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DISTRIBUTION AND CONTROL OF SOYBEAN CYST NEMATODE, *Heterodera glycines* Ichinohe (Tylenchida: Heteroderidae) IN OHIO

DISSEPTION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By
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*****

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2001

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ABSTRACT

Between 15 Aug 98, and 8 Jun 99, 4,500 soil samples from agronomic soils were sent to the C. W. Ellett Plant and Pest Diagnostic Clinic. Approximately 57.6% of the samples contained no soybean cyst nematodes (SCN), *Heterodera glycines* Ichinohe. SCN infestations were found in Franklin, Holmes, Lorain, Marion, Van Wert, Wayne, and Williams Co., previously not known to have SCN. As of 8 Jun 99, 59 Ohio counties were known to have SCN infestations.

Low (1.27 g) and high (6.35 g) rates of N-Viro Soil ® (NVS) (N-Viro International Corporation of Toledo, OH) were applied to the surface or mixed with 150 mL construction sand in cone-tainers ® C-10 (Stuewe&Sons, Inc., 2290 SE Kiger Island Dr., Corvalis, OR). One seed of soybean [*Glycine max* L. (Merr.)] ‘Corsoy 79’ was planted per cone-tainer. Treatments were replicated five times. Surface application and sand amended with low and high rates of NVS significantly \((P\leq 0.05)\) suppressed cyst, egg and *Rhizobium* nodule numbers on soybean roots compared to no NVS treatment. Plants receiving only the high rates were significantly \((P\leq 0.05)\) taller than those receiving no NVS with both application types. Low and high rates of NVS significantly increased in fresh shoot, dry root weight compared to the control with both application methods, although only high rates of NVS caused a significant increase in fresh root weight in surface application.
Egg numbers extracted from SCN cysts were significantly fewer in soil samples from the plots receiving 5 and 25 T NVS/A compared to the control. Although midseason egg population of SCN (Log 10) was significantly low from plots receiving 25 T NVS/A, postharvest egg population (Log 10) was significantly lower from plots receiving either 5 or 25 T NVS/A compared to the control. Harvest egg population of SCN was also significantly low in soil samples from plots where resistant soybean was grown.

Leachates of 0, 1 and 3 mo-old NVS significantly inhibited egg hatch of SCN compared to distilled water at 72 hr. Leachates of 6 and 12 mo-old NVS and 3 mM zinc sulfate significantly stimulated egg hatch compared to distilled water at 72 hr. Ammonia at 0.001, 0.01, 0.1 M in 0.02 M phosphate buffer significantly suppressed the egg hatch of SCN at 72 hr compared to distilled water and 0.02 M phosphate buffer. Three mM concentration of ZnSO₄ solution stimulated egg hatch compared to distilled water and 0.02 M phosphate buffer.
Dedicated to:
The memory of my father, my mother,
my brothers and sisters and
my nieces and nephews
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Economically important plant parasitic nematodes belonging to 24 nematode
genera cause about 10% of crop losses worldwide (Whitehead, 1998). Depending on
circumstances, losses due to plant parasitic nematodes may vary from negligible to total.
Left unchecked, nematodes can cause severe crop losses on crop plants (Whitehead, 1998).

The Soybean Cyst Nematode (SCN), *Heterodera glycines* Ichinohe, a devastating
root pathogen of soybean was first reported from Japan in 1915 (Riggs, 1977) although a
disease caused by SCN, but attributed to *H. schachtii*, had been known as “Moon Night
Disease” since 1881. SCN was reported from Korea in 1936, Manchuria in 1938,
Taiwan in 1958, Republic of Egypt in 1968, the Soviet Union in 1978, Java in 1984
and Brazil in 1993 (Mendes and Dickson, 1993) where it is considered to be major threat
to soybean production.

In the USA, SCN was first detected in a bulb-growing area in North Carolina in
1954 (Winstead et al., 1955). SCN was detected in southeastern Missouri and Tennessee
in 1956 (Epps, 1956), Arkansas, Kentucky and Mississippi in 1957, Virginia in 1958,
Illinois in 1959, Florida and Louisiana in 1967, Indiana in 1968, South Carolina in 1971,
Alabama in 1973, Oklahoma in 1975, Georgia in 1976, Delaware, Iowa (Noel, 1992),
Maryland (Sardenelli et al., 1982) and Minnesota in 1978 (MacDonald et al., 1980). SCN was found in North and South Dakota and Pennsylvania in 1992 (Noel, 1992). SCN was found in Ohio in 1980 in soil on tomato transplants (Hammond et al., 1981), Wisconsin in 1981, New Jersey in 1982, Texas in 1983, Kansas in 1985 (Noel, 1992), Nebraska in 1986 (Powers and Wysong, 1987) and Michigan in 1987 (Noel, 1992). SCN was first detected in Ohio on soybean in Sandusky Co. and Scioto Co. in 1987 (Riedel and Golden, 1988). Twenty-nine soybean-producing states are now infested by SCN (Noel, 1992).

Symptoms of SCN can be confused with the symptoms of nutrient deficiencies, chemical injury and other soybean disorders. Heavily infected plants may exhibit chlorosis or be stunted when the nematode population density is high, and the ameliorating conditions such as nutrient supply and ample water are favorable to the pathogen (Kinloch, 1998). Chlorosis is caused primarily due to N deficiency as a result of suppression of *Rhizobium* nodule formation by the nematode infection (Ichinohe and Asai, 1956).

Rotation with nonhost crops, use of nematicides and resistant cultivars are the important tools to manage SCN in the infested soybean fields. The volatile chemicals, D-D (dichloropropene-dichloropropane mixture) and DBCP (1,2-dibromo-3-chloropropane) have been used to control soil-borne plant diseases since 1943 and 1954, respectively (Thomason et al., 1983). Granular organophosphate and carbamate nematicides were introduced into the market for farmers’ use in 1960’s and in 1970’s, respectively. Although many granular nematicides can move from roots to foliage, a few (oxamyl, phenamiphos, and carbofuran) have some capacity to move from foliage
to roots in some plants (Thomason et al., 1983). However, many effective nematicides, which are not environmentally friendly and are not readily biodegradable, are banned and not available to growers anymore. DBCP (1,2-dibromo-3-chloropropane) and aldicarb (Temik®) (Thomason et al., 1983) were found in deep water wells in the central valley of California and in ground water in Long Island, NY, respectively (Thomason et al., 1983).

Yields of resistant soybean cultivars were equal to or greater than susceptible cultivars in all SCN-infested production fields in Ohio (Wheeler et al., 1997). Frequent use of resistant soybean cultivars may increase the selection pressure on SCN, which leads to appearance of new SCN populations. New populations are able to reproduce on previously resistant soybean cultivars.

In southern states, failure of some resistant cultivars in reducing SCN losses suggests that genetic variability in SCN populations exists (Young, 1992). The greater diversity of races of SCN in southern United States could be due to a longer history of planting resistant cultivars (Kim et al., 1997). Rotation of different sources of resistance may overcome this problem. Employment of susceptible cultivars in rotation programs is advised for SCN management in order to limit shifts in feeding behavior of SCN populations, although this practice has not able to prevent shifts in feeding activity (Wrather et al., 1992). Ability of second stage (J2) juveniles of SCN to parasitize the resistant cultivars Bedford and J81-116 (resistant to race 14 of SCN) was not prevented by rotations of soybean cultivars with different sources of resistance, and rotations of resistant and susceptible cultivars (Forrest, J82-21, and Peking x Centennial breeding line) with a nonhost (maize, *Zea mays* L.). However, the shift toward greater parasitism
of resistant cultivar (Bedford and J81-116) was slowed and greater mean soybean yields were produced by rotation of resistant and susceptible cultivars with a nonhost crop which makes this practice feasible (Young, 1998). In general, rotation of two consecutive years of nonhost crops such as maize and grain sorghum (*Sorghum bicolor*) also reduces SCN populations below damaging levels (Riggs and Schmitt, 1989).

More alternative control practices must be available to soybean growers to manage SCN and to achieve the best economic returns for their farming operations. Black velvetbean (*Mucuna aterrima*) and pigeon pea (*Cajanus cajan*) could also be used as trap plants because they attract a high number of J2 juveniles and few females develop on these plants. Few or no females were found in pigeon pea roots and black velvetbean, respectively (Valle et al., 1997). In another study, velvetbean effectively reduced yield loss caused by mixed populations of *Meloidogyne arenaria* and *H. glycines*, regardless of genetic resistance of soybean cultivar (Weaver et al., 1993).

Although various organic and inorganic soil amendments are known to reduce various plant parasitic nematode populations in soil, effects of N-Viro Soil (NVS) on parasitic nematodes have received recent attention. NVS is a municipal biosolid product in which human pathogens are killed by an alkaline stabilization process combining alkaline pH, drying, high temperature, high ammonia and salts (Burnham et al., 1992a and 1992b). A Cement Kiln Dust amended sewage sludge (CKDS) application of approximately 8 T/A affects soybean yield equivalent to fertilizer application of 200 kg N/ha (Logan et al., 1988). Although NVS is used in land reclamation, landfill cover, ocean fill or applied to agricultural land as a fertilizer (Logan and Harrison, 1995, Logan and Burnham, 1995), its potential to suppress parasitic nematode populations in
soil was not studied in detail. In the research reported here, effects of NVS on reproduction of SCN and host growth in the greenhouse and the field studies were investigated. Effects of NVS and ammonia on egg hatch of SCN were also investigated \textit{in vitro}.

Clear understanding of the mechanism of egg hatch and environmental and chemical factors that influence egg hatch must be known to develop an effective management tactic for SCN. Hatching of cyst nematodes (Clarke and Perry, 1977) and host plant influences on hatching have been reviewed. Factors such as temperature, moisture, aeration and diapause that influence hatching have also been discussed (Perry and Gaur, 1996).

The eggshells of \textit{Heterodera glycines} consist of a chitinous layer which comprises an osmophilic outer portion and a more substantial, less densely staining inner portion, and a lipid layer which is composed of a thin, amorphous outer layer external to a predominantly tetra- or pentalaminate inner layer (Perry and Trett, 1986). The epicuticle is formed by a secondary viteline membrane that is initially indistinguishable from the inner lipid layer, which separates as the first stage (J1) juvenile cuticle secreted during embryonation (Perry and Trett, 1986).

Permeability of the lipoprotein membranes of the eggshell is changed when the eggs of \textit{Globodera rostochiensis} are stimulated to hatch in potato root diffusate (PRD) (Perry et al., 1982). Hatching factor found in potato root diffusate has some of the properties of the cardiac glycosides and simpler lactones that may influence ion transport (Ellenby and Gilbert, 1957). Changes in permeability that may involve structural alterations of the lipoprotein membranes of the eggshell can be caused by
Ca\(^{2+}\) ion (Clarke and Perry, 1985). Emergence of J2 juveniles of *Heterodera rostochiensis* Woll. was significantly higher in solutions of hatching factor containing calcium ions than in those containing potassium ions (Ellenby and Gilbert, 1958). Clarke and Hennessy (1983), however, demonstrated that presence of free Ca\(^{2+}\) in the hatching medium is not essential for egg hatch of *G. rostochiensis*. Among the Na, K\(^{+}\), Ca\(^{2+}\), and Mg\(^{2+}\) chlorides they tested, only solutions containing 0.5 mM CaCl\(_2\) stimulated the emergence of significantly more juveniles from eggs in cysts than the exudate alone. They suggested that hatching of *G. rostochiensis* eggs involve changes in eggshell permeability caused by the effect of the hatching factor on bound Ca\(^{2+}\). In another study, egg hatch of *H. glycines* was not affected by calcium sulfate and calcium chloride compared to water (Teft and Bone, 1984). Clarke and Perry (1985) observed that Ca\(^{2+}\) content of intact eggs of *Heterodera schachtii* was not decreased by the treatment of eggs first with EDTA then with HCl compared to the untreated eggshells. However, they observed a decline in Ca\(^ {2+}\) content after treatment of empty-eggshells with EDTA/HCl. They suggested that structural changes in the eggshell occur and lead to altered eggshell permeability to solutes when hatching agents bind to or replace membrane bound cations.

Perry and Feil (1986) developed a novel hatching bioassay technique for *G. rostochiensis* based on the hypothesis that eggshell permeability changes and water uptake of juveniles is induced by hatching agents such as potato root diffusate, which may allow the passage of selected fluorochrome through the eggshell and into the unhatched juvenile. They observed that the percentage of juveniles fluorescing after PRD/acridine orange treatment was greater than ZnSO\(_4\)/acridine orange and distilled
water/acridine orange treatments, and correlated well with 80% egg hatch obtained from routine hatching test. Five minutes per week immersion of *G. rostochiensis* eggs in potato root diffusate for five weeks stimulated hatching from free eggs (Perry and Beane, 1982). Use of fresh diffusate and repetition of stimulus enhanced greater hatch of *G. rostochiensis* free eggs (not in cysts) than single exposures to diffusate or when the same diffusate was used each week (Perry and Beane, 1982). Cysts and free eggs of *G. rostochiensis* exposed to PRD before subjecting them to desiccation resulted in less hatch than those exposed to soil leachate when the cyst and eggs were stimulated to hatch after rehydration (Perry, 1983). Similarly, in another study, as desiccation time of cysts increased, egg hatch from cysts declined (Perry, 1983). Hatching rate of 3-year-old potato cyst nematode populations was more than double (mean=44.5%) than that shown by newly reared populations (mean=19.1%) (Gonzales and Phillips, 1996). Root diffusates of cereals and grasses, however, did not stimulate eggs of *H. rostochiensis* to hatch in vitro (Hesling et al., 1961). Emergence of juveniles from *Globodera pallida* eggs was significantly higher and earlier than *G. rostochiensis* (Gonzales and Phillips, 1996).

Forrest and Phillips (1984) reported that the hatching activity of root diffusate of partially resistant *Solanum tuberosum* X *Solanum vernei* clones was less than *S. tuberosum* cultivars in stimulating hatch of *G. pallida* eggs in pots in greenhouse. They, however, concluded that reduced hatching activity was not totally due to the overall resistance of the *S. vernei* hybrids. The least, fewer, and the greatest egg hatch of *G. pallida* were obtained when cysts were soaked in root diffusates of *S. vernei*, *S. vernei* X *S. tuberosum* hybrids, and *S. tuberosum* cv. Pentland Crown, respectively (Farrer and
Phillips, 1983). Atkinson et al. (1987) purified a hatching agent from potato root diffusate, 437 Da molecular weight, 60% anionic in nature and polar which was able to influence the nucleolus of dorsal pharyngeal gland cell nucleus of *G. rostochiensis*. The diameter of nucleolus of J2 juveniles of *G. rostochiensis* increased after soaking eggs in potato root diffusate for 3 to 4 days (Atkinson et al., 1987). The change in diameter of the nucleolus was synchronized with accumulation of secretions in this gland cell before hatching (Atkinson et al., 1987).

Lipid utilization of unhatched J2 juveniles of *G. rostochiensis* was increased after stimulation of hatch by potato root diffusate at eclosion (hatch), and reduced lipid content and delayed development in J2 juveniles were observed in late hatching juveniles (Robinson et al., 1985). Reduced motility and infectivity of hatched *G. rostochiensis* juveniles were correlated in depletion of lipid reserves during a longer storage period in a test and were significant when lipid reserves fell below approximately 65% of the original level (Robinson et al., 1987). In *G. rostochiensis* juveniles, no lipase activity was detected during hatching (Perry et al., 1992) and probably lipase does not play a major role in hatching.

Laughlin et al. (1974) suggested that the stylet does not play a primary role in the hatching of *Heterodera iri*. They drew this conclusion from the fact that *H. iri* emerged tail-first. On the other hand, the eggshell could be broken by the stylet and then this possibly allowed J2 juveniles to hatch tail-first.

A higher number of eggs of SCN hatched in leachate from 8-week-old susceptible soybean cv. Williams 82 and A3127 than in leachates from susceptible A2525 and resistant Fayette. The greatest egg hatch was observed during the initial 12
days of the experiment (Sikora and Noel, 1996). Teft et al. (1982) reported that as concentration of soybean root leachate increased, the mean rate of egg hatch of SCN increased. Soybean root leachate from Hartwig, a highly resistant variety, stimulated the greatest egg hatch (50.8%). A greater egg hatch occurred in root diffusates from a susceptible variety Essex than that in distilled water when free eggs were used in total volume of 10 mL treatment (Anand and Handoo, 1997). In another study, emergence of J2 juveniles of SCN increased further when root exudate solution was filtered with a 0.22 µm pore filter compared to that in nonfiltered root diffusate (Levene et al., 1998). In several tests, however, soybean root diffusate did not stimulate egg hatch of SCN (Skotland, 1957 and Slack and Hamblen, 1961).

Root diffusates from vegetative and reproductive stages of soybean plants increased hatching of SCN eggs compared to that from senesced soybean plants (Teft and Bone, 1985). Hill and Schmitt (1989) observed that pod-producing soybeans stimulated more eggs to hatch and supported greater reproduction of nematodes than those remaining vegetative.

Induced hatching of SCN eggs in soybean root diffusates was not affected by addition of the plant growth regulators, gibberellins or kinetin to root diffusate (Teft and Bone, 1985). Root diffusates with EDTA significantly stimulated hatching compared to normal root diffusate (Teft and Bone, 1985). Root leachates of pigeon pea or black velvet bean (Mucuna atrrima) stimulated the hatching of SCN eggs (Valle et al., 1997).

Emergence of J2 juveniles of Heterodera cajani, pigeon pea cyst nematode from mature cysts was stimulated 15-20% in root exudates from 15-day-old pigeon pea (Cajanus cajan) cv. ICPL 87 (Singh and Sharma, 1996). Hatching of H. cajani eggs
declined but was not completely inhibited when cysts were transferred to cowpea root
diffusate after up to 12 months desiccation (Gaur et al., 1996).

Root diffusates of certain plants within families of Chenopodiaceae and
Cruciferae, and zinc sulfate caused a stronger stimulation of hatch of H. schachtii eggs
than tap water, although their effects on Heterodera trifolii eggs were less evident
(Steele et al., 1982).

Leachates collected from 25-day-old or older corn plants growing in sand or
sandy field soil stimulated 22-24% more egg hatch of H. zeae from cysts after 28 days
than occurred in tap water. Emergence of J2 juveniles was, however, inhibited in the
leachates stored for 30 days at 4 C, and leachates of sand, sandy field soil, and silty field
soil compared to tap water (Hashmi and Krusberg, 1995).

Root diffusates of 22 summer weeds (except those of Rumex dentatus)
belonging 15 plant families and 12 winter weeds from 8 plant families stimulated egg
hatch of H. zeae. Of the summer weeds and winter weeds, root diffusates of Echinocloa
colonum and Melilotus indica most effectively stimulated egg hatch (Ismail and Hasaba,
1995).

Ibrahim et al. (1993) evaluated the effects of rice root diffusate, banana root
diffusate, soil leachate and distilled water on hatching of the rice nematodes,
Heterodera sacchari and Heterodera oryzicola using cysts collected from rice plants.
They found that the dependence of H. oryzicola on root diffusate was higher than that of
H. sacchari. The number of J2 juveniles hatched from H. sacchari eggs in cysts did not
differ significantly when the eggs were incubated in sugarcane root diffusate and in
water (Garabedian and Hague, 1984).
Egg hatch of *Heterodera crucifera* from field cysts and from eggs in egg masses was faster in oilseed rape root diffusate than that from white and brown females without egg masses. Optimum hatching temperature was 16°C (Koshy and Evans, 1986).

Egg hatch of tobacco cyst nematode, *Globodera tabacum solanacearum* was stimulated more by root exudates from resistant NC567 and susceptible K326 cultivars of flue-cured tobacco, *Nicotiana tabacum*, than by deionized water at 25°C (Wang et al., 1997).

Increase in juvenile activity and plasticity of the eggshell leads to breakdown of a lipid layer during hatching of *Meloidogyne javanica* eggs (Bird, 1968). No significant differences in cumulative egg hatch of *Meloidogyne hapla* and *M. chitwoodi* were observed in root leachates of potato cv. Russet Burbank, tomato cv. Columbian, and wheat (*Triticum vulgare*) cv. Hyslop and in distilled water (Inserra et al., 1983). Hatching of J2 juveniles of three root-knot nematodes, *M. hapla*, *M. incognita*, and *M. javanica* from egg masses significantly increased due to emanations from young seedling roots of Rutgers tomato (Viglierchio and Lownsbery, 1960). Root diffusates from 3 to 10 wk-old Pangola digitgrass (*Digitaria decumbens* Stent.) stimulated a greater number of eggs of *M. incognita* to hatch than plants 11 to 14 wk-old (Haroon and Smart, 1983).

Of 283 compounds tested, 31 resulted in hatching of *Heterodera schachtii* eggs equal to or greater than beet (*Beta vulgaris*) root diffusate. Nicotinic acid and picric acid were the most active compounds (Clarke and Shepherd, 1964). Clarke and Shepherd (1965) included metallic ions not tested previously, $\text{Ba}^{2+}$, $\text{Al}^{3+}$, $\text{Pb}^{2+}$, $\text{MoO}_4^{2-}$, $\text{Co}^{2+}$,
Zn$^{2+}$, and Cd$^{2+}$, and reevaluated the effect of MgCl$_2$, KCl, NaCl, HgCl$_2$, and FeCl$_3$. At ~3mM, zinc sulfate, zinc nitrate, and zinc and cadmium chlorides were more active in stimulating \textit{H. schachtii} egg hatch than the other twelve moderately or weakly active and ten inactive or inhibitory inorganic hatching agents. They observed little egg hatch with \textit{Heterodera goettingiana} in zinc chloride, more egg hatch in zinc chloride than in water with \textit{H. schachtii}, \textit{H. avena}, \textit{H. carotae}, \textit{H. cruciferae}, \textit{H. glycines}, \textit{H. rostochiensis}, \textit{H. tabacum}, and \textit{H. trifolii}.

Anhydrotetronic acid is a good synthetic hatching agent (Bishop, 1955, Winslow, 1959). Only 3 mM aqueous solution of anhydrotetronic acid, but not other tetronic acid derivatives, and potato root diffusate hatched similar number of the potato cyst nematode, \textit{H. rostochiensis}, eggs (Calam et al., 1949). Picric acid and potassium permanganate (Doliwa, 1956) stimulate egg hatch of \textit{H. rostochiensis}. Concentrations of 0.4 to 4 mM picrolonic acid were as effective as potato root diffuse to hatch eggs of \textit{H. rostochiensis} (Clarke and Shepherd, 1966). It also stimulated egg hatch of SCN and the tobacco cyst nematode, \textit{H. tabacum}, but none of the cabbage cyst nematode, \textit{H. cruciferae}, eggs. Flavianic acid at 0.6 to 3 mM concentration hatched a greater number of \textit{H. schachtii} and \textit{H. trifolii} eggs and more eggs of SCN than root diffusates did. The hatching activity of 0.3 M sucrose and inositol in 2 mM picric acid was not significantly different for \textit{H. schachtii} eggs than distilled water, although 0.6 M solution of sucrose completely inhibited egg hatch compared to distilled water (Perry et al., 1980b).

Zinc salts were reported to be very active hatching stimulants for SCN \textit{in vitro} (Clark and Shepherd, 1966). Teft and Bone (1984) reported that zinc chloride and zinc sulfate elevated hatching of SCN eggs. In another study, reagent grade zinc sulfate and
zinc chloride and fertilizer grade zinc sulfate stimulated significantly greater number of SCN eggs to hatch than deionized water while zinc chelate fertilizer significantly inhibited egg hatch compared to deionized water. However, in a field experiment, no differences between SCN egg population densities and corn yields were observed among plots that received 0, 11.2, and 22.4 kg Zn/ha rates of zinc chelate in a rotation study (Behm et al., 1995). A wide variation in egg hatch of SCN was observed in response to hatching stimuli when free and encysted eggs were used (Casta et al., 1997). A larger number of J2 juveniles of SCN emerged from free eggs than from encysted eggs when they were treated with equal concentrations (3 mM) of zinc chloride (Casta et al., 1997).

Application of 150 mg amino acid antimetabolites, DL alanine, DL valine and DL methionine, two week after inoculation significantly decreased the number of H. avenae Woll. on the roots of wheat, Triticum aestium L. (Prasad and Webster, 1967). Among the several analogs of glycinoeclepin, a hatching stimulus of SCN eggs at concentration above 10-12 g/mL, a keto diacid was found to be a hatch inhibitor (Kraus et al., 1996).

Perry and Beane (1988) reported that activated charcoal delayed hatching of J2 juveniles from G. rostochiensis eggs in cysts in charcoal/loam mixtures in pots. In contrast, they observed that yield of potato was significantly increased when plants were grown in pots where charcoal had either been incorporated in the soil or used as a substrate in outdoor pot experiments.

Hatch of J2 juveniles from encysted SCN eggs was inhibited by compounds containing NH$_4^+$ and NO$_3^-$ ions compared to phosphate buffer (Lehman et al., 1971).
Zheng and Ferris (1991) proposed four types of hatching pattern of *H. schachtii* eggs: 1) nondormant eggs that hatch readily in water (40-50%); 2) eggs, called obligately quiescence, that hatch readily in host root diffusate (10%); 3) eggs that hatch over a long period of time in water (10%), a phenomenon called delayed egg hatch; and 4) eggs that hatch over a long period in host root diffusate (30%), host-mediated, obligate diapause. Gaur et al. (1995) demonstrated the presence of three kinds of eggs in *Heterodera sorghi* cysts: the first type of eggs hatch readily in soil leachates; the second require stimulation from host root diffusates to hatch; and the third type a large percentage, do not hatch immediately. They stated that the proportions of these types of eggs differ from one generation to another.

Egg hatch of SCN race 3 was optimal at 25% soil moisture and pH 6. pH over 6.0 caused a decrease in hatching rate, and pH 8 caused a 100% reduction in hatching (Teft et al., 1982). In another test, pH of hatching solution around 5.3 to 6.1 elevated hatching of SCN eggs (Teft and Bone, 1984). In hatching tests with SCN eggs, Lehman et al. (1971) observed the greatest emergence of J2 juveniles from encysted eggs without egg masses at pH 3.5. Emergence was depressed at pH 5.5. In contrast, they did not see any pH effect on hatching of J2 juveniles from eggmass eggs.

The percent hatch of J2 juveniles of *H. rostochiensis* from free eggs was not different than that of encysted eggs (Den Ouden, 1963). Hatch of egg-mass eggs located at the posterior end of the female SCN was greater than hatch of encysted eggs starting from day 2 to day 18 of an *in vitro* test (Thomson and Tylka, 1997). A greater number of J2 juveniles of *H. cajani* emerged from egg sacs than from white or brown cysts (Singh and Sharma, 1996). A greater number of J2 juveniles emerged from eggs from
light brown cysts than from shrunken dark brown cysts or white or from yellow females of *H. glycines* (Slack and Hamblen, 1961).

J2 juveniles of SCN in cysts survived up to 11 months at 8.5% relative humidity and higher, when the cysts in soil were desiccated before the test (Endo, 1962).

Hatching of *H. cajani* eggs form cysts was delayed as storage time increased in 0 and 60% relative humidity compared to 80% and 100% relative humidities (Gaur et al., 1996).

Pre-incubation of cysts for a minimum period of 8 weeks at 0°C to 7°C is required to induce emergence of juveniles of *H. avenae* Woll. from eggs in the cysts (Fushtey and Johnson, 1965). Greatest hatching rate of SCN eggs were obtained when the eggs were pre-soaked in water at 25°C or 30°C for 1 to 4 weeks before they were soaked in the kidney bean (*Phaseolus vulgaris*) root diffusate at 25°C (Okada, 1971). The greatest number of SCN, race 1 eggs hatched at 26 day and 22°C night temperatures. Intermediate and fewest egg hatches were obtained at 22 and 18°C, and 18 and 14°C, respectively (Hill and Schmitt, 1989). Bird (1974) reported that brief heat treatment (46°C for 10 min) of eggs of *M. javanica* suppressed embryogenesis and hatching. Incubation of cysts at 40°C for 10 days impaired the emergence of J2 juveniles from eggs after transfer of eggs to 24°C (Slack and Hamblen, 1961).

Hatch of a southern population of *H. avenae* at Villasavary, France, in a Mediterranean type climate occurs during winter, while maximum hatching takes place in spring in northern populations at Nuisement-sur-cole in a continental climate (Rivool, 1986). Hatching of *G. rostochiensis* eggs was slow and prolonged in tests in autumn and early winter, and it was rapid in spring and summer (Hominick et al., 1985). Soil
samples collected in spring resulted in larger numbers of eggs and second stage juveniles of SCN than those samples collected in autumn (Warner et al., 1997). Female nematode and eggs may perceive signals from plant during growing season that leads to the diapause (Hominick et al., 1985). Egg hatch and infectivity of J2 juveniles of SCN eggs from cysts collected from field microplots in Missouri was highest in July-August, decreased to a low level by October and remained low until May or June of following year (Yen et al., 1996).

Hatch of SCN eggs was greater in soybean root exudates collected after postemergence application of acifluorfen, bentazon and lactofen to foliage of soybean plants than in deionized water, although the largest number of egg hatch was recorded in 3 mM ZnSO₄ solution (Levene et al., 1998a,b). Alachlor, atrazine, ethalfluralin, and trifluralin except acifluorfen (50 and 500 µ/mL solution) had no effect on hatch of *H. glycines* eggs compared to distilled water, although all the herbicides tested at 25 °C yielded significantly less hatch than zinc sulfate. However, acifluorfen inhibited egg hatch compared to distilled water (Wong and Tylka, 1992). Atrazine, bentazon, cyanazine, clomazone, alacor, ethalfluralin and trifluralin had no significant effect on egg hatching of SCN (Wong et al., 1993). Blazer (acifluorfen) at 50 and 500 µg/mL concentration hatched significantly fewer eggs of SCN than deionized water and zinc sulfate (3.14 mM) (Wong et al., 1993). SCN eggs could be induced to hatch by stimulatory herbicides in the absence of soybeans and conversely, it could be suppressed by inhibitory herbicides when soybeans were grown (Wong et al., 1993).

Egg hatch from cysts of *G. rostochiensis* was significantly reduced by chloridazon and tri-allate in potato root diffusate *in vitro* albeit the hatch from eggs in
cysts of G. rostochiensis and G. pallida were not affected by the same herbicides in 10 cm diameter pots in which either potato cv. Désirée or sugarbeet, cv. Monoire were grown (Beane and Perry, 1990). Tri-allate enhanced host invasion by Heterodera schachtii although it decreased host invasion by H. rostochiensis (Beane and Perry, 1990).

The insecticides / nematicides, aldicarb 10G, oxamyl 10G and carbofuran 5G equally prevented egg hatch of H. sacchari in cysts (Garabedian and Hague, 1984). Hatching of H. schachtii encysted eggs was inhibited by aqueous solution of 5-500 μg / mL aldicarb. Inhibition of hatching was not decreased by adding hatching agents, zinc chloride or sugarbeet root diffusate, to the aldicarb solution, although transfer of cysts to sugarbeet root diffusate and incubation of them there for 4 weeks led to resumption of juvenile emergence (Steele and Hodges, 1975).

Emergence of J2 juveniles of SCN increased in phenamiphos (0.5 μg/mL) + alachlor (0.063, 0.125, or 1 μg/mL) solution compared to untreated controls at 15 days, although it was suppressed by a higher concentration of phenamiphos (1 μg/mL) alone and in combination with alachlor (1 μg/mL) for 21 days (Bostian et al., 1984).

LITERATURE CITED


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CHAPTER 1

DISTRIBUTION OF SOYBEAN CYST NEMATODE, *Heterodera glycines*

Ichinohe, IN OHIO, 1998 AND POPULATIONS ASSOCIATED WITH DIFFERENT ROTATION SYSTEMS
INTRODUCTION

From 1989 to 1991, approximately 80% of total soybean [Glycine max (L.) Merr.] production in the United States was harvested in the North Central region. Six of the most productive states, and 10 of the top 12, are located in this region (Doupnik, 1993). In 1999, soybeans were grown on about 4.5 million acres in Ohio (Anonymous, 1999).

The Soybean Cyst Nematode (SCN) is probably the most serious and the most economically important disease-causing organism of soybeans in the north central United States (Doupnik, 1993). Average disease loss was estimated at 12.78, 16.50 and 10.10% in 1989, 1990 and 1991, respectively (Doupnik, 1993). Overall yield loss due to the SCN has been estimated to be about 15% although damage may vary from slight to near-total losses depending on population density of the nematode, soybean variety and other factors (Venkatesh et al., 1999). SCN causes severe soybean yield losses in western, central and northwestern parts of Ohio (Willson et al. 1996).

SCN was first discovered in Ohio near Tipp City (Miami Co.) in 1980 in soil accompanying the roots of tomato transplants shipped from Tennessee (Hammond et al. 1981). It was found on soybean cv. Williams 82 growing in sandy loam soil in Sandusky Co. in north-central Ohio and on soybean in Scioto Co. in south-central Ohio in August 1987 (Riedel and Golden, 1988). Of 33 counties sampled by the Ohio Cooperative
Agriculture Pest Survey in 1991 (Willson et al. 1996) Darke, Erie, Fulton, Huron, Preble, Seneca, Union and Wood counties were found to be infested. In a survey between 1992 and 1995, 93 fields (of 667 soybean fields surveyed in 63 counties) from 43 soybean-producing counties in Ohio were found to be infested with SCN (Willson et al., 1996). SCN was found in approximately 1434 samples of 3764 soil samples sent to the C. Wayne Ellett Plant and Pest Diagnostic Clinic. Positive samples represent approximately 40% of the total samples, which came from 60 counties in Ohio in 1998 (Alptekin et al., 1999).

As a part of a general education program, a soil sampling program was funded by the Ohio Soybean Council using soybean checkoff money to determine distribution of SCN in Ohio in 1998. This report gives the results of this survey.

**MATERIAL AND METHODS**

1. **Survey.** Farmers were asked to fill out the Ohio Soybean Cyst Nematode Sampling Program Form (see Appendix 1), which includes information such as name, address, phone, fax, and e-mail number of farmer and/or company, previous crop history of field, county, field name and location. Forms and soil samples were sent to the C. Wayne Ellett Plant and Pest Diagnostic Clinic. Samples were stored at 10 C until they were processed.

   Farmers were asked to use a soil probe (1” diameter X 6” length) to collect 10-20 soil cores per 10-20 A. Soil cores are combined to make one 500 mL composite sample. A 200 mL subsample was added to 5 L of water in a 10-L bucket and stirred vigorously
by hand to suspend cysts in the mixture. The water was poured over a nested 850 \(\mu\text{m}-\) pore (20 mesh) over 250 \(\mu\text{m}-\)pore (60 mesh) sieves 10 sec after stirring. Cysts collected on a 250 \(\mu\text{m}-\)pore-sieve were washed into a 250 mL glass beaker.

Cysts were crushed in a 40 mL Ten Broeck tissue grinder. The debris was poured onto nested 250 \(\mu\text{m}-\)pore sieve over a 75 \(\mu\text{m}-\)pore-sieve over 25 \(\mu\text{m}-\)pore sieve. The top sieve was removed and uncrushed cysts were placed in the tissue grinder a second time. The 25 \(\mu\text{m}-\)pore-sieve collected the eggs and J2 juveniles of SCN, which were later, washed back into a 250 mL glass beaker with 50 mL water. One mL acid fuchsin dye was added to 50 mL egg suspension in a 250 mL glass beaker and heated to boiling in a microwave oven. The volume of the suspension was brought to 200 mL by adding 150 mL tap water. Suspension was stirred to increase the accuracy (Krusberg et al., 1994) of egg counts with a magnetic stirrer, and 5 mL of suspension was removed with an automatic pipetter following a spiral pattern starting one cm above from the bottom of beaker and ending one cm below the surface. Eggs and J2 juveniles of SCN stained with acid fuchsin were counted with a dissecting microscope at 40X.

Longitudes and latitudes for each SCN positive field address were identified using Street Map Plus ® Soft Ware. Map Pro ® was used for each SCN positive field to place a filled triangle onto the state map at that street address.

2. Effect of Crop Rotation on SCN Reproduction. Only samples that were SCN positive and had four years continuous crop history on the form submitted by farmers, were analyzed. Samples were categorized to 16 crop rotations representing various combinations of soybean and nonhost crops for years 1995, 1996, 1997 and 1998.
Nonhost-nonhost-nonhost-nonhost rotation system was not included in ANOVA due to the very small sample size in this category.

Egg counts along with different soybean and nonhost rotation systems for four years were entered into MINITAB ® worksheet. Using box and dot plot of data, extreme variables having 25,000 or more eggs and J2 juveniles per 200 mL soil were deleted. ANOVA was performed on the data, and means were separated by Fisher’s pairwise comparisons using MINITAB software.

RESULTS AND DISCUSSION

1. Survey. A total of 4,569 soil samples were processed from 18 Aug. 98 to 5 Aug. 99. SCN was found in approximately 1,946 of 4,569 soil samples sent to the C. Wayne Ellett Plant and Pest Diagnostic Clinic. Positive samples represented approximately 42.6% of the total samples, which came from 64 counties in Ohio (Figure 1 and 2). Based on county acreage, the 64 counties sampled represent approximately 99% of Ohio’s total soybean acreage.

Of the positive samples, 29.6 % were below levels which generally cause significant damage to SCN-susceptible soybeans (<2000 eggs/200 mL of soil) (Figure 3 and 4); 6.1 % were at levels recommended for planting SCN-resistant soybeans (2001-5000 eggs/200 mL soil) (Figure 5); 6.9 % had extremely high population level (>5000 eggs/200 mL soil) recommended for planting a nonhost crop (Figure 6). Egg counts in positive samples from those counties ranged from 40 to 108,800 / 200 mL soil in this survey.
Over fifty percent of soil samples from Brown (53%), Clermont (88.2), Darke (68.1), Fayette (58.3), Fulton (59.3), Hamilton (100), Lucas (73), Marion (58), Medina (60), Miami (63.4), Monroe (75), Paulding (55.6), Richland (100), Sandusky (64.1), Wayne (53.8) and Wood (51.2) counties were infested with SCN. As a result of this survey, SCN infestations were found in 7 counties previously not known to have SCN, Franklin, Holmes, Lorain, Marion, Van Wert, Wayne and Williams (Figure 1). At the completion of this survey, 58 counties in Ohio were SCN positive. Sixty-four (of 88) Ohio counties are known to have SCN infestations including almost all major soybean-producing counties in 1999 (Figure 1).

SCN was not evenly distributed in the Ohio counties surveyed. In general, western and central Ohio (but not eastern Ohio, where soybean production is less intensive), were found to be infested heavily with SCN. In the northwest quadrant heavily infested fields (over 5,000 eggs per 200 mL soil) were found throughout Sandusky, western Seneca, western Ottawa, central Wood, eastern Fulton, and western Lucas counties. These high populations could indicate that SCN has been established for a long time in these counties and/or relatively sandy soil type and continuous soybean planting helped the nematode to build up the very high population level since the first discovery of the nematode in the state. Both could be true for Sandusky Co. where the nematode was first discovered in 1987. For the other counties a combination of the factors above might play a role in development of very high population level of SCN, but we do not have enough information about the first introduction of SCN to those counties. SCN could have been present for a long time, but continuous soybean planting might have helped to build up high population in those soils. It is possible that samples from
these counties were too few and did not represent of the whole county. Furthermore, soil samples were not randomly taken from fields in Ohio counties, but were submitted by growers interested in having soil tested or by growers having field consultants. These factors could have an effect on the distribution of SCN in those counties. It is also possible that SCN cysts could be concentrated on low lying parts of a field after heavy rain during flooding.

These results demonstrate that SCN is widespread throughout the major soybean growing areas of Ohio. Very high egg and second stage juvenile numbers per unit volume of soil indicate that SCN is well established in soybean growing areas of Ohio.

2. Effect of Different Rotation Systems on SCN Reproduction. Four year continuous soybean (SSSS) resulted in significantly \( P < 0.05 \) higher number of eggs than all other crop rotation types (Figure 7). Soybean, soybean, nonhost, soybean (SSNS), NSSS, SNSS and NNSS rotation types were the second highest in egg count per 200 mL soil. The other types of soybean nonhost crop rotation did not significantly \( P > 0.05 \) increase the egg population although average number of eggs for each rotation type was slightly different.

In a rotation study, population density of SCN eggs and eggs plus J2 juveniles decreased to almost nondetectible levels after two years of nonhost culture, and significantly \( P < 0.05 \) higher yield was obtained in the rotation system in which soybean followed one or more years of nonhosts than in monoculture (Koening et al., 1993). The primary benefit of corn in the rotation system has been emphasized in another study where corn and soybean yields were increased 14 and 11%, respectively (Howard et al., 32
Soybean yields were also significantly greater in soybean after one year corn rotation than soybean after soybean (Koening et al., 1995). Rotation of resistant and susceptible soybean cultivars with a nonhost crop could increase mean soybean yields and slow the shift toward greater parasitism of the resistant cultivar which leads to appearance of new populations of the nematode. Twelve year-mean yields of soybean rotation systems of Bedford soybean, corn, and Essex soybean were significantly greater than continuous Bedford soybean or Forrest soybean (Young, 1998). Hershman and Bachi (1995) recommended the use of SCN resistant cultivars alone or in a 75:25 % mixture with a susceptible cultivar (25%) as an SCN population management tactic to delay population shifts and prolong effectiveness of the resistant cultivar.
Figure 1.1 Ohio counties tested for *Heterodera glycines* (SCN) from soil samples submitted in response to a survey supported by Ohio Soybean Council in 1998.
Figure 1.2 Distribution of soybean cyst nematode positive fields in Ohio, 1998. = One positive field.
Figure 1.3 Distribution of soybean cyst nematode positive fields in Ohio having 40-200 eggs/200 mL of soil, 1998. = One positive field.
Figure 1.4 Distribution of soybean cyst nematode positive fields in Ohio having 201-2,000 eggs/200 mL of soil, 1998. = One positive field.
Figure 1.5 Distribution of soybean cyst nematode positive fields in Ohio having 2,001-5,000 eggs/200 mL of soil, 1998. = One positive field.
Figure 1.6 Distribution of soybean cyst nematode positive fields in Ohio having over 5,000 eggs/200 mL of soil, 1998. = One positive field.
Figure 1.7 Average number of soybean cyst nematode (SCN), *Heterodera glycines* eggs/200 mL soil sample collected in Ohio from all possible 4 years (1995, 1996, 1997 and 1998) soybean vs. nonhost crop rotation (S = Soybean, N = Nonhost). Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher's least significant difference test (P ≤ 0.05).
Table 1. Distribution of *Heterodera glycines* in Ohio by Counties, 1998-99.

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<th>Low (200-1999)</th>
<th>Moderate (2000-4999)</th>
<th>High (5000-Over)</th>
<th>Low (%)</th>
<th>Moderate (%)</th>
<th>High (%)</th>
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LITERATURE CITED


CHAPTER 2

CONTROL OF SOYBEAN CYST NEMATODE (SCN), Heterodera glycines, Ichinohe IN GREENHOUSE AND FIELD TESTS WITH N-VIRO® SOIL
INTRODUCTION

A wide range of interactions takes place between pathogens and their hosts. In these interactions, the form of nitrogen (N) available to the host or pathogen rather than the amount of N determines disease severity or resistance (Huber and Watson, 1974). Amendment (20% w:w) of citrus soil with composted municipal waste (CMW) reduced the incidence of infection of 5-week-old susceptible citrus seedlings by Phytophthora nicotianae from 95% to as low as 5% (Widmer et al., 1998). In another study, biological oxidation of ammonium to nitrate N was suggested for the reduction of the severity of bean root rot (Huber and Watson, 1970). Ammonia (NH₃) and nitrous acid (HNO₂) released from the organic amendments, meat and bone meal (MBM; 8% N), a by-product of animal rendering, killed microsclerotia of the soilborne pathogen, Verticillium dahliae (Tenuta and Lazarovits, 1999). Number of microsclerotia of V. dahliae were reduced by both fresh (8.7%) and dry (1% w:w) broccoli amendments in soil at 10, 15, 20, 25, 30 and 35 C compared to that in unamended soil (Subbarao and Hubbard, 1996). The soil amendments with blood meal, fishmeal, and ammonium sulfate that are high in nitrogen resulted in significant reduction in the inoculum potential of Verticillium albo-atrum (Wilhelm, 1951). Nitrogenous compounds, such as anhydrous ammonia, ammonium sulfate, ammonium nitrate, ammonium bicarbonate, and calcium nitrate were
among the most often pronounced amendments to reduce disease severity. Ammonium salts and carbonate and bicarbonate salts had fungicidal effect on sclerotia of *Sclerotium rolfsii* due to the dominance of free NH$_3$, and CO$_3$ and –HCO$_3$, respectively at high pH (8.6) (Punja and Grogan, 1982). Reduction in sporangial germination of *Phytophthora cinnamomi*, *P. parasitica* and reduction in chlamydospore germination of *P. parasitica* were correlated with the prevalence of NH$_3$ and HNO$_2$ in the ammonium/ammonia and nitrite/nitrous acid solutions (Tsao and Oster, 1981).

Anhydrous ammonia significantly decreased species of *Hoplolaimus*, *Criconemoide*, *Trichodorus*, and *Belonolaimus* (Eno et al., 1955). In a greenhouse experiment, anhydrous ammonia at 62 mg N/kg soil or higher reduced populations of *Tylenchorhynchus claytoni* and *Helicotylenchus dihystera* in soil, although only 125 mg N/kg soil rate significantly reduced root populations of *H. dihystera* or *Hoplolaimus galeatus* (Rodriguez-Kabana et al., 1981). However, Rodriguez-Kabana et al. (1982) reported that application of anhydrous ammonia of 0 and 224 kg N/ha rates at planting did not reduce late-season juvenile populations of *Meloidogyne arenaria* (Neal) Chitwood in three field experiments albeit ammonia at 224 kg N/ha rate significantly increased soybean yield in one experiment. In another field experiment, J2 juvenile populations of SCN decreased following the ammonia application at 56 and 112 kg N/ha 14 days after planting (Rodriguez-Kabana, 1981). Planting time applications of ammonia (56 or 112 kg N/ha) in combination with ethylene dibromide (4.7-18.6 L/ha) resulted in greater soybean yield and better control of *M. arenaria* and *Heterodera glycines* (SCN) than their individual application (Rodriguez-Kabana et al., 1981 and 1982).
The chemical composition of N-Viro soil (NVS), a municipal biosolids, has been recently identified (Yamakawa, 1999). The potential of NVS to suppress diseases caused by plant parasitic nematodes has yet to be explored.

The objectives of this study were 1) to evaluate the effects of NVS on SCN reproduction and on growth of soybean ‘Corsoy 79’ grown in sand amended with NVS and grown in sand that was surface applied with NVS, in response to the different application rates of NVS in the greenhouse and 2) to evaluate the effects of NVS on SCN egg and J2 juvenile populations from resistant (to SCN race 3) and susceptible soybean receiving 0, 5 and 25 T NVS/A in field.

MATERIALS AND METHODS

Source of SCN Inoculum for Greenhouse Tests. Four seeds of the SCN-susceptible soybean ‘Corsoy 79’ were sown 1 cm deep in each of 6, 15-cm diameter clay pots containing sterile construction sand. Ten days after planting, each pot was inoculated with 100,000 eggs and second stage juveniles of SCN, race 3. The pots were kept at 25-30 C in a greenhouse. Plants were fertilized with 400 ppm 15:15:15 (N:P:K) and watered from the shoot daily. Six weeks after SCN infestation, the shoots of the plants were cut one cm above soil, and the roots were pulled out. The cysts on the roots were returned to the soil. Soil was mixed thoroughly and additional sand was added if necessary. New ‘Corsoy 79’ seeds were sown. The procedure above was repeated to obtain a heavy SCN population in the pots.
**Preparation of Inoculum for Greenhouse Tests.** A 200 mL aliquot of infested soil was dumped on to a sheet of newspaper and large clumps of soil were broken by hand. Soil was dumped into 5 L of water in a 10-L bucket. The water was stirred vigorously by hand. Then soil particles were allowed to settle to the bottom of the bucket for 10 sec. Supernatant was poured through a 850 μm pore (20 mesh) sieve nested over 250 μm-pore (60 mesh) sieve. The 850 μm-pore sieve was removed, and the cysts collected on the 250μm-pore sieve and washed into a 250 mL glass beaker with 30 mL of tap water. At this step, the cysts in the tubes were stored in the refrigerator at 10 C until used.

To release eggs, cysts were crushed in a 40 mL Ten-Broeck cyst homogenizer. The suspension was poured on to nested 250 μm-pore (60 mesh) over 75 μm-pore (200 mesh) over 25 μm-pore (500 mesh) sieves, and the pestle was rinsed on the sieve. Material on the top sieve (250μm-pore) was rinsed back into the tube and was crushed again. The 25 μm-pore (500 mesh) sieve collected the nematode eggs and J2 juveniles. SCN eggs and J2 juveniles on the 25-μm-pore (500-mesh) sieve were washed back into the beaker. The volume was brought to 200 mL with tap water and stirred with a magnetic stirrer. A 5 mL aliquot of stirred suspension was poured into another 250 mL beaker, one mL of acid fuchsin dye was added, and the contents of beaker were heated in microwave oven to boiling. The volume was brought to 200 mL with cold tap water. The suspension was stirred for about 5 min. Five mL aliquots were poured into a petri dish with grid and eggs were counted to determine population level. Eggs numbers in the
stock solution were standardized to 2,000 eggs and J2 juveniles per two mL in 200 mL water in 250 mL glass beaker.

1. Effect of Surface Applied N-Viro Soil on SCN Reproduction and Host Growth

Experimental Design. The bottom of cone-tainers were lined with one cm of gravel and then 150 mL construction sand was added on shoot of gravel. One seed of soybean variety ‘Corsoy 79’ was planted in each cone-tainer® C-10 (Stuewe & Sons, Inc., 2290 SE Kiger Island Dr., Corvalis, OR) and watered from the top to promote emergence. Seven-day-old plants were infested with 2,000 SCN eggs and second stage juveniles in 2 mL water by pipetting the inoculum around the stem of soybean plants. Treatments of 0, 1.27, and 6.35 g NVS were surface-applied to the designated cone-tainers. There were 4 replicates for each treatment. The treatments were randomized within three blocks.

The cone-tainers were placed in racks in an Ebb and Flow pan. The plants were watered from the bottom by filling the pan with water at 25+/-1C. Five week after the treatment, the shoots of the plants were cut one cm above soil level. Roots were washed with tap water. Cysts on the roots as well as in the soil were counted. Cysts in soil and on the roots were crushed. Eggs from cysts were extracted and counted under a stereomicroscope at 40X.

After shoot and root fresh weights and the number of nodules on each root system were recorded, shoots and roots were placed in a paper bag individually, and labeled according to the treatment and replicate number. The paper bags were placed in an oven at 58 C for 48 hr. Bags were taken from oven and shoot and root dry weights
were recorded for each replicate. The experiment was repeated two times. A one-way ANOVA was performed on data obtained from the experiments.

2. Effect of Two Rates of N-Viro Soil Mixed with Construction Sand on SCN Reproduction and Host Growth. Four replicates of 0, 1.27 g and 6.35g N-Viro soils were mixed with 150 mL construction sand. One cm of gravel was placed in the bottom of cone-tainers. The rest of the space in cone-tainers C-10 was filled with N-Viro soil and construction sand mix. Two seeds of soybean Corsoy 79 were sown in each cone-tainer. Seven days after planting, plants were thinned to one per cone-tainer. Each cone-tainer was inoculated with 2000 SCN eggs and J2 juveniles. Plants were watered daily from the bottom with water 25+/-1 C and fertilized weekly with 400 ppm of 15-15-15 (N:P:K). Height of each plant was measured at inoculation and weekly until plant shoots were harvested. Cysts on the roots and in the soil were counted. Cysts from both soil and roots were combined and crushed as outlined above. Eggs and second stage juveniles were counted under stereomicroscope.

Fresh weight of shoot and root were recorded. Shoots and roots were dried at 58 C to a constant weight, prior to dry weight determination. The experiments were repeated two times. A one-way ANOVA was performed on data obtained from the experiments.

3. Effect of two Rates of N-Viro Soil on Reproduction of SCN on Resistant and Susceptible Soybeans in the Field. Experimental plots were established in a commercial field at the NE corner of Bays and Cloverdale Road (OR-Win Farms, 7678
Huffinan Rd) in Wood Co., OH. The soil type was a combination of Wauseon fine sandy loam and Ottooee and Spinks loamy fine sand.

N-Viro soil (NVS) produced at Toledo, OH on 23 Dec 98 was shipped to the field site and spread on the plots on 2 Feb 99. Treatments were 0, 5, and 25 T NVS/A replicated four times. NVS was applied with a Knight side-slinger manure spreader. Soil was not tilled after application of NVS. Each treatment was applied to 40x500 ft. plots. The NVS was applied perpendicular to the direction of planting. Center of the first replication of control was located 30-ft from end rows. Soybean cv. 92B91, resistance to race 3 derived from PI 88788, and soybean cv 2730, susceptible to race 3 were planted in alternating strips across the plots. Plots of each cultivar were 20 ft wide.

**Sampling.** Pre-plant soil samples were taken on 15 Apr 99. Cores, 1 in. X 6 in. were taken every 6 ft using a zigzag pattern. Soil cores were mixed thoroughly and retained as a single sample for each treatment replicate. Soil samples from plots where resistant and susceptible soybeans were to be grown were mixed in the pre-plant samples and analyzed together since resistant and susceptible soybeans were not planted yet. Midseason soil samples were taken on 24 Jun 99. Soil cores from resistant and susceptible cultivars were separated in mid-season and post-harvest samples. Soil cores were taken for the 450 ft length of the treatments. The soil cores were then mixed thoroughly and retained as two separate samples for each treatment replicates. Plots were harvested on 24 Sep 99. Post-harvest soil samples were taken on 28 Sep 99.

SCN eggs were extracted from 200 mL aliquots of mixed field samples according to the previously described method.
RESULTS

1. Effect of Surface Applied N-Viro Soil on SCN Reproduction and Host Growth. In the first experiment, significantly ($P \leq 0.05$) lower cyst population of SCN on the roots of soybean 'Corsoy 79' was found in the with N-Viro soil treatments at 6.35 g/150 mL sand rate compared to that receiving no NVS treatment. Cyst numbers on the root system of 'Corsoy 79', however, were not significantly ($P > 0.05$) different between 1.27 g NVS / 150 mL sand treatment and untreated control (Table 2.1, Exp. 1). Average cyst numbers in the first experiment were 243.0, 123.3 and 65.3 on the root system of soybean 'Corsoy 79' and in sand receiving surface application of 0, 1.27 and 6.35 g NVS/150 mL sand, respectively.

In the second experiment, SCN produced an average of 466.0, 226.0 and 25.0 cysts on the roots of soybean 'Corsoy 79' and in sand treated with 0, 1.27 g and 6.35 g NVS/150 mL sand, respectively (Table 2.1, Exp. 2). NVS at 6.35 g caused the greatest ($P \leq 0.05$) decline in the number of cysts on the roots compared to untreated check, although NVS at 1.27 g rate resulted in significantly greater decline in cyst numbers compared to no NVS treatment (Table 2.1, Exp. 2).

When the data from experiment 1 and 2 were pooled and analyzed together, significantly ($P \leq 0.05$) fewer (44.9 cysts/root system) cysts numbers were obtained on
the roots of soybean ‘Corsoy 79’ grown in sand surface applied with 6.35 g NVS compared to the untreated check (339 cysts/root system) and 1.27 g NVS/150 mL sand. Cysts were significantly \( (P < 0.05) \) fewer on soybean roots grown in 1.27 g NVS and sand mix (181.7 cysts/plant) than those from control (Table 2.1).

Significantly \( (P < 0.05) \) fewer eggs (5,002 eggs/plant) were associated with soybean plants receiving 6.35 g NVS /150 mL sand surface applied compared to control plants (57,750 eggs/plant) which received no NVS (Table 2.2, Exp. 1)). However, no significant \( (P < 0.05) \) difference in egg number was observed between 1.27 g NVS treatment (30,100 eggs/plant) and untreated check (5,002 eggs) (Table 2.2, Exp. 1)).

In the second experiment, number of eggs obtained by crushing the cysts formed on the roots of soybean ‘Corsoy 79’ and in sand treated with 0, 1.27 and 6.35 g NVS/ 150 mL sand were 82,220, 19,213, and 3,380, respectively. The greatest suppression of egg production was correlated \( (P < 0.05) \) with 6.35 g NVS/150 mL sand application compared to the untreated control (Table 2.2, Exp. 2). A significant \( (P < 0.05) \) suppression of egg population was observed when sand was surface applied with 1.27 g NVS (Table 2.2, Exp. 2).

Pooled data from the experiments indicates that the applications of 6.35 g NVS caused significantly \( (P < 0.05) \) greater depression of egg numbers than the application of 1.27 g NVS. Egg numbers on the roots of control soybeans were significantly greater than those eggs produced in cysts on the roots of soybeans from the two treatments. Egg numbers were averaged as 68,237, 24,657, and 4,191 for 0, 1.27 and 6.35 g NVS/150 mL sand applications (Table 2.2).
## Average Cyst Number

<table>
<thead>
<tr>
<th>NVS (g/150 mL sand)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Pooled Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>243.0 a b</td>
<td>466.0 a</td>
<td>338.6 a</td>
</tr>
<tr>
<td>1.27</td>
<td>123.3 a</td>
<td>225.5 b</td>
<td>181.7 b</td>
</tr>
<tr>
<td>6.35</td>
<td>65.3 b</td>
<td>24.5 c</td>
<td>44.9 c</td>
</tr>
</tbody>
</table>

**LSD$_{(0.05)}$**

138.8 87.8 104.3

---

*a* Average Cyst Number was obtained by counting the cysts collected from each replicate and averaging the total cyst number by the number of replicates for each treatment.

*b* Within columns, means followed by the same letter are not significant according to LSD$_{(0.05)}$.

*c* Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher’s least significant difference test ($P \leq 0.05$).

**Table 2.1** Number of soybean cyst nematode (SCN), *Heterodera glycines*, cysts on the roots of soybean ‘Corsoy 79’ five wk after surface application of 0, 1.27 g and 6.35 g N-Viro Soil (NVS)/150 mL sand in soil infested with 2,000 eggs and J2 juveniles of SCN race 3 per cone-tainers in greenhouse.
### Average Egg Number

<table>
<thead>
<tr>
<th>NVS (g/150 mL sand)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Pooled Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>57,750 a</td>
<td>82,220 a</td>
<td>68,237 a</td>
</tr>
<tr>
<td>1.27</td>
<td>30,100 ab</td>
<td>19,213 b</td>
<td>24,657 b</td>
</tr>
<tr>
<td>6.35</td>
<td>5,002 b</td>
<td>3,380 c</td>
<td>4,191 c</td>
</tr>
</tbody>
</table>

$LSD_{(0.05)}^e = 40,805$  
14,678  
19,124

*a Average egg number was obtained by crushing the cysts collected from each replicate and averaging the total egg number by the number of replicates for each treatment.

*b Within columns, means followed by the same letter are not significant according to LSD_{(0.05)}.

*c Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher's least significant difference test ($P \leq 0.05$).

Table 2.2 Average number of eggs extracted from cysts of soybean cyst nematode (SCN), developed on the roots of soybean 'Corsoy 79' five wk after surface application of 0, 1.27 g and 6.35 g N-Viro Soil (NVS) /150 mL sand infested with 2000 eggs and J2 juveniles of SCN race 3 per cone-tainers in greenhouse.
Height of soybean ‘Corsoy 79’ grown in surface applied NVS at 6.35 g / 150 mL sand rate was the greatest \( (P \leq 0.05) \) in experiment 1 (Table 2.3). Height of soybean plant receiving 1.27 g NVS/150 ml sand was significantly \( (P \leq 0.05) \) greater than that of soybean plants receiving no NVS application in experiment 1 (Table 2.3). NVS at 0, 1.27 and 6.35 rate surface application resulted in 15.2, 16.0 and 17.1 cm average height per plant.

In the second experiment, soybean treated with 6.35 g NVS/150 mL sand was significantly \( (P \leq 0.05) \) taller than soybean treated with either 0 or 1.27 g NVS / 150 mL sand (Table 2.3, Exp. 2). Average height soybean plants grown in sand surface applied with 0, 1.27 and 6.35 g NVS was 13.0, 13.3 and 15.5 cm/plant.

Pooled data showed that surface application of 6.35 g NVS caused significant \( (P < 0.05) \) increase in heights of soybeans compared to control, although 1.27 g NVS had not have any affect on height. Average heights of soybeans were 14.1, 14.5 and 16.3 cm when they received 0, 1.27 and 6.35 g NVS/ 150 mL sand, respectively (Table 2.3).

The greatest fresh shoot weight, 2.2 g, was obtained when soybeans were treated with 6.35 g / 150 mL sand. Application of 1.27 g NVS/150 mL sand resulted in the second highest fresh shoot weight, 1.9 g, compared to control, 1.6 g (Table 2.4, Exp. 1) \( (P \leq 0.05) \).

In the second experiment, application of both 1.27 g and 6.35 g NVS/150 ml sand caused a higher production of fresh shoot weight of soybean compared to untreated control albeit the difference among the two treatments was not significant \( (P > 0.05) \) (Table 2.4, Exp.2). Average fresh shoot weight of plants receiving 0, 1.27 and 6.35 g NVS surface application was 1.4, 1.9 and 2.2 cm, respectively.
<table>
<thead>
<tr>
<th>NVS (g/150 mL sand)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Pooled Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15.2 a&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.0 a</td>
<td>14.1 a</td>
</tr>
<tr>
<td>1.27</td>
<td>16.0 b</td>
<td>13.3 a</td>
<td>14.5 a</td>
</tr>
<tr>
<td>6.35</td>
<td>17.1 c</td>
<td>15.5 b</td>
<td>16.3 b</td>
</tr>
</tbody>
</table>

LSD<sub>(0.05)</sub><sup>c</sup>  
0.7  
1.4  
1.5

<sup>a</sup> Average height was obtained by measuring the height of each plant in a treatment and averaging the total height by the number of replicates.

<sup>b</sup> Within columns, means followed by the same letter are not significant according to LSD<sub>(0.05)</sub>.

<sup>c</sup> Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher’s least significant difference test (<i>P</i>≤0.05).

Table 2.3 Height of soybean ‘Corsoy 79’ five wk after surface application of 0, 1.27 g and 6.35 g N-Viro Soil (NVS)/150 mL sand infested with 2,000 eggs and J2 juveniles of soybean cyst nematode (SCN), race 3 per cone-tainers in greenhouse.
Average Fresh Shoot Weight (g)\textsuperscript{a}

<table>
<thead>
<tr>
<th>NVS (g/150 mL sand)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Pooled Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.6 \textsuperscript{a,b}</td>
<td>1.4 \textsuperscript{a}</td>
<td>1.5 \textsuperscript{a}</td>
</tr>
<tr>
<td>1.27</td>
<td>1.9 \textsuperscript{b}</td>
<td>1.9 \textsuperscript{b}</td>
<td>1.9 \textsuperscript{b}</td>
</tr>
<tr>
<td>6.35</td>
<td>2.2 \textsuperscript{c}</td>
<td>2.2 \textsuperscript{b}</td>
<td>2.2 \textsuperscript{c}</td>
</tr>
</tbody>
</table>

LSD (0.05)\textsuperscript{c} 0.2 0.3 0.2

\textsuperscript{a} Average fresh shoot weight was obtained by weighing each plant shoot from each replicate and averaging the total fresh shoot weight by the number of replicates.

\textsuperscript{b} Within columns, means followed by the same letter are not significant according to LSD\textsuperscript{(0.05)}.

\textsuperscript{c} Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher’s least significant difference test (\(P \leq 0.05\)).

Table 2.4 Fresh shoot weight of soybean 'Corsoy 79' five wk after surface application of 0, 1.27 g and 6.35 g N-Viro Soil (NVS)/150 mL sand infested with 2,000 eggs and J2 juveniles of soybean cyst nematode (SCN) race 3 per cone-tainers in greenhouse.
Application of either 1.27 or 6.35 g NVS/150 mL sand resulted in significantly ($P \leq 0.05$) greater fresh root weight of soybean than untreated control although there was no significant ($P > 0.05$) difference between the two rates of NVS (Table 2.5, Exp. 1). Average fresh root weight of 1.7, 2.1, and 2.4 g was obtained from plants grown in sand receiving 0, 1.27, and 6.35 g NVS surface applied.

In the second experiment, fresh root weight of soybean receiving 6.35 g NVS/150 mL sand were significantly ($P < 0.05$) greater than that of soybean receiving 0, and 1.27 gNVS/150 mL sand (Table 2.5, Exp. 2). Surface application of 0, 1.27 and 6.35 g NVS caused production of 1.5, 1.7 and 2.2 g fresh root weight.

Surface application of only 6.35 g NVS caused significant ($P = 0.03$) increase in fresh root weight compared to check (pooled data in Table 2.5). The low rate (1.27 g NVS) did not have any affect on fresh root weight compared to the check. Fresh root weights of 1.6, 1.9, and 2.3 g were obtained when data from soybean plants received 0, 1.27 and 6.35 g surface application of NVS were analyzed together, respectively.

Dry shoot weights of soybeans were also positively correlated ($P \leq 0.05$) with the rate of NVS treatment. NVS at 1.27 g and 6.35 g /150 mL sand significantly increased dry shoot weights of soybeans, 0.45 and 0.48 g/plant, compared to untreated control, 0.40 g/plant (Table 2.6, Exp. 1).

In the second experiment, albeit dry shoot weight of soybean was significantly ($P \leq 0.05$) greater in NVS application at a rate of 6.35 g/150 mL sand compared to untreated control, it was not significantly ($P > 0.05$) different between plants receiving 0,
and 1.27 g NVS / 150 mL sand (Table 2.6, Exp.2). NVS at 0, 1.27 and 6.35 g rate resulted in 0.40, 0.43, and 0.45 g dry shoot weights of soybean, respectively.

As can be seen from pooled data in table 2.6, dry shoot weights of soybean plants receiving 0, 1.27 and 6.35 g NVS/150 mL sand were significantly (P < 0.05) greater than the control although there was no significant difference among the treatments.

The effects of different application rates of NVS on average dry root weights of soybean plants inoculated with 2,000 eggs and J2 juveniles of SCN are shown in Table 2.7. Application of 6.35 g NVS/150 mL sand significantly (P<0.05) increased dry root weight of soybean compared to untreated check. Application of 1.27 g NVS/ 150 mL sand did not cause any increase in dry root weight compared to untreated control (Table 2.7, Exp. 1). Average dry root weight of 0.2, 0.3 and 0.4 g was produced from plants receiving 0, 1.27 and 6.35 g surface application of NVS.

In the second experiment, dry root weights of soybean plants receiving 6.35 g NVS/150 mL sand were significantly (P≤0.05) higher than that of soybean receiving no NVS (Table 2.7, Exp. 2). Dry weights of roots were 0.2, 0.3, and 0.3 g from soybean plants receiving 0, 1.27 and 6.35 g NVS surface application.

A greater (P≤0.05) dry root weight, 0.3 g was obtained when soybean plants were treated with 6.35 g NVS/150 mL sand compared to untreated check, 0.2 g (Pooled data in table 2.7). NVS at 1.27 g rate also caused significant increase in dry root weight, 0.3 g compared to the check.
<table>
<thead>
<tr>
<th>NVS (g/150 mL sand)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Pooled Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.7 a b</td>
<td>1.5 a</td>
<td>1.6 a</td>
</tr>
<tr>
<td>1.27</td>
<td>2.1 b</td>
<td>1.7 a</td>
<td>1.9 a</td>
</tr>
<tr>
<td>6.35</td>
<td>2.4 b</td>
<td>2.2 b</td>
<td>2.3 b</td>
</tr>
</tbody>
</table>

LSD\(_{(0.05)}\) = 0.3 0.5 0.3

\(^a\) Average fresh root weight was obtained by weighing washed, fresh roots cut from 1 cm above soil line, removing excess water with a paper towel, and averaging the total fresh root weight by the number of replicates for each treatment.

\(^b\) Within columns, means followed by the same letter are not significant according to LSD\(_{(0.05)}\).

\(^c\) Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher’s least significant difference test (P≤0.05).

**Table 2.5** Fresh root weight of soybean ‘Corsoy 79’ five wk after surface application of 0, 1.27 g and 6.35 g N-Viro soil (NVS)/150 mL sand infested with 2,000 eggs and J2 juveniles of soybean cyst nematode (SCN) race 3 per cone-tainers in greenhouse.
### Table 2.6

<table>
<thead>
<tr>
<th>NVS (g/150 mL sand)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Pooled Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.40 a&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.40 a</td>
<td>0.40 a</td>
</tr>
<tr>
<td>1.27</td>
<td>0.45 ab</td>
<td>0.45 ab</td>
<td>0.45 b</td>
</tr>
<tr>
<td>6.35</td>
<td>0.48 b</td>
<td>0.45 b</td>
<td>0.46 b</td>
</tr>
</tbody>
</table>

LSD<sub>(0.05)</sub><sup>c</sup>  
0.07  
0.05  
0.01

---

**Average Dry Shoot Weight (g)<sup>a</sup>**

**a** Average dry shoot weight was obtained by drying shoots cut 1 cm above the soil line at 58°C in an oven for 48 hr averaging the total dry weight by the number of replicates.

**b** Within columns, means followed by the same letter are not significant according to LSD<sub>(0.05)</sub>.

**c** Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher's least significant difference test (P<0.05).

**Table 2.6** Dry shoot weight of soybean 'Corsoy 79' five wk after surface application of 0, 1.27 g and 6.35 g N-Viro Soil (NVS)/150 mL sand infested with 2,000 eggs and J2 juveniles of soybean cyst nematode (SCN) race 3 per cone-tainers in greenhouse.
<table>
<thead>
<tr>
<th>NVS (g/150 mL sand)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Pooled Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.23 a</td>
<td>0.22 a</td>
<td>0.22 a</td>
</tr>
<tr>
<td>1.27</td>
<td>0.26 a</td>
<td>0.29 a b</td>
<td>0.28 b</td>
</tr>
<tr>
<td>6.35</td>
<td>0.36 b</td>
<td>0.32 b</td>
<td>0.34 c</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td>0.09</td>
<td>0.08</td>
<td>0.05</td>
</tr>
</tbody>
</table>

a Average dry root weight was obtained by weighing the roots from each replicate after drying in an oven at 58 C for 48 hr and dividing the total dry root weight by the number of replicates.

b Within columns, means followed by the same letter are not significant according to LSD(0.05).

c Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher's least significant difference test ($P \leq 0.05$).

Table 2.7 Dry root weight of soybean 'Corsoy 79' five wk after surface application of 0, 1.27 g and 6.35 g N-Viro Soil (NVS)/150 mL sand infested with 2,000 eggs and J2 juveniles of soybean cyst nematode (SCN) race 3 per cone-tainers in greenhouse.
Application of both 1.27 g and 6.35 g NVS/150 mL sand significantly ($P \leq 0.05$) decreased the number of nodules on the roots of soybean compared to control (Table 2.8, Exp. 1). Nodule numbers on roots of plants surface applied with 0, 1.27 and 6.35 g NVS were 44.33, 6.33 and 0.50, respectively.

In the second experiment, NVS at 6.35 g / 150 mL sand rate caused a significant decrease in number of nodules formed on the roots of soybean compared to untreated check. Nodule production was not significantly ($P > 0.05$) affected by 1.27 g NVS application compared to control (Table 2.8, Exp. 2). Average nodule numbers were 2.50, 1.00, and 0.25 from roots of plants grown in sand surface applied with 0, 1.27 and 6.35 g NVS, respectively.

The highest number of nodules formed on the roots was associated with those plants receiving no NVS. Significantly fewer nodules, 0.38 and 3.29, were formed on the plant roots treated with 1.27 and 6.35 g NVS/150 ml sand, respectively, compared to the check (pooled data in Table 2.8). NVS at 0, 1.27 and 6.35 g NVS caused production of 20.43, 3.29 and 0.38 nodules, respectively.
Table 2.8 Number of nodules on the roots of soybean ‘Corsoy 79’ five weeks after surface application of 0, 1.27 g and 6.35 g N-Viro Soil (NVS)/150 ml sand infested with 2,000 eggs and J2 juveniles of soybean cyst nematode (SCN) race 3 per cone-tainers in greenhouse.
2. Effect of two Rates of N-Viro Soil Mixed with Construction Sand on SCN Reproduction and Host Growth. SCN formed an average of 73 cysts/plant on checks, in comparison to 23 and 15 cysts on the roots of plants treated with 1.27 g and 6.35 g N-Viro Soil/150 ml construction sand, respectively (Table 2.9, Exp. 1). Cyst formation was suppressed significantly \((P \leq 0.05)\) by 32 % and 21 % on the roots of soybean treated with 1.27 g and 6.35 g N-Viro/150 ml construction sand, respectively, compared to the check.

In the second experiment NVS at 1.27 and 6.35 g NVS /150 ml significantly \((P \leq 0.05)\) reduced the number of cyst on the roots of ‘Corsoy 79’ plants compare to untreated check (Table 2.9, Exp. 2). Soybeans grown in sand amended with 0, 1.27 and 6.35 g NVS had 95, 21 and 21 cysts, respectively.

Sand amended with 1.27 and 6.35 g NVS significantly \((P \leq 0.05)\) suppressed the cyst formation on the roots of susceptible soybean ‘Corsoy 79’ compared to check (pooled data in Table 2.9). However, no significant differences were observed on number of cysts formed on the roots of soybeans grown in sand amended with 1.27 and 6.35 g NVS in the pooled data. Average cyst numbers of 90, 24, and 18 were obtained when plants were grown in sand amended with 0, 1.27 and 6.35 g NVS in the pooled data.

The average number of eggs was 23,733, 6,880, and 3,320 for the treatments of 0, 1.27 and 6.35 g NVS/150 mL sand, respectively (Table 2.10 (Exp.1)). Average number of eggs for 1.27 and 6.35 g NVS/150 mL sand were 28 % and 14 % lower than the check \((P \leq 0.05)\).
Average Cyst Number

<table>
<thead>
<tr>
<th>NVS (g/150 mL sand)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Pooled Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>83 a b</td>
<td>95 a</td>
<td>90 a</td>
</tr>
<tr>
<td>1.27</td>
<td>26 b</td>
<td>21 b</td>
<td>24 b</td>
</tr>
<tr>
<td>6.35</td>
<td>16 b</td>
<td>21 b</td>
<td>18 b</td>
</tr>
</tbody>
</table>

LSD\(_{0.05}\)^c 17 37 19

\(^a\) Average Cyst Number was obtained by counting the cysts collected from each replicate and averaging the total cyst number by the number of replicates for each treatment.

\(^b\) Within columns, means followed by the same letter are not significant according to LSD\(_{0.05}\).

\(^c\) Data were analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher’s least significant difference test (\(P \leq 0.05\)).

**Table 2.9** Number of cyst on the roots of soybean ‘Corsoy 79’ grown in sand amended with 0, 1.27 and 6.35 g N-Viro soil (NVS)/150 mL sand and infested with 2,000 eggs and J2 juveniles of soybean cyst nematode (SCN) race 3 per container 5 wk after treatments in greenhouse.
In the second experiment, NVS at 1.27 and 6.35 g caused production of significantly ($P \leq 0.05$) fewer eggs on the roots compared to the check, although the effects of low and high rates of NVS on egg numbers were not significantly separated (Table 2.10, Exp.2). Plants grown in 0, 1.27 and 6.35 g NVS and 150 mL sand mix resulted in 22,610, 5,420 and 2,990 average eggs, respectively.

Soybean plants grown in sand amended with 1.27 and 6.35 g NVS had fewer ($P \leq 0.05$) eggs, 6,150 and 3,155, respectively, on their roots than those grown in sand with no NVS, 23,091 eggs, in pooled data (Table 2.10).

NVS at 6.35 g /150 ml rates significantly ($P \leq 0.05$) increased the height of soybean plants compared to the control. In contrast, no significant ($P < 0.05$) difference was observed between 0 and 1.27 g NVS /150 ml sand applications (Table 2.11, Exp. 1). Average heights of the plants grown in sand amended with 0, 1.27 and 6.35 g NVS/150 mL sand were 18.1, 20.4, and 21.7 cm, respectively.

In the second experiment, however, there was no significant ($P > 0.05$) difference in the height of the plants. Heights of plants receiving 0, 1.27 and 6.35 g NVS/150 ml construction sand were 18.3, 19.1, and 21.3 cm, respectively (Table 2.11, Exp. 2).

The height of soybean plants grown in sand amended with 6.35 g NVS was significantly ($P \leq 0.05$) greater than the control. However, no significant ($P > 0.05$) difference in heights was observed among the treatment 1.27 g NVS and the check. Average heights of soybeans were 18.21, 19.71 and 21.43 cm when they were grown in sand amended with 0 and 1.27 and 6.35 g NVS, respectively (pooled data in Table 2.11).
## Average Egg Number

<table>
<thead>
<tr>
<th>NVS (g/150 mL sand)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Pooled Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23,733 a</td>
<td>22,610 a</td>
<td>23,091 a</td>
</tr>
<tr>
<td>1.27</td>
<td>6,880 b</td>
<td>5,420 b</td>
<td>6,150 b</td>
</tr>
<tr>
<td>6.35</td>
<td>3,320 b</td>
<td>2,990 b</td>
<td>3,155 b</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td>5,859</td>
<td>7,609</td>
<td>4,173</td>
</tr>
</tbody>
</table>

* Averagé egg number was obtained by counting the eggs at 40 X collected by crushing cysts from soil and root systems of plants from each replicate and averaging the total egg number by the number of replicates for each treatments.

* Within columns, means followed by the same letter are not significant according to LSD(0.05).

* Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher's least significant difference test (P≤0.05).

**Table 2.10** Number of eggs extracted from cysts from the roots of soybean 'Corsoy 79' grown in sand amended with 0, 1.27 and 6.35 g N-Viro soil (NVS)/150 mL sand and infested with 2000 eggs and J2 juveniles of soybean cyst nematode (SCN) race 3 per cone-tainers 5 wk after treatments in greenhouse.
Average height was obtained by measuring the height of plants from each replicate and dividing the total height by the number of replicates for each treatment.

Within columns, means followed by the same letter are not significant according to LSD (0.05).

Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher's least significant difference test (P≤0.05).

Table 2.11 Height of soybean ‘Corsoy 79’ grown in sand amended with 0, 1.27 and 6.35 g N-Viro soil (NVS)/150 mL sand and infested with 2,000 eggs and J2 juveniles of soybean cyst nematode (SCN) race 3 per cone-tainers 5 wk after treatments in greenhouse.
NVS at 1.27 and 6.35 g NVS /150 mL sand significantly \((P \leq 0.05)\) increased fresh shoot weight, 2.77 and 3.36 g, respectively, compared to check, 1.92 g, in the first test (Table 2.12, Exp.1).

In the second experiment, NVS at 6.35 g significantly \((P \leq 0.05)\) increased fresh shoot weight, 3.47 g, compared to 0, 2.10 g, and 1.27 g NVS, 2.43 g, (Table 2.12, Exp. 2).

Fresh shoot weight of soybeans treated with 6.35 g NVS were significantly \((P \leq 0.05)\) greater than that of those treated with 1.27 g NVS compared to the check (pooled data in Table 2.12). The treatments, 0, 1.27 and 6.35 g NVS / 150 mL sand had average fresh shoot weights of 2.02, 2.57 and 3.42 g, respectively.

In the first experiment, average fresh root weight of plants treated with NVS at 0, 1.27 and 6.35 g NVS/150 ml sand was 1.14, 1.46 and 3.31 g, respectively (Table 2.13, Exp. 1). NVS at 6.35 g / 150 mL sand significantly \((P \leq 0.05)\) increased fresh root weight of soybean compared to no NVS treatment. However, NVS at 1.27 g rate had no significant \((P \geq 0.05)\) effect on fresh root weight (Table 2.13, Exp. 1).

In the second experiment, average fresh root weights of 1.62, 1.41 and 3.27 g were obtained from NVS treatments at 0, 1.27 and 6.35 g NVS at 6.35 g, respectively. The 6.35 g rates of NVS significantly \((P \leq 0.05)\) increased fresh root weight of soybean compared to no NVS amendment (Table 2.13, Exp. 2). There was no significant \((P \geq 0.05)\) difference in average fresh root weights of soybeans grown in 0, and 1.27 g NVS and sand mix treatments.
Average Fresh Shoot Weight (g)\(^a\)

<table>
<thead>
<tr>
<th>NVS (g/150 mL sand)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Pooled Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.92 (^b)</td>
<td>2.10 (a)</td>
<td>2.02 (a)</td>
</tr>
<tr>
<td>1.27</td>
<td>2.77 (b)</td>
<td>2.43 (a)</td>
<td>2.57 (b)</td>
</tr>
<tr>
<td>6.35</td>
<td>3.36 (b)</td>
<td>3.47 (b)</td>
<td>3.42 (c)</td>
</tr>
</tbody>
</table>

\(^c\) LSD\(_{0.05}\)

|            | 0.80 | 0.81 | 0.50 |

\(^a\) Average fresh shoot weight was obtained by weighing fresh shoot weight of each plant shoot cut 1 cm above soil line from each replicate and dividing the total fresh shoot weight by the number of replicates for each treatment.

\(^b\) Within columns, means followed by the same letter are not significant according to LSD\(_{0.05}\).

\(^c\) Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher's least significant difference test (\(P\leq 0.05\)).

**Table 2.12** Fresh shoot weight of soybean 'Corsoy 79' grown in sand amended with 0, 1.27 and 6.35 g N-Viro soil (NVS)/150 mL sand and infested with 2,000 eggs and J2 juveniles of soybean cyst nematode (SCN) race 3 per cone-tainers \(5\) wk after treatments in greenhouse.
In the pooled data, the treatments, 0, 1.27 and 6.35 g NVS/150 mL sand had average fresh root weights of 1.41, 1.44 and 3.28 g, respectively (Table 2.13, Pooled data). Fresh root weight of soybean was significantly ($P \leq 0.05$) greater at 6.35 g rate treatment compared to untreated check and NVS at 1.27 g rate (Table 2.13).

In the first experiment, NVS at low and high rates significantly ($P \leq 0.05$) increased dry shoot weight of soybean, 0.81 and 0.98 g, respectively, compared to check, 0.55 g (Table 2.14 (Exp. 1)).

In the second experiment, dry shoot weight of soybean grown in control, 0.59 g was significantly ($P \leq 0.05$) less than the high rate, 0.97 g (Table 2.14, Exp. 2). However, the low rate treatment, 0.76 g was the same as control, 0.59 g.

In the pooled data, dry shoot weight of soybean was significantly ($P \leq 0.05$) greater in the treatment 1.27 and 6.35 g NVS compared to the check (Table 2.14). The high rate of NVS caused more increase in dry shoot weight of soybean than the low rate. Dry shoot weights in no NVS, the low and high rates of NVS were 0.57, 0.79 and 0.97 g, respectively (Table 2.14).

In the first experiment, low and high rate treatment significantly ($P \leq 0.05$) increased dry root weight of soybean, 0.34 and 0.37 g, respectively, compared to check, 0.27 g (Table 2.15, Exp. 1).

In the second experiment, the high rate treatment significantly ($P \leq 0.05$) increased the dry root weight of soybean, 0.42 g, compared to control, 0.26 g, although the low treatment did not cause any significant increase in dry root weight, 0.29 g, compared to check, 0.26 g (Table 2.15, Exp. 2).
<table>
<thead>
<tr>
<th>NVS (g/150 mL sand)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Pooled Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.14 a b</td>
<td>1.62 a</td>
<td>1.41 a</td>
</tr>
<tr>
<td>1.27</td>
<td>1.46 a</td>
<td>1.41 a</td>
<td>1.44 a</td>
</tr>
<tr>
<td>6.35</td>
<td>3.31 b</td>
<td>3.27 b</td>
<td>2.28 b</td>
</tr>
</tbody>
</table>

LSD\(_{(0.05)}\)^c 0.55 0.63 0.34

\(^a\) Average fresh root weight was obtained by weighing washed, fresh roots of soybeans stem of which were cut 1 cm above soil line, removing excess water with a paper towel and dividing the total fresh root weight by the number of replicates for each treatment.

\(^b\) Within columns, means followed by the same letter are not significant according to LSD\(_{(0.05)}\).

\(^c\) Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher’s least significant difference test (\(P\leq0.05\)).

**Table 2.13** Fresh root weight of soybean ‘Corsoy 79’ grown in sand amended with 0, 1.27 and 6.35 g N-Viro soil (NVS)/150 mL sand and infested with 2,000 eggs and J2 juveniles of soybean cyst nematode (SCN) race 3 per cone-tainers 5 wk after treatments in greenhouse.
### Average Dry Shoot Weight (g)\(^a\)

<table>
<thead>
<tr>
<th>NVS (g/150 mL sand)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Pooled Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.55 (^a)</td>
<td>0.59 (^a)</td>
<td>0.57 (^a)</td>
</tr>
<tr>
<td>1.27</td>
<td>0.81 (^b)</td>
<td>0.76 (^{ab})</td>
<td>0.79 (^b)</td>
</tr>
<tr>
<td>6.35</td>
<td>0.98 (^b)</td>
<td>0.97 (^b)</td>
<td>0.97 (^c)</td>
</tr>
<tr>
<td>LSD(^{(0.05)})(^c)</td>
<td>0.18</td>
<td>0.27</td>
<td>0.15</td>
</tr>
</tbody>
</table>

\(^a\) Average dry shoot weight was obtained by drying each plant shoots cut 1 cm above soil line at 58° C in an oven for 48 hr from each replicate and dividing the total dry weight by the number of replicates.

\(^b\) Within columns, means followed by the same letter are not significant according to LSD\(^{(0.05)}\).

\(^c\) Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher’s least significant difference test (\(P \leq 0.05\)).

**Table 2.14.** Dry shoot weight of soybean ‘Corsoy 79’ grown in sand amended with 0, 1.27 and 6.35 g N-Viro soil (NVS)/150 mL sand and infested with 2,000 eggs and J2 juveniles of soybean cyst nematode (SCN) race 3 per cone-tainers 5 wk after treatments in greenhouse.
6.35 g NVS caused significantly ($P < 0.05$) greater increase in dry weight of roots than 1.27 g NVS and the untreated check (Table 2.15). Average dry root weights were 0.27, 0.31 and 0.40 when plants were grown in sand amended with 0, 1.27 and 6.35 g NVS, respectively (pooled data in Table 2.15).

In a first experiment, low and high rate treatments significantly ($P < 0.05$) reduced number of nodules per root systems, 3.25 and 1.33, respectively, of soybean plants compared to control, 9.25, (Table 2.16, Exp. 1). Low and high rates were not significantly different from each other.

In the second experiment, number of nodules formed on the roots of soybean was significantly ($P < 0.05$) reduced by low and high rates, 1.25 and 1.25 nodules, respectively, compared to check, 8.75 nodule (Table 2.16, (Exp. 2). Nodule numbers on roots of soybean plants receiving the low rate was not significantly different from high rate treatment, 1.25 and 1.25 nodules, respectively.

NVS at 0, 1.27 and 6.35 g rates had 9.00, 2.25 and 1.29 nodules per root systems of soybeans. Only 6.35 g NVS treatment significantly suppressed nodule numbers on the roots of soybean in greenhouse tests (Table 2.16). No significant difference in nodule numbers was observed among 1.27 g NVS and the control.
### Table 2.15

Average Dry Root Weight (g)\(^a\)

<table>
<thead>
<tr>
<th>NVS (g/150 mL sand)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Pooled Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.27 ( a^b )</td>
<td>0.26 ( a )</td>
<td>0.26 ( a )</td>
</tr>
<tr>
<td>1.27</td>
<td>0.34 ( b )</td>
<td>0.29 ( a )</td>
<td>0.31 ( a )</td>
</tr>
<tr>
<td>6.35</td>
<td>0.37 ( b )</td>
<td>0.42 ( b )</td>
<td>0.40 ( b )</td>
</tr>
<tr>
<td>LSD(_{0.05}^c)</td>
<td>0.07</td>
<td>0.11</td>
<td>0.06</td>
</tr>
</tbody>
</table>

\(^a\) Average dry root weight was obtained by drying each plant roots cut from 1 cm above soil line, placed in a paper bag and dried at 58°C in an oven for 48 hr, dividing the total dry root weight by the number of replicates.

\(^b\) Within columns, means followed by the same letter are not significant according to LSD\(_{0.05}\). 

\(^c\) Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher's least significant difference test \((P \leq 0.05)\).

*Table 2.15* Dry root weight of soybean 'Corsoy 79' grown in sand amended with 0, 1.27 and 6.35 g N-Viro soil (NVS)/150 mL sand and infested with 2,000 eggs and J2 juveniles of soybean cyst nematode (SCN) race 3 per cone-tainers 5 wk after treatments in greenhouse.
Table 2.16 Number of nodules on the roots of soybean ‘Corsoy 79’ grown in sand amended with 0, 1.27 and 6.35 g N-Viro soil (NVS)/150 mL sand and infested with 2,000 eggs and J2 juveniles of soybean cyst nematode (SCN) race 3 per cone-tainers 5 wk after treatments in greenhouse.
3. Effect of two Rates of N-Viro Soil on Reproduction of SCN on Resistant and Susceptible Soybeans in the Field. Pre-plant egg population density of SCN from plots receiving 5 and 25 T NVS /A, were 340, and 320 eggs and J2 juveniles/200 mL soil, respectively, compared to the untreated control, 960 eggs and J2 juveniles, 73 days after application of N-Viro soil to the plots (Table 2.17) \( (P < 0.05) \) Low and high rate treatments were not significantly \( (P > 0.05) \) different (Table 2.18). In other words, low and high rate of NVS equally suppressed pre-plant egg population of SCN compared to control. Since pre-plant soil samples from resistant and susceptible variety were mixed together, the effects of those varieties on soil egg and J2 juveniles population densities of SCN were not analyzed for pre-plant SCN egg population.

The high rate had significantly lower egg population of SCN compared to control during midseason (Table 2.18). The low rate had no effect on egg population during midseason compared to control (Table 2.18).

Significantly lower post-harvest egg populations of SCN were obtained from plots receiving the high rate of NVS surface application (Table 2.18). The post harvest egg populations from the low rate of NVS were significantly lower than the control (Table 2.18).

Mid-season egg populations (Log 10) of SCN did not significantly \( (P > 0.05) \) differ among the treatments of susceptible and resistant varieties (Table 2.19). Post-harvest egg populations of SCN were significantly lower in samples from resistant variety plots than those from susceptible ones.
### Table 2.17 Pre-plant egg population of soybean cyst nematode (SCN) race 3, 53 days after surface application of N-Viro soil (NVS) at 0, 5, and 25 T/A rate.

<table>
<thead>
<tr>
<th>N-Viro soil (T/A)</th>
<th>Average Egg Count (/200 mL soil)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>960 $^a$ $^b$</td>
</tr>
<tr>
<td>5</td>
<td>340 $^b$</td>
</tr>
<tr>
<td>25</td>
<td>320 $^b$</td>
</tr>
</tbody>
</table>

$^a$ Average egg number was obtained by crushing the cysts collected from each replicate, counting the eggs at 40 X and dividing the total egg number by the number of replicates.

$^b$ Within columns, means followed by the same letter are not significant according to LSD($0.05$).

$^c$ Data was analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher’s least significant difference test ($P \leq 0.05$).
### Table 2. 18 Egg population (Log 10) of soybean cyst nematode (SCN) in response to surface application of N-Viro soil (NVS) at 0, 5 and 25 T/A rate during growing season and at post harvest.

<table>
<thead>
<tr>
<th>N-Viro soil (T/A)</th>
<th>Average Egg Count (Log 10) (/200 mL soil)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Midseason</td>
</tr>
<tr>
<td>0</td>
<td>2.77 (^a) (^b)</td>
</tr>
<tr>
<td>5</td>
<td>2.79 (^a)</td>
</tr>
<tr>
<td>25</td>
<td>2.45 (^b)</td>
</tr>
<tr>
<td></td>
<td><strong>LSD(_{0.05})^c</strong></td>
</tr>
</tbody>
</table>

\(^a\) Average egg number was obtained by crushing the cysts collected from each replicate, counting the eggs at 40 X and dividing the total egg number by the number of replicates.

\(^b\) Within columns, means followed by the same letter are not significant according to LSD\(_{0.05}\).

\(^c\) Egg numbers from each replicate were converted to Log 10 values. Data were analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher’s least significant difference test \((P = 0.05)\).
<table>
<thead>
<tr>
<th>Soybean</th>
<th>Average Egg Count (Log 10) (/200 mL soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mid-season</td>
</tr>
<tr>
<td></td>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>Resistant</td>
<td>2.65 a b</td>
</tr>
<tr>
<td>Susceptible</td>
<td>2.69 a</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD_{0.05}</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*a Average egg number was obtained by crushing the cysts collected from each replicate, counting the eggs at 40 X and dividing the total egg number by the number of replicates.

*b Within columns, means followed by the same letter are not significant according to LSD_{0.05}.

c Egg numbers from each replicate were converted to Log 10 values. Data were analyzed by ANOVA and if a significant F-value was obtained, means were separated by Fisher's least significant difference test (P \( < 0.05 \)).

**Table 2.19** Egg population (Log 10) of soybean cyst nematode (SCN) on resistant (to race 3 of SCN) and susceptible soybean variety during mid-season and at post-harvest.
DISCUSSION

Surface applications of NVS or the mixing NVS with sand at 1.27 and 6.35 g NVS /150 mL sand rate significantly suppressed cyst and egg numbers of SCN in greenhouse experiments. Similarly, NVS at 5 and 25 T/A had significantly less pre-plant egg numbers (/200 soil) 53 days after application of NVS in the field experiment. Mid-season egg population of SCN was significantly reduced compared to control. NVS at 5 T/A rate had no effect on midseason egg population of SCN compared to control in the field. The lowest and a reduced post-harvest egg population of SCN was present in samples receiving 25 and 5 T/A NVS, respectively, compared to control.

Mid-season egg population of SCN was not affected by resistant and susceptible soybean varieties. Egg population of SCN at post-harvest was successfully suppressed by resistant soybean variety. In addition, interactions of soybean variety vs. NVS treatment had no significant effect on egg population of SCN during growing season and harvest.

Incidence of *Verticillium* wilt, potato scab and populations of lesion nematode, *Pratylenchus penetrans*, pin nematode, *Pratylenchus projectus*, and root knot, *Meloidogyne hapla* were reduced to near zero levels by the application of very high rates of soybean meal, and meat and bone meal in two commercial potato fields, and ammonia
was the major cause of this decline in pathogen populations in soil (Lazarovits et al., 1999). Similarly, major organic N source are from proteins in the biosolids (NVS), and fresh NVS contains 8967.92 mg N/kg NVS (or 10.33 mg organic N/L, NVS extracts) total organic nitrogen per kilogram (Yamakawa, 1999). Ammonia concentration is very high in fresh NVS (194.24 mg NH$_3$/L, NVS extracts) (Yamakawa, 1999). It is possible that toxic substances such as ammonia are involved in suppressing SCN egg populations in greenhouse experiments in which NVS was surface applied or mixed with 150 mL sand in cone-tainers, and in suppressing pre-plant egg population of SCN at 25 T/A rate in the field where NVS was surface-applied to the plots. Another possibility for fewer eggs from surface application plots of NVS at 25T/A is that eggs in cysts could decay in soil (Shepherd, 1960) after being killed by ammonia resulting in fewer egg numbers.

Mid-season egg populations of SCN were relatively higher than pre-plant egg populations. However, there was no significant difference in mid-season egg population of SCN between 5 and no NVS applications or resistant and susceptible soybeans, or interaction of soybean variety versus NVS treatments. Application of NVS at 25 T/A rate resulted in significantly fewer egg numbers compared to the control in mid-season.

SCN population in the field fluctuates depending on the over-wintering egg population, crop variety and environmental and soil factors such as soil structure, moisture, pH, soil temperature (Alston and Schmitt, 1987) and presence of weed hosts of SCN (Venkatesh et al., 2000). Noel and Barker, (1985) found that variation in soil parameters such as clay, sodium and copper contents, accounted for over 50% of the spatial variation in nematode counts. In a field study, the best correlation was obtained between the initial numbers of eggs in the soil and soil and root nematode populations.
and 30 days later, although no correlation was found between egg and J2 juvenile numbers in soil at harvest and numbers that over-wintered (Bonner and Schmitt, 1985). Increase in SCN populations was more rapid in low initial populations (Pi) of SCN than high Pi during growing season (Alston and Schmitt, 1987).

In this study, NVS applied at 5 T/A, planting resistant and susceptible soybean variety had no affect on egg numbers of SCN during growing season. Surface application of NVS in field study caused significant declines in preseason SCN egg population 73 days after application. Zn content (500 mg/kg) in NVS is relatively high (Yamakawa, 1999) and Zn is a natural hatching stimulus of SCN eggs. It is possible that surface application of NVS in field stimulated more SCN eggs to hatch and resulted in lower egg populations later since NVS has high Zn contents. Another possibility is that embryos in encysted eggs of SCN could be killed by toxic volatiles (e.g. ammonia) released from NVS. As a result of this, fewer J2 juveniles, the only infective stage of SCN, would be present when soybeans were planted.

NVS at 5 and 25 T/A rate significantly reduced SCN egg population at harvest along with planting a resistant variety. This suggests that the effect of NVS on SCN persists until harvest. NVS at 25 T/A also significantly suppressed the egg population of SCN on a susceptible variety at harvest. This suggests that susceptible soybean cultivar could be planted along with the application of NVS at 25 T/A even though pre-plant egg populations of SCN are high. This practice could be employed in soils where preseason egg populations of SCN are high and when soybean crop price is high. We do not know if surface application of NVS at 25T/A effectively suppresses SCN populations in soil for more than one season.
Growth of soybean ‘Corsoy 79’ as expressed by shoot and root fresh and dry weights, was significantly enhanced by both 1.27 and 6.35 g NVS compared to that in unamended sand when it was applied to the surface or incorporated into sand in presence of 2,000 eggs and J2 juveniles of SCN. Although 6.35 g NVS appeared to reduce the nematode populations most effectively, it also significantly enhanced plant yield more efficiently than 1.27 g NVS/150 mL sand application in greenhouse experiments. NVS is high in N that comes from proteins. Improved soybean growth in this study is contributed mostly to high N level in NVS and suppression of SCN reproduction on the roots.

In an experiment under controlled conditions in a phytotron, soybean shoot and root weights were significantly lower when they were infested with *H. glycines* than uninfested control (Hussey and Barker, 1976). A 90% inhibition of nodule weight/plant as a result of SCN inoculation with 12,500 eggs corresponded to a 55% suppression of plant growth (Ko et al., 1984). As is clear from the results of previous research, SCN causes significant growth suppression in soybean plant. Soybean growth could be effectively improved by application of NVS into soil in the presence of SCN. Improved plant growth could be due to the presence of some organic and inorganic materials in NVS, which supply a more complete source of nutrients and other growth promoters. Soybean plant may synthesize higher photosynthetic products when there are fewer SCN feeding on the roots due to suppression of cyst and egg populations in sand amended with NVS.

Soil amendment with NVS significantly suppressed nodule formation and nodule development compared to unamended sand on the ‘Corsoy 79’ roots 5 weeks after
inoculation with 2000 eggs and second stage juveniles of SCN. The process of nodule formation by Rhizobia on the soybean roots is complex and first starts with the attachment of infective rhizobia to soybean root hairs. Infection threads are formed by host cells in response to infection by rhizobia where rhizobia multiply. Finally nodules are formed by invaded and unininvaded, and dividing and expanding host cells (Bauer, 1981). The entry of rhizobial infection threads into the suitable cortical cells may be prevented by localized destruction of cortical cells by the nematode, which in turn prevents the symbiosis (Ko et al., 1984). Soybean lectin also plays a major role in binding *Rhizobium japonicum* to soybean roots, and SCN infection suppresses the binding between roots and rhizobia due to interference of the nematode with soybean lectin metabolism (Huang et al., 1984). Similarly, first nodules were observed on the roots of control soybeans 9 days after rhizobial inoculation while these did not appear on the SCN infected soybean roots until 12 days later (Ko et al., 1985). As a result, nodule formation by *Rhizobium* spp. can be reduced by SCN infection (Riggs and Schmitt, 1993). High nematode population densities and favorable environmental conditions for SCN may cause stunting and chlorosis symptoms on infected soybeans. These symptoms are caused primarily by nitrogen deficiency as a result of suppression of nodulation.

Leghemoglobin (Lb) are red-pigmented hemoproteins consisting of an iron porphyrin and a peptide and they are found in root nodules. Nodules from nematode infected soybeans yielded lower fresh weight per plant, lower specific nitrogenase activity (micromoles of C$_2$H$_4$ formed per gram of nodules per hour) as assayed by the acetylene reduction procedure and lower Lb content per gram of nodule (Huang and Barker, 1983).
SCN severely inhibited nodule formation (expressed as nodules per g root) compared to nematode free plants (Hussey and Barker, 1976). In a split-root experiment, where one half of the plant root system was inoculated with SCN and the other half kept free from SCN, nodulation of plant as a whole was suppressed progressively as the level of SCN inoculum increased from 0 to 12,500 eggs (Ko et al., 1984). Nodule number, nitrogen fixing capacity, and the average nodules weights decreased 86%, 83%, and 43%, respectively, as compared to the control at the 12,500 eggs inoculum level (Ko et al., 1984).

In this study, further decline in nodule number on soybean roots grown in sand amended with 1.27 and 6.35 g NVS/150 ml sand could be explained by the presence of high nitrogen in this amendment compared to unamended control. In another study, the application of nitrogen fertilizer, NH$_4$NO$_3$ decreased SCN damage and improved growth of diseased plants (Ross, 1959). Nitrogen such as nitrate at high levels significantly inhibited nodule formation (Ross, 1959). In this study, presence of a high level of organic and inorganic nitrogen sources in NVS may compete with Rhizobia and result in a decline in nodule number.

Satisfactory control of soybean cyst nematode could be obtained when the numbers of cysts per unit volume of soil is reduced to a below a threshold level at which the value of the damage caused is equal to the cost of control (Ferris, 1978) rather than its complete eradication. Surface application of NVS at 25 T/A could be involved in effective management of SCN as a soil amendment.


CHAPTER 3

EFFECT OF N-VIRO SOIL AND AMMONIA (NH₃) ON EGG HATCH OF
SOYBEAN CYST NEMATODE (SCN), *Heterodera glycines* Ichinohe
INRODUCTION

*In vitro* research to manipulate egg hatch of soybean cyst nematode (SCN), *Heterodera glycines* has had much attention for decades. Several swine manure products and breakdown products, such as indole, 4-ethyl phenol, butylated hydroxytoluene, and 4-amino acetophenone at one mM and 3-methyl indole and 4-methyl phenol at 2 mM, were evaluated to determine the effects on eggs and J2 juveniles of *H. glycines* in airtight containers (Reynolds et al., 1998). In the study above showed that contact with 3-methyl phenol and indole inhibited the egg hatch, but volatiles from indole, 4-ethyl and 4-methyl phenol stimulated the hatch and body movement of J2 juveniles after the hatch. Breakdown products of secondary plant metabolites, glucosinolates such as cyanohydroxipropene and cyanohydroxipropene propionate at 10 and 100μg/mL resulted in 0.4% and 4.6% hatch of *H. glycira* eggs of that occurring in distilled water after 24 days (Tylka et al., 1997). In the work above, egg hatch of SCN was irreversibly inhibited because transfer of eggs from glucosinolate breakdown products to deionized water or 3 mM zinc sulfate did not cause any further eggs to hatch after 24 days. In another study Kraus et al. (1996) reported that several analogs of glycinoeclepbin A, a natural hatching stimulus of SCN inhibited the egg hatch of SCN. Thus, they suggested that inhibition of egg hatch was due to maximum functionality of a keto diacid.

Zinc salts have been reported to be very active hatching stimulants for SCN eggs in *vitro* (Clarke and Shepherd, 1965 and 1966). Three milimolar concentration of zinc
sulfate stimulated emergence of J2 juveniles of SCN from encysted eggs and free eggs compared to distilled water (Casta et al., 1997). In another study, strong and moderate increase in egg hatch of SCN was observed when free eggs were incubated in zinc chloride and zinc sulfate solutions compared to distilled water (Teft and Bone, 1984).

N-Viro Soil (NVS) is a product of an advanced alkaline stabilization process for municipal biosolids (Logan and Burnham, 1995). A great majority of inorganic nitrogen in NVS is in the form of ammonium (NH₄⁺), though oxidized forms such as nitrate(NO₃⁻) are low. Although 99% of an NH₃-NH₄ mixture is in the form of NH₄⁺ at neutral pH or lower, above pH 11 over 98% of the mixture is free NH₃ at equilibrium (Warren, 1962, Yamakawa, 1999). Due to pH decrease and volatilization with time in NVS, free NH₃ drops rapidly. As a result, NH₃ is converted into NH₄⁺ and immobilized by actively growing microorganisms (Yamakawa, 1999).

Free NH₃ dissolved in water (ammonia) is very toxic to most soil microorganisms (Logan and Burnham, 1995) and aquatic animals (Warren, 1962; Kathleen et al., 1955). In a factorial experiment, cations NH₄⁺, Ca⁡⁺⁺, and Mg⁡⁺⁺ were used in combination with each of the anions NO₃⁻, SO₄²⁻, and Cl⁻ to evaluate the effects of those inorganic ions on egg hatch of SCN (Lehman et al., 1971). In the experiment above, NH₄⁺ or NO₃⁻ containing compounds inhibited egg hatch compared to phosphate buffer. The results of our previous experiments in the greenhouse showed that roots of soybean 'Corsoy 79' grown in soil infested with 2,000 eggs and J2 juveniles of SCN race 3 formed fewer cysts and eggs than the control plants when they were treated with 1.27 and 6.35 g NVS per 150 mL sand. These results led to further investigation of the
(1) effects of the N-Viro leachates extracted from varying ages of N-Viro soils and the
(2) impact of different concentrations of ammonia on the egg hatch of SCN in vitro.

MATERIALS AND METHODS

Materials. N-Viro Soil (NVS) was obtained from the Bayview N-Viro facility, Toledo, OH.

Source of SCN. Soybean Cyst Nematode (SCN), Heterodera glycines race 3 was maintained on the roots of soybean (Glycine max) ‘Corsoy 79’ grown in 15 in. pots in greenhouse and used as a source of eggs for hatching experiments.

Extraction of N-Viro Leachate. NVS was dumped in the field at the Waterman Farm, The Ohio State University, Columbus, in June, 1997. The pile was 2 X 1 X 0.5 m high. The NVS soil cores were collected from the interior of the pile, 15 cm deep, at 4 different places, 0, 1, 3, 6, 12 mo later (Yamakawa, 1999). The NVS samples were kept at 5 C until used.

Water extract of NVS samples were collected with a mechanical extractor (Model 24-01, Centurion International Inc., Lincoln, NE) using a 60 ml syringe (Jaymes and Bigham, 1986) on 5 Jan 2000. Ten g of NVS was placed in the sample tube. Approximately 55 mL of water extract was collected over 12 hr. The NVS extract was kept at 5 C to use in future experiments.
1. **pH of N-Viro Soil Leachate.** pH of NVS extracts was determined with a digital pH meter (Model 430, Corning Incorporated, Science Product Division, Corning, NY) by inserting an electrode into the extracts in 100 ml plastic bottle. Due to the very limited amount of NVS leachates, 2mL/replicate, NVS leachates from the same treatments were collected and pH of the total sample was determined. However, statistical analysis was not performed on pH of NVS extracts because there were not enough samples to be analyzed.

2. **Effect of the Leachates Collected from Various Ages of N-Viro Soil.** Fifty mL of soil containing cysts of SCN were removed from root zones of actively growing soybean ‘Corsoy 79’. Soil was dumped in 5 L water in a 10 L plastic beaker. The water was stirred vigorously by hand in order to resuspend the cysts. The soil was allowed to settle in the bottom of the beaker for 10 sec. Supernatant water was poured through an 850 μm-pore (20 mesh) sieve nested over 250 μm-pore (60 mesh) sieve. The 850 μm-pore sieve was removed, and cysts collected on the 250 μm-pore sieve washed into a 250 mL glass beaker with 30 mL tap water. The cysts were rinsed into a 40 mL Ten-Broeck cyst homogenizer and crushed. The suspension was poured onto a nested 250 μm-pore (60 mesh) sieve over 75 μm-pore (200 mesh) sieve over 25 μm-pore (500 mesh) sieve, and the pestle was rinsed onto the 250 μm-pore sieve. The top sieves were removed. Eggs and J2 juveniles of SCN on the 25 μm-pore (500 mesh) sieve were washed in to a clean 250 mL glass beaker. Concentration of the suspension was standardized to 65 eggs / mL suspension by adding tap water to the suspension.
One mL aliquots containing approximately 65 eggs from continuously stirred suspension in 250 mL beaker were poured into 3 cm-diam plastic petri dishes. Eggs were allowed to settle in the bottom of the petri dishes for 10 min. Water in the dishes was removed carefully with a Pasteur pipette without disturbing the eggs on the bottom. Petri dishes were filled with 2 mL leachate of 0, 1, 3, 6, 12-mo-old N-viro soil, distilled water or 3 mM zinc sulfate, pH 7. Final volumes of the treatment solutions and checks were 2mL. Treatments were replicated 5 times. There were a total of 35 petri dishes for each experiment. Petri dishes were placed into two plastic trays on tissue paper separately for each experiment. The petri dishes were kept at laboratory condition under 8/16 hr light/dark illumination at 25 C on a lab bench. Percent egg hatch were determined by counting the second stage juveniles using a stereomicroscope (40X) at the beginning of test, 24, 48 and 72 hr after the test started. Data were reported as cumulative hatch at 0, 24, 48 and 72 hr.

3. Effect of Ammonia (NH₃) on Egg Hatch of SCN. Ammonia solutions, 0.001, 0.01, and 0.1 M and 0.02 M phosphate buffer solution and 3 mM ZnSO₄ solution were prepared in advance.

Eggs and J2 juveniles of SCN were extracted from 200 mL soil infested with cysts as described above. Concentration of eggs in the 200 mL tap water was adjusted to 80 eggs/mL suspension. One mL from continuously stirred egg suspension was pipetted into a 3-cm-diam plastic petri dish. The eggs were allowed to settle in the bottom of the petri dish for about 20 min. One mL tap water in the dishes were removed and replaced by 2 mL solution of the treatments 0.1, 0.01, 0.001 M ammonia, 0.02 M phosphate
buffer, 3mM ZnSO₄ and distilled H₂O with a Pasteur pipette. There were seven replicates of each treatment and positive and negative controls, 3mM zinc sulfate and distilled water, respectively.

Eggs were counted immediately in each replicate. Emerged J2 juveniles of SCN were counted at time 0, 24, 48 and 72 hr. Each treatment had approximately 50-100 eggs per hatching dish. Data were reported as cumulative hatch at 0, 24, 48 and 72 hr.

ANOVA was performed on the means of each treatment and checks. Treatment means were separated by Fisher’s Pairwise Comparison Test.

RESULTS

1. pH of N-Viro Soil Leachate. pH of leachates from 0 and 1 mo-old NVS declined from 13.0 and 12.4 to 8.2 and 8.3, respectively (Table 3.1). Change between initial pH and final pH of leachates from 3, 6, and 12 mo-old NVS was less than one pH level (pH initial, 8.2, 8.1 and 8.1 and pH final, 8.9, 8.9 and 8.8, respectively) at 72 hr. pH of 3 mM zinc sulfate in 0.02 M phosphate buffer was the most consistent and did not change significantly at the end of the experiment (pH initial, 7.0 and pH final 7.1) although pH of distilled water showed a 0.9 unit increase at the end of experiment (Table 3.1).

2. Effect of the Leachates Collected from Various Ages of N-Viro Soil. Age of N-Viro soil was positively correlated with percent egg hatch of SCN in vitro (Table 3.2) (P < 0.05). Egg hatch of SCN was suppressed by the leachates from 0 and 1 mo-old
NVS in which cumulative percent egg hatch was 0, and 1.48, respectively. Egg hatch was not affected in leachates from 3, 6, and 12 mo-old NVS or in 3 mM zinc sulfate solution compared to distilled water at 24 hr (Table 3.2). Average cumulative percent egg hatch was 3.36, 3.52, and 4.60 in leachates of 3, 6, and 12 mo-old NVS, respectively, and 3.16 and 5.02 in distilled water and in 3 mM zinc sulfate, respectively, at 24 hr.

Egg hatch was suppressed in leachates from 0 and 1 mo-old NVS but was not affected in leachate from 3, 6, 12 mo-old NVS compared to distilled water at 48 hr. However, egg hatch was significantly stimulated by 3 mM zinc sulfate solution at 48 hr. Cumulative percent egg hatch was 0, 1.64, 3.55, 5.02, and 5.56 in leachates from 0, 1, 3, 6, and 12 mo-old NVS, respectively. It was 3.29 and 6.32 in distilled water and 3 mM zinc sulfate solution, respectively, at 48 hr (Table 3.2).

A greater suppression of emergence of J2 juveniles of SCN was observed in leachate from 0 than in leachate from 1 and mo-old NVS compared to distilled water at 72 hr. Leachates of 6 and 12 mo-old NVS significantly stimulated egg hatch compared to distilled water at 72 hr. Three mM zinc sulfate solution caused the greatest stimulation of egg hatch compared to distilled water at 72 hr. Leachates of 0, 1, 3, 6, and 12 mo-old NVS, and distilled water and 3 mM zinc sulfate solution resulted in 0, 2.57, 4.74, 7.47, 7.58, 4.32 and 9.02 % cumulative egg hatch at 72 hr (Table 3.2).
<table>
<thead>
<tr>
<th>NVS leachates*</th>
<th>pH Initial (0 hr)$^b$</th>
<th>pH Final (72 hr)</th>
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<tr>
<td>0</td>
<td>13.0</td>
<td>8.2</td>
</tr>
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<td>1</td>
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<tr>
<td>6</td>
<td>8.1</td>
<td>8.9</td>
</tr>
<tr>
<td>12</td>
<td>8.1</td>
<td>8.9</td>
</tr>
<tr>
<td>distilled H$_2$O</td>
<td>7.0</td>
<td>7.9</td>
</tr>
<tr>
<td>3 mM ZnSO$_4$</td>
<td>7.0</td>
<td>7.1</td>
</tr>
</tbody>
</table>

$^a$ Water extract of 0, 1, 3, 6, and 12 mo-old NVS samples were collected with a mechanical extractor (Model 24-01, Centurion International Inc., Lincoln, NE) using a 60 ml syringe (Jaynes and Bigham, 1986) on 5 Jan. 2000.

$^b$ pH of NVS extracts were determined with a digital pH meter (Model 430, Corning Incorporated, Science Product Division, Corning, NY 14831) by inserting an electrode into the extracts in 100 mL plastic bottle at the beginning (0 hr) and end of the experiment (72 hr).

Table 3.1 pH of leachates of 0, 1, 3, 6, and 12 mo-old N-Viro soil and positive and negative control solutions determined at 0 and 72 hr (3mM ZnSO$_4$ and distilled water, respectively).
<table>
<thead>
<tr>
<th>Treatments</th>
<th>Average Cumulative Egg Hatch (%)&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>distilled H&lt;sub&gt;2&lt;/sub&gt;O</td>
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<tr>
<td>3 mM ZnSO&lt;sub&gt;4&lt;/sub&gt;</td>
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</tbody>
</table>

LSD<sub>(0.05)</sub> 0 1.89 2.22 1.53

<sup>a</sup> Average cumulative egg hatch (%) was calculated by adding J2 juvenile numbers at that particular time dividing it by the total number of eggs at the beginning of the experiment for each replicate and multiplying it by 100. Then, average percent cumulative egg hatch for each replicate was added up and divided by number of replicates for each experiment.

<sup>b</sup> Number of emerged J2 juveniles of SCN was counted under a stereomicroscope at 40 X at 0, 24, 48 and 72 hr and recorded.

**Table 3.2** Average cumulative hatch (%) of SCN eggs incubated in leachates of 0, 1, 3, 6, and 12 mo-old N-Viro (NVS) soil, distilled water and ZnSO<sub>4</sub> solution at 25C.
3. Effect of Ammonia (NH₃) on Egg Hatch. Egg hatch of SCN was not suppressed by 0.001, 0.01 and 0.1 M ammonia solutions at 24 hr (Table 3.3). However, egg hatch of SCN was significantly stimulated in 3 mM zinc sulfate solution compared to that in distilled water and in 0.02 M phosphate buffer solution at 24 hr. Cumulative egg hatch was 1.68, 2.11, and 1.47 in 0.001, 0.01 and 0.1 M NH₃ solutions. It was 1.98, 1.85, and 4.55 in distilled water, 0.02 M phosphate buffer and 3 mM zinc sulfate solution, respectively, at 24 hr (Table 3.3).

All the concentrations of ammonia (0.001, 0.01, and 0.1 M) significantly (P < 0.01) suppressed emergence of J2 juveniles from SCN eggs compared to distilled water and 0.02 M phosphate buffer at 48 hr. Emergence of J2 juveniles increased significantly (P < 0.01) in 3 mM zinc sulfate compared to that in distilled water and in 0.02 M phosphate buffer at 48 hr. Cumulative percent egg hatch was 2.58, 2.55, 1.90, 3.43, 3.19, and 5.87 in 0.001, 0.01 and 0.1 M ammonia, distilled water, 0.02 M phosphate buffer and 3 mM zinc sulfate solution, respectively, at 48 hr (Table 3.3).

The greatest suppression of emergence of J2 juveniles occurred in 0.01 and 0.1 M ammonia solution in 0.02 M phosphate buffer although a greater suppression of egg hatch was observed in 0.001 M ammonia solution compared to distilled water and 0.02 M phosphate buffer solution at 72 hr. Egg hatch of SCN was significantly (P < 0.01) induced by 3 mM zinc sulfate solution compared to distilled water and 0.02 M phosphate buffer at 72 hr. Cumulative egg hatch at 72 hr was 3.91, 3.50, 2.23, 5.54, 5.46, 8.93 in 0.001, 0.01 and 0.1 M ammonia solutions and in distilled water, 0.02 M phosphate buffer and in 3 mM zinc sulfate solution, respectively, at 72 hr (Table 3.3).
<table>
<thead>
<tr>
<th>Treatments</th>
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<tr>
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<td>0.02 M Phosphate Buffer</td>
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<tr>
<td>3 mM ZnSO(_4)</td>
<td>0 a</td>
</tr>
</tbody>
</table>

**LSD\(_{(0.05)}\)**

| 0 | 1.02 | 1.27 | 1.54 |

\(^a\) Average cumulative egg hatch (%) was calculated by adding the total egg hatch at that particular time dividing it by the total number of eggs at the beginning of the experiment for each replicate and multiplying it by 100. Then, average egg hatch for each replicate was added up and divided by number of replicates for each experiment.

\(^b\) Number of emerged J2 juveniles of SCN was counted under a stereomicroscope at 40 X at 0, 24, 48 and 72 hr.

**Table 3.3** Average cumulative hatch (%) of SCN eggs incubated in 0.1, 0.01, 0.001 M concentrations of ammonia, 3 mM ZnSO\(_4\) and distilled water at 25 C.
DISCUSSION

In this study, egg hatch of SCN was suppressed by leachates from 0 and 1 mo-old NVS at 24, 48, and 72 hr. In another study, 0 (fresh), 1, 3, 6 mo-old NVS contained 194.2, 46.6, 8.0 and 2.3 mg/L ammonia, although no ammonia was detected in leachate of 12 mo-old NVS (Yamakawa, 1999). Anhydrous ammonia is very toxic to soil microorganisms and animals (Warren, 1962) since un-ionized ammonia crosses cell membranes freely and causes irreversible death. Because leachates from 0 and 1 mo-old NVS has relatively higher amounts of ammonia than the leachates of 3 mo-old or older NVS, it is possible that high ammonia concentration caused the death of J2 juveniles inside eggs and thus inhibited egg hatch. There is also a small possibility that high pH of leachates from 0 and 1 mo-old NVS, 13 and 12.39, respectively, might account for suppression of egg hatch at 24, 48, and 72 hr in vitro in the experiments.

Although egg hatch was not affected by leachates of 1, 3, 6, 12 mo-old NVS, and 3 mM zinc sulfate, it was suppressed by leachates of 0 and 1 mo-old NVS at 24 hr (Table 3.2). Egg hatch of SCN was suppressed by leachates from 0 and 1 mo-old NVS although leachate from 3, 6 and 12 mo-old NVS did not have any affect on egg hatch compared to distilled water at 48 hr. Zinc sulfate at 3 mM cocentration significant increase in egg hatch at 48 hr.
NVS has a relatively high Zn content (500 mg/L). In general, at higher pH, solubility of Zn is limited by absorption on oxides and aluminosilicates, and precipitate as Zn oxide, hydroxide, and hydrocarbonate. Since leachates from 0 and 1 mo-old NVS had relatively high pH (13 and 12.39, respectively) Zn cannot be detected. More Zn can be detected at lower pH. Relatively higher mobility of Zn in leachates of 6 and 12 mo-old NVS possibly hatched more SCN eggs since zinc is a natural hatching stimulus for SCN.

All three different concentration of ammonia (0.001, 0.01 and 0.1 M) did not have any effect on egg hatch of SCN compared to distilled water and 0.02 M phosphate buffer, although 3 mM zinc sulfate significantly increased egg hatch at 24 hr. Ammonia solutions at 0.001, 0.01 and 0.1 M caused an equal suppression of egg hatch compared to distilled water and 0.02 M phosphate buffer at 48 hr albeit 3 mM zinc sulfate solution significantly induced egg hatch of SCN at 48 hr.

At 72 hr, ammonia at 0.1 and 0.01 M concentrations caused a greater suppression of egg hatch than at 0.001 M concentration compared to distilled water and 0.02 M phosphate buffer although 3 mM zinc sulfate significantly stimulated egg hatch of SCN at 72 hr.

All the three concentrations of ammonia were found to be suppressive to SCN eggs at 48 and 72 hr in vitro. Toxicity of anhydrous ammonia to microorganisms and animals has been reported (Warren, 1962). In a factorial experiment, Lehman et al. (1971) used cations NH4\(^+\), Ca\(^{++}\), and Mg\(^{++}\) in combination with each of the anions NO3\(^-\), SO4\(^{2-}\), and Cl\(^-\) to evaluate the effects of those inorganic ions on egg hatch of SCN, H. glycines. In that experiment, NH4\(^+\) or NO3\(^-\) containing compounds significantly inhibited egg hatch compared to phosphate buffer. Inhibition of egg hatch of SCN in three
different ammonia concentrations in our in vitro experiments agrees with results of Lehman et al. (1971).

**LITERATURE CITED**


BIBLIOGRAPHY


11. Behm, J. E., Tylka, G. L.,


APPENDIX
A. Chemical Characteristics of N-Viro Soil

<table>
<thead>
<tr>
<th>Element</th>
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Table A.1 Average concentrations of macro elements in N-Viro soil (Yamakawa, 1999).

<table>
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<tr>
<th>Element</th>
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<tr>
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Table A.2 Average concentrations of trace elements in N-Viro soil (Yamakawa, 1999)