COMBINATIONS OF ANHYDROUS AMMONIA AND ETHYLENE DIBROMIDE FOR CONTROL OF NEMATODES PARASITIC OF SOYBEANS

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ABSTRACT

Rodríguez-Kábana, R., P. S. King, and M. H. Pope. 1981. Combinations of anhydrous ammonia and ethylene dibromide for control of nematodes parasitic of soybeans. Nematropica 11: 27-41.

In a greenhouse test NH_3 was applied to a sandy loam soil infested with *Tylenchorhynchus claytoni* Steiner, 1937, *Helicotylenchus dihystera* (Cobb) Sher, *Pratylenchus brachyurus* (Godfrey) Goodey and *Hoplolaimus galeatus* (Cobb) Sher at rates of 0, 7.8, 15.6, 31.2, 62.5, and 125.0 mg N/500 gm soil. NH_3 applications reduced soil populations of *T. claytoni* and *H. dihystera* at the three highest rates. Numbers of *P. brachyurus* in Ransom soybean roots were not reduced by any NH_3 treatment. Root populations of *H. dihystera* and *H. galeatus* were reduced only with the 125 mg rate. Two field experiments demonstrated that planting-time applications of anhydrous ammonia at rates ranging from 0-224 kg N/ha were relatively ineffective in reducing late season larval numbers of *Meloidogyne arenaria* (Neal) Chitwood although significant yield increases were obtained in response to the treatments in one experiment. Results from a third field experiment demonstrated that at plant applications of anhydrous ammonia at rates of 56 and 112 Kg/ha reduced numbers of larvae of *Heterodera glycines* (race 3) Ichinohe in soil samples collected 14 days after planting. Planting-time applications of ethylene dibromide (4.7-18.6 L/ha) combined with anhydrous ammonia (56 and 112 Kg N/ha) resulted in yield increases and a degree of control of *M. arenaria* and *H. glycines* superior to results obtained when each chemical was applied singly.
Additional key words: nitrogenous amendments, biological control, soybean cyst nematode, root-knot nematodes, Glycine max.

RESUMEN

Rodríguez-Kábana, R., P. S. King, y M. H. Pope 1981. Combinaciones de amoniaco anhidro con bibromuro de etileno para el combate de nematodos parásitos de la soya. Nematropica 11: 27-41.

En un experimento de invernadero se fumigó con NH_3 un suelo limo arenoso infestado con *Tylenchorhynchus claytoni* Steiner, *Helicotylenchus dihystera* (Cobb) Sher, *Pratylenchus brachyurus* (Godfrey) Goodey y *Hoplolaimus galeatus* (Cobb) Sher con dosis de 0, 7.8, 15.6, 31.2, 62.5, 6 125 mg N/500 gm de suelo. Los tres tratamientos más concentrados de NH_3 redujeron las poblaciones del suelo de *T. claytoni* y *H. dihystera.* El número de *P. brachyurus* en las raíces de soya (cv = Ransom) no fue afectado por ninguna de las dosis de NH_3 pero los de *H. dihystera* y *H. galeatus* si fueron reducidos con la dosis de 125 mg. Los resultados de dos experimentos de campo con soya demonstraron que las inyecciones al suelo durante la siembra de amoniaco anhidro a niveles de 0-224 kg N/ha fueron relativamente ineficaces para reducir el número de larvas de *Meloidogyne arenaria* (Neal) Chitwood en muestras de suelo tomadas cerca del tiempo de cosecha, aunque se registraron aumentos significativos en rendimientos de soya en uno de los experimentos. Los resultados de un tercer experimento de campo señalaron que las aplicaciones de amoniaco anhidro a niveles de 56 y 112 Kg N/ha redujeron los números de larvas de *Heterodera glycines* (raza 3) Ichinohe en muestras de suelo tomadas 14 días después de la siembra. Las aplicaciones durante la siembra de combinaciones de bibromuro de etileno (4.7-18.6 L/ha) con amoniaco anhidro (56 y 112 Kg N/ha) dieron aumentos de rendimientos y grados de combate de *M. arenaria* y *H. glycines* superiores a los obtenidos cuando se utilizaron esos materiales individualmente.

Palabras claves adicionales: enmiendas nitrogenadas, combate biológico, nematodo enquistador, nematodos noduladores, Glycine max.

INTRODUCTION

The addition of organic manures to soil has often been reported to reduce numbers of plant parasitic nematodes (5, 6, 7, 9, 11, 12, 13, 14, 15, 19, 20, 23). Most efficacious of these amendments have been those with low C/N ratios which result in the release of NH_3 into the soil (11, 12, 13, 15, 19, 20, 23). In spite of repeated successes in controlling nematodes with these manures under laboratory or greenhouse conditions, their use under field conditions has not been practical. The difficulty in translating this knowledge to the field lies in the large volume of amendment (10-20 MT/ha) necessary to attain effective kill of nematodes. In addition, organic manures are difficult to standardize so that oftentimes results cannot be repeated. Mineral fertilizers that generate NH_3 on addition to soil have also been reported effective against nematodes (1, 3, 20, 22).

Recently, we presented evidence that high levels of urea combined with a source of easily oxidizable organic carbon (molasses) could be used to control nematodes in soil (16). The addition of urea to soil resulted in release of NH_3 and formation of nitrate nitrogen. The levels of urea required for nematode kill resulted in accumulation of NH_4^+-N and NO_3^--N to levels that were phytotoxic to the test plant (squash). Mixing urea with oxidizable organic carbon removed the phytotoxic effect while retaining the nematicidal activity of the amendment. Apparently, the active nematicidal agent in these amendments is NH_3, thus it is not suprising that anhydrous ammonia applied to soil has been reported to be nematicidal. As early as 1955, Eno *et al* (3) reported that under Florida conditions anhydrous ammonia afforded some control of ectoparasitic nematodes. Later Vassalo (22) and Birchfield *et al* (1) reported on the nematicidal activity of NH_3. In spite of these studies there has been no comprehensive evaluation of the nematicidal activity of anhydrous ammonia under field conditions. Because of its relatively low cost in comparison with standard nematicides, we decided to determine whether anhydrous ammonia could be used either alone or in combination with standard nematicides for control of nematodes on soybean.

MATERIALS AND METHODS

a. *Greenhouse Experiment.* A greenhouse experiment was conducted with Ransom soybeans to determine the effect of NH_3 on plant parasitic nematodes. A silt loam soil was collected in a cotton field near Tallassee, Alabama, infested with *Helicotylenchus dihystera* (Cobb) Sher, *Hoplolaimus galeatus* (Cobb) *Sher, Pratylenchus brachyurus* (Godfrey) Godey and *Tylenchorhynchus claytoni* Steiner. The soil was passed through a 2-mm mesh sieve to remove crop debris and large soil particles and was then apportioned in 500 gm amounts. Each amount was placed into a 7.6 cm diam cylinder made from PVC pipe that was 17.5 cm long (Fig. 1, A). A 1-mm mesh fiberglass screen overlaid with a disc of Whatman No. 1 filter paper prevented the soil from falling through the cylinder. The cylinder was glued to a second cylinder 4.5 cm long (Fig. 1, B), and the whole structure was glued with silicone rubber to a square, wide mouth (5.5 cm diam) 900 ml capacity glass jar (Fig. 1, C). The jar contained 10 ml of water or the appropriate aqueous solution of $(NH_4)_2SO_4$. After placement of the soil in A of the apparatus, 10 ml of 3N NaOH were delivered into the jar with a curved syringe needle through a 2 mm hole in B which was covered with silicone rubber. The addition of NaOH resulted in the release of NH_3 from the $(NH_4)_2SO_4$ solution and difussion of the gas through the soil column. The ammonium sulfate solutions were prepared to deliver: 0, 7.8, 15.6, 31.2, 62.5, or 125 mg of NH_3-N per 500 gm of soil. Five soybean seeds were planted into each cylinder with soil immediately after addition of NaOH. Each concentration of NH_3-N was represented by eight cylinders (replications) arranged in a completely randomized design on a

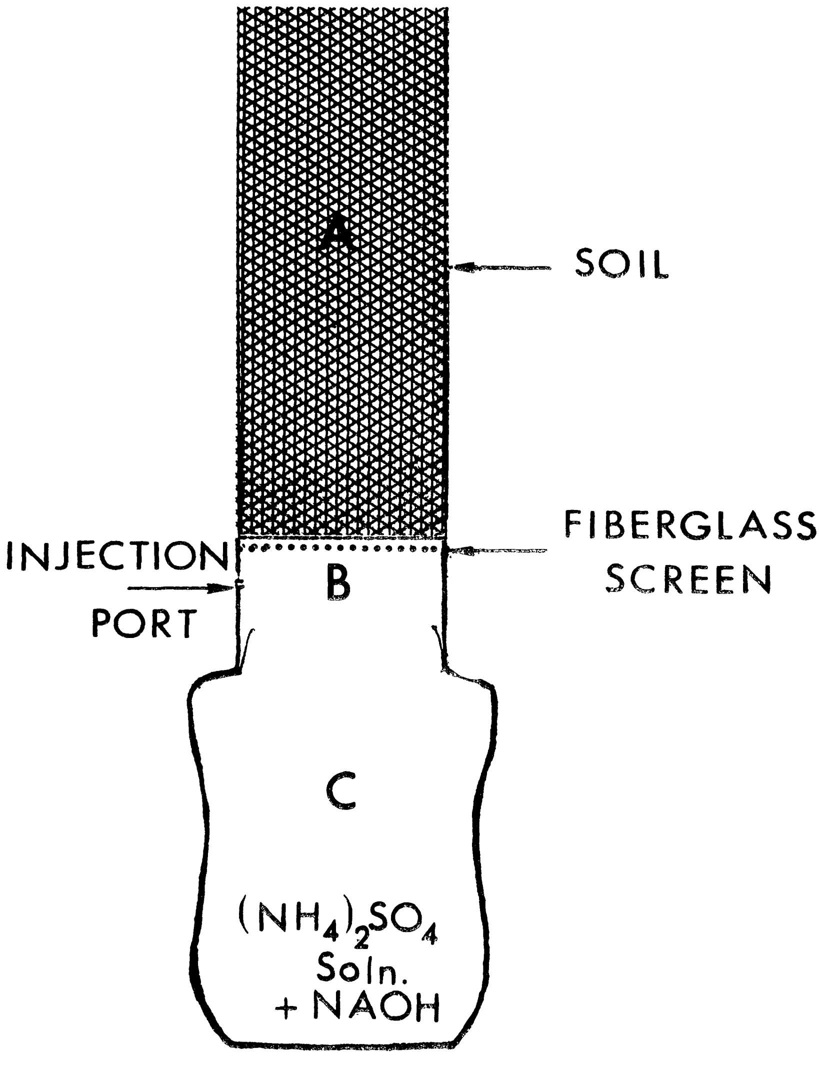

Fig. 1. Apparatus used to treat soil with NH_3 in a greenhouse experiment with Ransom soybeans.

greenhouse bench. Soybeans were allowed to develop for 6 weeks, then soil samples and roots were collected for nematode analysis. Nematodes in the soil were extracted by the molasses flotation sieving technique (17). Nematodes in the roots were extracted using a modified Baerman procedure. The roots were spread evenly over a 15-cm diam PVC sieve with a 1-mm mesh fiberglass screen covered with tissue paper. The sieve with roots was immersed in a bowl with ca. 1 L of water and incubated for 72 hr when the sieve was removed and nematodes in the water were collected by sieving through a 38 um mesh stainless steel screen.

Shoot heights and fresh weights of shoots and roots were also determined. Relative appearances of root systems were estimated using a subjective scale in which 1 represented healthy and vigorously growing roots and 5 poor root development with the roots showing considerable necrosis and blackening. Incidence of *Rhizobium* nodules was also recorded.

b. *Field Experiments.* The effect of anhydrous ammonia on *Meloidogyne arenaria* (Neal) Chitwood was studied in 1979 in a field with a silt loam soil heavily infested (ca. 50 larvae/50 cm^3 soil, at planting time) with the nematode. The field had been in potatoes the preceeding winter and in soybean culture for the past 5 years. NH_3 was applied at planting time at rates of 0, 56, 112, 168, and 224 Kg N/ha. A planting time treatment with Soilbrom 90 (EDB90) at 19 L/ha was included to serve as positive control. NH_3 and Soilbrom 90 were injected into the soil to a depth of 20 cm using two injectors per row, each 10 cm (Soilbrom 90) or 14 cm (NH_3) on each side of the seed furrow. The statistical design was a randomized complete block with seven replications. Plots (replications) were 6.7 M long with two 90 cm wide rows. Cultural practices and control of foliar diseases, insect and weeds were according to recommendations for the area (4). The soybean cultivar used in this experiment was Bragg.

Two experiments were conducted in 1980 to evaluate the efficacy of combinations of NH_3 and Soilbrom 90 for control of nematodes. One experiment with Ransom soybeans was established at the Gulf Coast substation near Fairhope, Alabama, in a field heavily infested with *M. arenaria.* The experiment studied the effect of Soilbrom 90 at 0 and 14 L/ha on the nematicidal activity of anhydrous ammonia applied at 0, 56, 84, and 112 Kg N/ha. Statistical design, size of plots and method and time of application of these materials were as described for the 1979 experiment, except that there were eight replications per treatment.

The other experiment in 1980 was established near Summerdale, Alabama, with Bragg soybeans, in a field with a loamy sand heavily infested with *M. arenaria* and race 3 of *Heterodera glycines* Ichinohe (ca. 30 larvae/100 cm^3 soil at planting time). The experiment studied the effects of three levels of NH_3(0, 56, and 112 Kg N/ha) and four dosages of Soilbrom 90 (0, 4.7, 9.3, 18.6 L/ha). Each treatment was represented by eight replications and the statistical design, method and time of application of materials and practices followed were as described for the other field experiments.

Soil samples for nematode analysis from field experiments were collected just prior to harvest for *M. arenaria*, or two weeks after planting, for *H. glycines*, to coincide with the periods most likely to relate populations to yield

Table 1. Effect of NH_3 on soil populations of plant parasitic nematodes and those in the roots of Ransom soybeams in a greenhouse experiment.

mg NH_3-N per 500 gm soil	Numbers per 50 cm ^{3} soil ^{x}		Nematodes per gram of fresh root^x		
	Tylenchorhynchus claytoni	*Helicotylenchus dihystera*	*Pratylenchus brachyurus*	*Helicotylenchus dihystera*	*Hoplolaimus galeatus*
0.0	89.0 A	44.0 A	14.7 A	46.4 A	48.5 A
7.8	95.1 A	48.7 A	31.7 B	55.2 A	59.2 A
15.6	108.2 A	52.5 A	29.2 B	69.2 A	77.8 B
31.2	50.2 B	29.8 B	22.8 AB	53.2 A	51.4 A
62.5	29.1 B	21.2 B	20.7 AB	55.4 A	57.8 A
125.0	1.0 C	0.5 C	5.4 A	4.4 B	6.7 C

x/ Figures for variables represent the averages of eight replications; those within the same column with common letters were not statistically different (P = 0.05).

losses (*unpublished data*). Each sample consisted of soil cores 2.5 cm in diam obtained from the root zone to a depth of 15-20 cm along the center of each plot to provide a total of 15-18 cores per plot. The cores from each plot were composited and a 100 cm^3 subsample was used to extract nematodes with the modified Baerman method described earlier in this paper.

Statistical analyses. All data were analyzed following standard procedures for analysis of variance (21). When applicable, the data were treated as for factorial analysis. Differences between means were evaluated for significance following a modified Duncan's multiple range test. Correlation analyses were also performed according to standard techniques. Unless otherwise specified, all differences in the text were significant to the 5% or lower level of probability.

RESULTS

a. *Greenhouse experiment.* Addition of NH_3 reduced soil populations of *T. claytoni* and *H. dihystera* at the three highest rates (Table 1). Numbers of *P. brachyurus* and *H. galeatus* in the soil were too low to detect significant changes. Numbers of *P. brachyurus* in the roots were higher in plants that had received the 7.8 or 15.6 mg N treatments than in roots of control plants; differences between other NH_3 treatments and the control were not significant. Numbers of *H. dihystera* in the root did not vary with nitrogen concentrations below the 125 mg level but were reduced by this dosage. Root populations of *H. galeatus* were lowest in plants from soils that received the 125 mg rate.

Shoot height was increased by the addition of 15.6 mg N to the soil but was reduced by the 125 mg treatment (Table 2). However, no significant effect was observed for fresh shoot weight in response to any of the NH_3 treatments. Fresh root weight and the root condition index indicated restricted root development in soils that received the two highest NH_3 levels. Roots in NH_3-treated and control soils showed similar levels of *Rhizobium* nodulation.

b. *Field Experiments.* Application of NH_3 at planting time in the 1979 experiment resulted in significant increases in larval populations in response to rates above 56 Kg N/ha (Table 3); larval populations in plots that received 56 Kg N/ha were not significantly different from those in control plots. All NH_3 treatments resulted in significant increases in yield. Yield differences between plots receiving NH_3 were not significant. Highest yields of the experiment corresponded to the EDB 90 treatment; however, the average yield for this treatment was not significantly different from those obtained with the 56, 112, or the 168 Kg N/ha treatments. The EDB 90 treatment did not significantly reduce larval populations below the numbers found in control plots. Larval populations in EDB-treated plots were lower than those in plots that received NH_3.

Table 1. Effect of NH_3 on soil populations of plant parasitic nematodes and those in the roots of Ransom soybeams in a greenhouse experiment.

mg NH_3-N per 500 gm soil	Numbers per 50 cm^3 soil ^{x}		Nematodes per gram of fresh root ^{x}		
	Tylenchorhynchus claytoni	*Helicotylenchus dihystera*	*Pratylenchus brachyurus*	*Helicotylenchus dihystera*	*Hoplolaimus galeatus*
0.0	89.0 A	44.0 A	14.7 A	46.4 A	48.5 A
7.8	95.1 A	48.7 A	31.7 B	55.2 A	59.2 A
15.6	108.2 A	52.5 A	29.2 B	69.2 A	77.8 B
31.2	50.2 B	29.8 B	22.8 AB	53.2 A	51.4 A
62.5	29.1 B	21.2 B	20.7 AB	55.4 A	57.8 A
125.0	1.0 C	0.5 C	5.4 A	4.4 B	6.7 C

x/ Figures for variables represent the averages of eight replications; those within the same column with common letters were not statistically different ($P = 0.05$).

Table 3. Effect of anhydrous ammonia applied at planting on populations of *Meloidogyne arenaria* and yield of Bragg soybeans in a field experiment at Fairhope, Alabama in 1979

NH_3-N (Kgs/ha)	Larvae per 100 cm^3 soil	Yield (Kgs/ha)
Control	96 AB	1139 A
56	233 BC	1668 BC
112	313 C	1709 BC
168	260 C	1709 BC
224	260 C	1546 B
EDB 90y/ (Soilbrom® 90) 19L/ha	37 A	1790 C

x/ Figures for variables represent averages of seven plots (replications); those within the same column followed by a common letter were not statistically different (P = 0.05).
y/ Applied at planting time to serve as positive control.

Data from the Fairhope experiment in 1980 are presented in Table 4. Application of NH_3 alone did not affect yields or larval populations of *M. arenaria.* However, a significant interaction was observed between NH_3 and EDB 90 for yield. The use of EDB 90 alone resulted in a significant increase in yield, however, additional yield increases were obtained by combining EDB with NH_3. Within these combinations, the dosage of NH_3 apparently did not affect yield since the lowest dosage of NH_3 combined with EDB 90 resulted in as high a yield as the other two NH_3 levels combined with EDB 90. All plots that received EDB 90 contained lower larval populations that those not treated with the fumigant. Differences in numbers of larvae between EDB 90 treatments were not significant.

Application of anhydrous ammonia reduced larval populations of *H. glycines* in the Summerdale test (Table 5). The reduction was only evident in the first sampling 14 days after planting. Numbers of *H. glycines* larvae were lower in the second sampling than on the first so that differences between treatments for the final sampling (just prior to harvest) were generally not

Table 4. Effect of combinations of planting time applications of anhydrous amonia and EDB 90 (Soilbrom® 90) on larval populations of *Meloidogyne arenaria* and yield of Ransom soybeans in a field experiment at Fairhope, Alabama in 1980

	Soilbrom 90 (L/ha)			
	0.0		14.0	
NH_3-N (Kg/ha)	Larvae per 100 cm^3 soil	Yield (Kg/ha)	Larvae per 100 cm^3 soil	Yield (Kg/ha)
0.0	103 A	1505 A	20 A	2197 A
56.0	87 A	1465 A	6 A	2441 B
84.0	120 A	1383 A	24 A	2360 B
112.0	132 A	1587 A	13 A	2319 B

x/ Figures for variables represent averages of eight plots (replications); those within the same column followed by a common letter were not statistically different (P = 0.05).

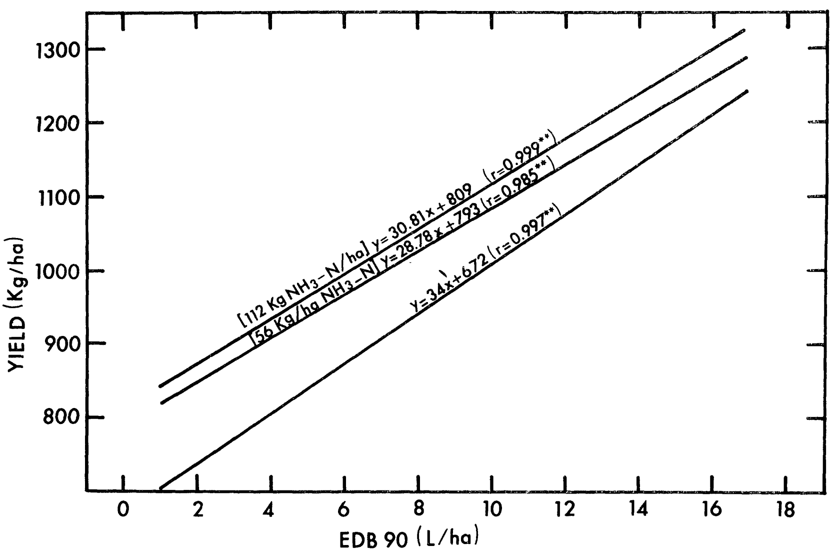

Fig. 2. Effect of three levels of anhydrous ammonia applied at planting time on the linear regression between soybean yields and at plant applications of ethylene dibromide (Soilbrom®90).

Table 5. Effect of combinations of planting time applications of anhydrous ammonia and Soilbrom®90 (EDB 90) on yield of Bragg soybeans and on larval populations (100 cm³ soil) of *Meloidogyne arenaria* and of *Heterodera glycines* (race 3) in a field experiment near Summerdale, Alabama, in 1980

	Anhydrous Amonia (Kg N/ha)											
	0.0				56.0				112.0			
Solibrom 90 (L/ha)	*M. arenaria*	*H. glycines* *I*y	*H. glycines* *II*	Yield (Kg/ha)	*M. arenaria*	*H. glycines* *I*	*H. glycines* *II*	Yield (Kg/ha)	*M. arenaria*	*H. glycines* *I*	*H. glycines* *II*	Yield (Kg/ha)
0.0	18 A	42 A	10 A	686 A	13 A	23 A	8 A	834 A	30 A	3 A	11 A	809 A
4.7	17 A	24 A	12 A	803 B	6 AB	19 AB	14 A	875 A	6 B	19 B	10 A	956 B
9.3	2 B	34 A	6 A	997 C	1 B	18 AB	7 A	1058 B	1 B	7 AB	22 B	1093 C
18.6	0 B	25 A	16 A	1297 D	0 B	7 B	10 A	1343 C	0 B	4 A	6 A	1383 C

x/ Figures for variables represent averages of eight replications (plots); those within the same column followed by a common letter were not statistically different (P = 0.05).

y/ I refers to the first sampling 14 days after planting and II to the final sampling just prior to harvest.

significant. Larval populations of *M. arenaria* were very low in soils from the first sampling (data not shown) but increased significantly by the second sampling. NH_3 had no effect on numbers of *M. arenaria* larvae. EDB 90 alone at dosages above 4.7 L/ha consistently reduced larval populations of *M. arenaria.* The interaction between NH_3 and EDB 90 was not significant for larval populations of *M. arenaria*; however, the interaction for larvae of *H. glycines* was significant. Use of NH_3 resulted in significant increases in yield but the differences in yield between the two levels of NH_3 were not significant. Aplication of EDB 90 resulted in a significant, positive linear response (r = 0.997**) between yield and EDB 90 dosage (Fig. 2). The interaction between NH_3 and EDB 90 for yield was not significant.

DISCUSSION

Results from the greenhouse study show that NH_3 is nematicidal against some plant parasitic nematode species in soil at concentrations of 31.2 mg NH_3-N/500 gm soil or higher. Also, the effect of NH_3 on endoparasitic nematodes is not pronounced since only the highest dosage studied resulted in any significant decrease in numbers of two of these species and populations of *P. brachyurus* were not reduced by NH_3 at any concentration. On a broadcast basis, field rate equivalents for the dosages used in the greenhouse experiment ranged from 35 kg N/ha for the 7.8 mg dose to 559 kg N/ha for the 125 mg treatment. This indicates that the effectiveness of NH_3 against nematodes is limited to very exaggerated rates. In this respect our results are well in accordance with those obtained by Eno *et al* (3) in Florida soils. These workers concluded that it was necessary to have dosages of 300-600 ppm of N in the soil before consistent nematicidal activity could be attained with anhydrous ammonia. In our greenhouse study only the highest dosage falls within this range. However, with soybeans our results also show that such high concentrations of NH_3 at planting time are detrimental for root development.

Results from field experiments show that considerable yield increases can be obtained with planting time applications of anhydrous ammonia at rates lower than 200 Kg N/ha. At these rates NH_3 is not particularly effective in reducing populations of *M. arenaria* but is very effective, at least early in the season, against *H. glycines.* The nematicidal activity of NH_3 in the field appears to be short-lived. Its mode of action may be as suggested by Eno *et al* (3), limited to the retention zone of the chemical when applied to soil. This suggests that for some time after application the developing soybean roots grow into a zone relatively free of nematodes but that later they grow into areas where the nematicidal effect of NH_3, because of its poor diffusion characteristics, is negligible. This explains the lack of reduction in numbers of larvae of *M. arenaria* and *H. glycines* in response to NH_3 applications observed in late season soil samples. Treatment of soil with a short-lived nematicide active early in the season can be expected to result in higher numbers of plant parasitic nematodes in samples taken late in the season. This can occur through increased availability of feeding sites in the treated plots and the destruction of root systems by the nematodes early in the season in untreated plots. Our results for *M. arenaria* agree somewhat with this interpretation. Lower numbers of *H. glycines* in late season samples when compared to those in samples taken 14 days after planting probably reflect poor development of the nematode when in competition with *M. arenaria* which does not occur in significant numbers until later in the season. There is also the possibility that as NH_3 is converted to nitrates in the soil it may have some nutritional value for soybeans. Although NH_3 did not affect *Rhizobium* nodulation it could have been of value to the plants early in the season before a significant degree of N-fixation took place.

Our field results indicate good compatibility of ethylene dibromide and anhydrous ammonia for simultaneous planting-time applications in soybeans. EDB was particularly effective against larvae of *M. arenaria* which agrees with previous reports (8, 18); however, in contrast to NH_3, EDB was not very effective in reducing larval populations of *H. glycines* in the early season samples. This difference in nematicidal activity perhaps explains why combined applications on NH_3 and EDB resulted in higher yields than those obtained when these materials were applied singly. The data suggest that it may be possible to optimize NH_3-EDB mixtures to obtain maximal yield and nematode control at lower cost than when EDB is used as the sole nematicide.

Although we believe the primary mode of action of NH_3 is based on its plasmolysing effect against nematodes (22), the data obtained for *H. glycines* suggest that there may be other mechanisms involved. NH_3 was effective against this nematode at rates lower than the rates necessary for effectiveness against other nematode species in the greenhouse study. It is possible that NH_3 could have exerted a selective influence for microbial antagonists of *H. glycines*, e.g., fungi. Several species of fungi have been reported parasites of *Heterodera* spp. (10). It is possible that since NH_4^+-N is the preferred source of N for many soil fungi (2) some fungal parasites of *H. glycines* could have increased in numbers following application of NH_3 to soil. Proliferation of such fungal parasites in turn could have resulted in low numbers of *H. glycines* larvae. This possibility is currently being explored in our laboratory.

CONCLUSIONS

1. Our results indicate that anhydrous ammonia is a weak nematicide at rates below 200 kg N/ha.

2. NH_3 is particularly effective against *H. glycines* in planting-time applications and, when combined with EDB, it can result in higher soybean yields and better nematode control than when each chemical is applied singly.

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