced the mobility of J2 relative to all other treatments. The overall average percent mobile J2 for all treatments except CHP was 27.0 % after one-hour incubation and 15.7 % after 24-hour incubation. Incubation of J2 in CHP for 1 or 24 hours reduced mobility to 1.1% and 0.2%, respectively. After incubation in the CHP treatment, immobile J2 appeared rigid in contrast to the flaccid appearance of immobile J2 for all other treatments. The effects of AA, Va, MP, and ZnSO₄ on J2 mobility were not different than that of sdw7 for both incubation periods (Fig. 2). Incubation of J2 in AA for one hour resulted in a greater percent of mobile J2 (Fig. 2), 31.5%, compared to 26.3% in water, but this difference was not significant.

LABORATORY HATCH STUDIES

CONTACT EFFECTS. Hatch was stimulated when eggs were incubated in ZnSO₄ and inhibited when eggs were incubated in each of the four compounds. At the end of the experiment, cumulative percent hatch for all treatments ranged between 1.3% for 1,000 µg a.i. MP/ml and 59.6% for the MP formulation blank treatment. Cumulative percent hatch of eggs incubated directly in AA, Va, CHP, and both concentrations of MP was less (α = 0.05) than that of eggs incubated in sdw7. These differences began at day six and continued through the transfer to ZnSO₄ on day 18 (Fig. 3A). Hatch in ZnSO₄ was greater (α = 0.05) than hatch in all other treatments through day 16 (Fig. 3A).
Transferring eggs to ZnSO$_4$ stimulated hatching after incubation in AA, but not after incubation in CHP or MP. After the transfer to ZnSO$_4$, eggs previously incubated in CHP and both concentrations of MP hatched less ($\alpha = 0.05$) than those incubated in sdw7, and hatch in 1,000 µg a.i./ml was less ($\alpha = 0.05$) than in all other treatments. Incubation in ZnSO$_4$ caused unhatched eggs previously incubated in AA, the formulation blank, and sdw7 to resume a level of hatch similar to that of eggs continuously incubated in ZnSO$_4$ (Fig. 3A).

**VOLATILE EFFECTS.** Two of the three compounds inhibited hatch by volatile activity. At the end of the experiment, cumulative percent hatch for all treatments ranged from 2.7% for 1,000 µg a.i./ml of MP to 63.0% for the AA treatment (Fig. 3B). There were no differences among egg hatch for the ZnSO$_4$, AA, MP formulation blank, and sdw7 treatments for the duration of the experiment. Cumulative percent hatch of eggs incubated in sdw7 in the presence of CHP and both concentrations of MP was less ($\alpha = 0.05$) than in the sdw7 treatment beginning on day four and continuing through the end of the experiment (Fig. 3B).

The volatile activity of CHP and MP continued to inhibit hatch after eggs were transferred to ZnSO$_4$. The cumulative percent hatch in CHP and both MP treatments remained less ($\alpha = 0.05$) than sdw7 after all sieves were transferred to ZnSO$_4$ (Fig. 3B).

**DISCUSSION**

This study evaluated the effects of several compounds on egg and juvenile stages of *H. glycines*. Our results support previous evidence of *H. glycines* hatch inhibition by
incubating eggs in AA, Va, and CHP (Kraus et al., 1996; Thompson and Tylka, 1997),
and provide the first evidence of *H. glycines* hatch inhibition by MP. The hatch
inhibition of eggs incubated in water in the presence of CHP and MP provides evidence
that the compounds also inhibit *H. glycines* egg hatch through volatile effects. After
contact and volatile inhibition by CHP and MP, a majority of the eggs did not respond to
incubation in ZnSO₄, suggesting both contact and volatile effects are at least partially
irreversible.

Volatility and irreversibility are two characteristics of effective nematicidal
compounds. Volatile nematicides are successful fumigants, rapidly dispersing in soil and
possibly improving penetration of nematode membranes and cyst walls (Metcalf, 1991).
Irreversible inhibition of egg hatch conceivably holds greater potential to affect *H.
glycines* population dynamics in a field environment than inhibition that is temporary.

In addition to the irreversible contact and volatile inhibition of egg hatch, CHP
also has a toxic effect on hatched J2. Very few nematodes moved from their initial
placement on the agar after they were incubated in CHP. This immobile state is not a
certain indication of nematode death (Chen and Dickson, 2000). However, our
observations of immobility up to 5 days after contact suggest that this impact adversely
affects the viability of J2. Affecting both egg and juvenile stages of *H. glycines* suggests
CHP could be a useful management tool if these effects occur in natural soil
environments.

Solutions of AA and Va did not have volatile or irreversible effects on hatch, nor
did they cause contact toxicity of J2. However, J2 were repelled by these two compounds
at some concentrations, and there was an indication of increased mobility of J2 after one hour incubation in AA. Incubation of *H. glycines* eggs in AA also has been shown to stimulate the hatch of *H. glycines* eggs at 1, 2.5, and 5 µg a.i./ml (Thompson, 1997). Possible stimulation of J2 mobility by AA and evidence of increased hatch (perhaps caused by stimulation of the developing J2) resemble similar responses of other nematodes to nematicides, such as the initial periods of hyperactivity of *M. incognita* and *C. elegans* in response to carbamate and organophosphate nematicides (Opperman and Chang, 1990). Further studies are needed to increase the understanding of these responses of *H. glycines* to glycinoeclepin A analogs.

The lack of differences in J2 mobility after incubation in AA, Va, MP, and sdw7 indicates that these compounds do not cause contact toxicity of J2. However, the possibility remains for these test compounds to affect the orientation of *H. glycines* J2 toward a soybean root without affecting general mobility. Such effects would not be determined in either the contact toxicity or mobility assays and should be examined in further studies.

In addition to the test compounds, ZnSO₄ was included as a treatment for each assay. Zinc sulfate has been included in past *H. glycines* laboratory hatch studies as a positive control treatment due to its consistent hatch-stimulation effects (Clark and Shepherd, 1966; Tefft and Bone, 1984). In addition to the hatch response of *H. glycines* eggs, studies suggest that *H. glycines* juveniles are attracted to ZnSO₄ and other ions. Papademetriou and Bone (1983) found a dosage-dependent attraction of *H. glycines* juveniles to ZnCl₂, ZnSO₄, CaSO₄, and MgCl. Tefft and Bone (1984) also reported an
observed attraction to ions including ZnCl₂. Other nematodes, including *Caenorhabditis elegans*, are also attracted to several anions and cations (Cl⁻, Br⁻, I, Na⁺, Li⁺, K⁺, and Mg⁺) (Ward, 1973). In our studies we found an attraction of *H. glycines* J2 toward ZnSO₄, and there were no contact toxicity effects on J2 after one- or twenty-four-hour incubation in ZnSO₄. A clear explanation of these behaviors does not exist at this time. The attraction of *H. glycines* to ZnSO₄ is of particular interest due to the stimulating effect of the compound on *H. glycines* hatch. Understanding the mechanisms by which ZnSO₄ affects nematode behavior would be an important addition to our understanding on the biology of this pathogen.

In conclusion, by combining the effects of test compounds on both egg and juvenile stages, this study demonstrated a useful approach for understanding the activities of these compounds. Understanding how AA, Va, CHP, and MP affect *H. glycines* may facilitate useful field application of these or similar compounds.

**Literature Cited**


Figure 1. Response of *H. glycines* J2 on agar plates treated with H$_2$O, ZnSO$_4$, 2-hydroxymethylene cyclopentanone (AA), 1-cyano-1-hydroxy-2-propene (CHP), and methyl pelargonate (MP). The experiment was repeated four times with two replications for each trial. Values are means of data taken at seven time periods for the four experiments. Comparisons are between each treatment and the internal standard of 0.5. The internal standard is represented by the dashed reference line. Avoidance factors greater than 0.5 indicate movement of nematodes away from the test compound. Error bars indicate absolute values of Fisher's LSD.
Figure 2. The percentage of mobile *H. glycines* J2 after incubation of the J2 for one or twenty-four hours in H₂O, ZnSO₄, 2-hydroxymethylenecyclopentanone (AA), 2-(1-ethoxycarbonyl-1-hydroxymethylene)cyclopentanone (Va), 1-cyano-1-hydroxy-2-propene (CHP), or methyl pelargonate (MP). Bars represent means of three trials consisting of four replications each. The values for CHP are different than all other treatments for each incubation period (α = 0.05). No other differences existed among treatments.
Figure 3. *Heterodera glycines* egg hatch in response to contact (A) and volatile (B) effects of ZnSO₄, H₂O, methyl pelargonate formulation blank (MP-FB), 2-hydroxymethylene cyclopentanone (AA), 1-cyano-1-hydroxy-2-propene (CHP), and methyl pelargonate (300 µg a.i./ml (MP 300), and 1,000 µg a.i./ml (MP 1000)). Error bars represent Fisher's LSD values at each day.
GENERAL DISCUSSION

The current *H. glycines* management recommendation integrates use of host resistance with crop rotation. Each of these two strategies is more effective when used in combination than alone. Chemical control is a common strategy in other pathosystems and a logical progression in *H. glycines* research that may further enhance management options. Integration of chemical control within the current management recommendation conceivably could multiply the benefits at each tactic of the management program. The research comprising this thesis examined the potential of synthetic compounds AA, Va, CHP, and methyl pelargonate to fill this need. Significant hatch reductions in the laboratory hatch studies were not mirrored in either growth chamber or field conditions, and the reason remains a question.

This discussion is intended to stimulate thought - why does the discrepancy exist between laboratory and field results and how can the approach to chemical testing be improved? The first half of the discussion looks at the responses of encysted eggs versus those of egg-mass eggs as a possible source of experimental error. The second half suggests an emphasis for chemical testing that integrates pathogen life cycle, host development, and chemical persistence.

A majority of the eggs in this study were obtained from within females or cysts by manual extraction. In nature, the *H. glycines* female deposits approximately one-third of the eggs in an external egg mass, and the remaining eggs are retained internally (Ishibashi et al., 1973). Under natural field conditions, eggs in the external egg masses would hatch first, whereas eggs inside cysts are the secondary source of hatch (Ishibashi et al., 1973).
Taking this into account, it becomes evident that the eggs used in these and other laboratory hatch studies do not represent natural field populations.

Studies suggest that the two egg sources have different responses to hatching cues. A study conducted by Lehman et al. (1971) showed different hatching responses at different pH levels by eggs inside cysts compared to inside egg masses. Hatch of eggs from cysts was stimulated between pH 2.5 and 3.5, while hatch from egg masses showed no preference. Also, the timing of hatch differed sharply. The majority of encysted eggs hatched between day six and day thirteen, while the majority of egg-mass eggs hatched in the first six days (Lehman et al., 1971). Different hatching responses from the two egg sources also were detected in a study by Thompson and Tylka (1997). Encysted eggs were inhibited at a greater rate than egg-mass eggs when incubated in a synthetic analog of glycinoeclepin A. Other analogs tested showed no differences.

The factors that contribute to these different responses have been considered. Thompson and Tylka (1997) state that hatch response of egg-mass versus encysted eggs is explained in part by developmental stages of the juvenile nematodes. Other possible contributing factors include dormancy effects, physical damage during the extraction process, and intrinsically different responses to hatching stimuli (Thompson and Tylka, 1997).

Whatever the cause of these different responses, it is important to determine the implications of this information for future *H. glycines* research. Assuming egg masses pose the initial threat in the field and are, therefore, the target of synthetic test compounds, one implication is that laboratory studies testing eggs extracted from cysts
become less valuable. If a different mechanism is responsible for hatch of egg-mass and encysted eggs, significant hatch inhibition from laboratory studies would have less relevance for the dynamics of a field-soil population of *H. glycines*. Hatch inhibition of encysted eggs would be an important tool for disease management, but only if the compound is able to penetrate the cyst wall and if the effects are irreversible. It is thought that encysted eggs are inhibited by natural compounds found in the cyst wall (Okada, 1972). Therefore, a temporary and artificial hatch inhibition of encysted eggs may not impact overall population densities in the presence of naturally maintained hatch inhibition levels.

The variability between egg-mass and encysted eggs is only one of the possible factors contributing to variable results. In addition to focusing on single factors, *H. glycines* research can be directed toward examining multiple-factor relationships that compose the “big picture” of chemical control. By emphasizing the relationships among characteristics of the pathogen, host, and chosen test compounds, possible constraints in the current approach to chemical control may be determined. The following example uses these factors and their relationships to analyze a single aspect of chemical control - the timing of nematicide applications.

Persistence of nematicidal compounds could be maximized at certain points in the growing season. Nematicide studies often involve one or two applications of chemical at the beginning of the growing season. Justification of early application is based on controlling nematode numbers before they have a chance to increase exponentially. Effectiveness of the compound in soil extends a characteristically brief period of time.
This window of effectiveness for early nematicide applications does not correspond to the population dynamics of *H. glycines*. Maximum hatch of *H. glycines* eggs incubated in soybean root diffusate occurs with soybeans at the R2 stage of development (Tefft and Bone, 1985). If a correlation can be made to hatch in a field environment, a roughly estimated time frame puts this maximum hatch at approximately 50 days after soybean emergence. On average, this leaves 40-50 days until the crop matures. During this time, there is the possibility of two additional nematode life cycles beyond the protection of early season nematicide applications.

Biology of the pathogen, especially as it affects host yield, is another important factor to consider for determining the timings of nematicide applications. The ability of *H. glycines* to reduce yield is attributed to reduced nodulation (Barker, et al., 1972; Huang et al., 1984; Ko et al., 1986) and interference with healthy root function (Radcliffe et al., 1990). Nodules begin to form only one week after a soybean seedling emerges, and 10-14 days later the nodules are providing the full nitrogen requirements of the plant (Scott et al., 1970). Nodulation, therefore, conceivably is impacted by early season nematode populations, which are most greatly affected by early season nematicide application. However, healthy root function is important throughout soybean development. Nematode interference with normal root function may affect yield through a larger portion of the growing season, and therefore could be affected by multiple nematicide applications.

And finally, nutrient demands of the host plant help determine effective timings of chemical applications as well. Soybeans require more attention to their development later
in the growing season when nutrient requirements are greatest (Fagaria et al., 1991). The growth stage of a soybean that is most susceptible to stress is between R4 and R6, roughly estimated to be 70-95 days after emergence (Herman, 1996). This information suggests that the focus on early season application may be too narrow. However, studies support the benefits of early-season nematicide applications (Wrather and Anand, 1988), and this analysis of application timings was meant simply to generate ideas.

In conclusion, the purpose of this discussion was not to suggest absolute answers, but rather to encourage integrative thinking that takes into account the decades of \textit{H. glycines} information that have already been gathered. By simultaneously considering characteristics of the pathogen, host, and test compound, the possibility of chemical control of \textit{H. glycines} can be evaluated thoroughly.

\textbf{Literature Cited}


