COMBINATIONS OF ANHYDROUS AMMONIA AND 1,3-DICHLOROPROPENES FOR CONTROL OF ROOT-KNOT NEMATODES IN SOYBEAN.

R. Rodríguez-Kábana, R. A. Shelby, P. S. King, and M. H. Pope
Department of Botany, Plant Pathology and Microbiology, Auburn University, Agricultural Experiment Station, Auburn University, Alabama 36849, U.S.A.

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ABSTRACT

Rodríguez-Kábana, R., R. A. Shelby, P. S. King, and M. H. Pope. 1982. Combinations of anhydrous ammonia and 1,3-dichloropropenes for control of root-knot nematodes in soybean. Nematropica 12: 61-69.

The efficacy of combination planting time treatments of anhydrous ammonia (NH_3) and 1,3-dichloropropenes (1,3-D) against species of *Meloidogyne* Goeldi was studied in two field experiments with Ransom soybean [*Glycine* max (L) Merr.]. One experiment was in a field infested with *M. incognita* (Kofoid & White) Chitwood and the other in a field with *M. arenaria* (Neal) Chitwood. NH_3 was applied at 0, 56, and 112 kg N/ha alone and in combination with the Telone® II formulation of 1,3-D at rates of 0, 18.7, 37.4, and 56.1 l/ha. NH_3 alone failed to reduce soil larval populations of *M. incognita* in soil but reduced numbers of *M. arenaria* when applied at the lowest rate. Factorial analysis of the larval data indicated no significant interaction between NH_3 and Telone II treatments. A significant reduction in larval numbers in response to Telone II applications was detected for *M. incognita* when the data were considered independently of the effect of NH_3; the effect of Telone II on *M. arenaria* larvae was not so definite. Applications of NH_3 alone did not result in increased yields in both tests; however, all combination treatments except one (NH_3 at 56 kg N/ha + Telone II at 18.7 l/ha) increased yields in the *M. incognita* experiment as did treatment with Telone II alone at rates above 18.7 l/ha. In the experiment with *M. arenaria* the only treatments that resulted in increased yields were those with Telone II at 56.1 l/ha alone and in combination with 112 kg N/ha of NH_3. Analysis of the yield data from the two experiments indicated that the effects of NH_3 and Telone II on the variable were synergistic.
Additional key words: chemical control, fertilizers, pest management, pesticide interactions, plant nutrition, oil crops.

RESUMEN

Rodríguez-Kábana, R., R. A. Shelby, P. S. King, y M. H. Pope. 1982. Uso the combinaciones de amoniaco anhidro y 1,3-dicloropropenos para combatir nematodos noduladores en soya. Nematropica 12: 61-69.

Se estudió la eficacia de tratamientos combinados de amoniaco anhidro (NH_3) con 1,3-dicloropropeno (1,3-D) para combatir nematodos noduladores (*Meloidogyne* Goeldi) en dos experimentos de campo con soya [*Glycine max* (L.) Merr.] cv. Ransom. Uno de los experimentos se efectuó en un campo infestado con *M. incognita* (Kofoid & White) Chitwood y el otro en un campo con *M. arenaria* (Neal) Chitwood. NH_3 se aplicó en dosis de 0, 56 y 112 kg N/ha sólo y en combinación con la formulación Telone® II de 1,3-D en dosis de 0, 18.7, 37.4 y 56.1 l/ha. Los tratamientos con NH_3 sólo no redujeron las poblaciones de larvas de *M. incognita* en el suelo, pero si las de *M. arenaria* en las parcelas que recibieron la dosis más baja del material. Un análisis factorial de lo datos sobre larvas demostró que no hubo interacción significativa entre los tratamientos de NH_3 y los de Telone II. El Telone II redujo las poblaciones de larvas de *M. incognita* cuando los datos se consideraron independientemente del efecto de NH_3 sobre la variable; el efecto del nematicida sobre larvas de *M. arenaria* no fué tan definido. Las aplicaciones con NH_3 sólo no dieron aumentos en rendimientos en ambos experimentos. Todos los tratamientos combinados con excepción de uno (NH_3 a 56 kg N/ha + Telone II a 18.7 l/ha) asi como los con Telone II sólo con las dos dosis más altas resultaron en aumentos significativos en producción en el experimento con *M. incognita*. Los únicos tratamientos que dieron aumentos en rendimientos en el experimento con *M. arenaria* fueron aquellos con Telone II a 56.1 l/ha sólo o combinado con 112 kg N/ha de NH_3. El análisis de los resultados sobre la producción de soya en los dos experimentos señaló que NH_3 y Telone II son sinérgicos sobre la variable.

Palabras claves adicionales: combate químico, fertilizantes, manejo de plagas, interacciones de pesticidas, nutrición vegetal, oleaginosas.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* Goeldi) are among the most important parasitic nematodes of soybean [*Glycine max* (L) Merr.]. Damage caused by these parasites on the crop in the southeastern United States is significant (4,16,17). Current practices for control of root-knot nematodes in soybean are based on the use of resistant (tolerant) cultivars in combinations with nematicide treatments (5,8). The number of nematicides presently available for use on soybeans in the United States is limited. Most effective among these are fumigants containing halogenated hydrocarbons (6,7,13,15). Fumigants with 1,3-dichloropropenes (1,3-D) as the active ingredient have been shown to be effective against root-knot nematodes in soybeans in preplant and planting time applications (6,7,13). Previous studies have shown that the effectiveness of ethylene dibromide (EDB) against *Heterodera glycines* Ichinohe and *M. arenaria* (Neal) Chitwood on soybean can be enhanced when its use is combined with applications of anhydrous ammonia (NH_3) to the soil (12). Since a similar effect could occur with other halogenated hydrocarbons we conducted a study to determine the relative effectiveness of NH_3 and 1,3-D mixtures against *M. incognita* (Kofoid & White) Chitwood and *M. arenaria* on soybean. This paper presents results obtained from field experiments in the study.

MATERIALS AND METHODS

The effect of treatments with anhydrous ammonia (NH_3) in combination with applications of Telone II (1,3-D) for control of root-knot nematodes on Ransom soybeans was studied in two field experiments in southern Alabama. One experiment was established in a field infested with *M. arenaria* which had been with soybean and hairy vetch (*Vicia villosa* Roth) in winter for the preceeding six years. The soil was a sandy loam with a pH of 6.2 and organic matter content of less than 1% (w/w). The other experiment was conducted in a field near Elberta infested with *M. incognita*. The field had been with soybean and winter fallow in the previous three years. Soil in this field was also a sandy loam with pH of 6.0 and organic matter content of less than 1% (w/w).

In each experiment NH_3 and Telone II were applied at planting time. NH_3 was delivered to a depth of 30 cm using a single standard injector per row offset 10 cm from the seed furrow. NH_3 was applied at rates of 56 and 112 kg N/ha. Telone II was applied to a depth of 20 cm using two injectors per row set 20 cm apart with the seed furrow in the middle. Telone II was applied at 18.7, 37.4 and 56.1 l/ha. In addition, each experiment contained treatments with NH_3 plus Telone II so as to have every combination of the rates described for each material. Plots receiving no treatment and others receiving 18.7 l/ha of EDB-90 (Soilbrom® 90) applied as described for Telone II were also included in each experiment to serve as controls.

Plots in each experiment were two-row (each 90 cm wide) x six m and there were eight plots (replications) representing each treatment arranged in a randomized complete block design.

Cultural practices and control of foliar diseases, insects and weeds were as recommended for the area (2).

Soil samples for nematode analysis were collected four weeks before harvest to coincide with the period of maximal larval population development of the nematodes (1,3). The samples were taken from the root zone to a depth of 20 cm using a standard 2.5 cm diam soil probe. A total of 16-20 cores were collected from each plot by sampling every meter from both rows along the length of the plot. The cores from each plot were composited and a 100 cm^3 subsample was used to determine nematode numbers with the "salad bowl" technique (14).

The relative subjective appearance of plants in each plot was determined 70 days after planting using an index scale that ranged in value from one to five. A value of one was assigned to plots with chlorotic plants showing stunted unthrifty growth and a value of five to plots with plants showing excellent and vigorous growth.

Yield was determined at maturity of the crop by harvesting the entire area of each plot.

Data were analyzed following standard procedures for analysis of variance (18). Factorial analysis to determine the effects of NH_3 and Telone II were also performed following standard procedures. Differences between means were evaluated for signficance following a modified Duncan's multiple range test (18). Unless otherwise stated differences referred to in the text were significant at the 5% or lower level of probability. Linear correlation analyses between yield data and nematode numbers were performed by the usual methods (18).

RESULTS

Meloidogyne incognita. Results obtained from the experiment with *M. incognita* are presented in Table 1. Highest yield in the experiment was obtained in response to applications of EDB 90. Injection of Telone II alone at rates above 18.7 l/ha resulted in increased yields but treatments with NH_3 alone did not improve yields. When NH_3 applications were combined with injections of Telone II significant yield improvements were obtained for all combinations except one (56 kg N/ha + Telone II at 18.7 l/ha). Factorial analysis of yield data from treatments with NH_3 and Telone II indicated that a significant ($P = 0.01$) interaction resulted from the use of NH_3 and Telone II suggesting that yield responses could not be attributed solely to the effect of NH_3 or Telone II. Thus, two combination treatments (NH_3 at 112 kg N/ha + Telone II at 18.7 l/ha, and NH_3 at 56 kg N/ha + Telone II at 56.1 l/ha) resulted in significantly higher yields than those resulting from treatments with NH_3 or Telone II alone at equivalent rates. The effects of NH_3 applications on yield were significant when the data were considered independently of the effects of Telone II; the converse consideration indicated that the effect of Telone II was also significant.

The only two treatments that significantly reduced the number of larvae were EDB 90 and treatments with NH_3 at 56 kg N/ha combined with Telone II at 56.1 l/ha. Factorial analysis of the data on larval populations evidenced no significant interaction between NH_3 and Telone II. The analysis also showed that injections of Telone II reduced larval numbers when the data were considered without the effect of NH_3; however, NH_3 had no effect on larval populations when results were considered without the action of Telone II on the larvae. Linear correlation analysis of the data on yield and larval numbers showed that the two variables were significantly ($P = 0.01$) correlated ($r = -0.781$).

Applications of EDB 90, and of Telone II alone at all rates, improved values for subjective appearance; however, treatments with NH_3 alone did not improve appearance. All combination treatments resulted in significant improvements in appearance when compared with that of control plots. Factorial analysis of the data for subjective appearance showed no significant interaction between NH_3 and Telone II, or any effect of NH_3 on index values when the data were considered without the effect of Telone II on the variable. The analysis revealed that applications of Telone II significantly improved subjective appearance of soybeans.

Meloidogyne arenaria. Data from the experiment with *M. arenaria* are presented in Table 2. Treatment with EDB 90, and application of Telone II

Table 1. Effect of at plant applications of anhydrous ammonia (NH_3) and Telone II (1,3-D) on *Meloidogyne incognita* and yield of Ransom soybeans in a field experiment near Elberta, Alabama.

NH_3 (Kg N/ha)	Telone II (l/ha)	Subjective[x] Appearance Index	Larvae per 100 cm^3 Soil	Yield (kg/ha)
0	0.0	3.9 C[y]	200 ABC	799 H
56	0.0	3.9 C	233 AB	785 H
112	0.0	4.1 BC	263 A	927 FGH
56	18.7	4.5 AB	151 ABCD	924 GH
112	18.7	4.6 A	152 ABCD	1119 EFG
56	37.4	4.5 AB	169 ABCD	1310 CDE
112	37.4	4.7 A	110 CDE	1241 DE
56	56.1	4.6 A	61 DE	1778 B
112	56.1	4.7 A	170 ABCD	1513 C
0	18.7	4.5 AB	140 BCDE	785 H
0	37.4	4.7 A	136 BCDE	1143 EF
0	56.1	4.5 AB	125 BCDE	1424 CD
EDB 90 (1/ha)	18.7	4.9 A	31 E	2815 A

[x]The subjective appearance index was based on a scale of 1-5 where a value of 1 represented plots with unthrifty chlorotic plants 70 days after planting and a value of 5 was ascribed to plots with plants showing excellent growth and no stunting.

[y]Means within the same column followed by a common letter were not statistically different ($P = 0.05$). Means are the averages of eight replications.

alone at 56.1 l/ha resulted in improved yields; the use of NH_3 alone did not improve yields. When NH_3 was applied at 112 kg N/ha in combination with 56.1 l/ha of Telone II a significant increase in yield was obtained but other combination treatments did not improve yields over the control. Factorial analysis of the yield data revealed no significant interaction between NH_3 and Telone II applications. Further, the analysis indicated that while the general effect of Telone II on yield was a significant improvement in yield, that of NH_3 was not significant.

Treatments with NH_3 alone or Telone alone at the lowest rates for each material resulted in significant reductions in larval numbers; all other simple treatments failed to reduce larval populations. The only combination treatments that reduced larval populations were the two NH_3 treatments with

Table 2. Effect of at plant applications of anhydrous ammonia (NH_3) and Telone II (1,3-D) on *Meloidogyne arenaria* and yield of Ransom soybeans in a field experiment near Fairhope, Alabama.

NH_3 (Kg N/ha)	Telone II (l/ha)	Subjective[x] Appearance Index	Larvae per 100 cm^3 Soil	Yield (kg/ha)
0	0.0	4.0 BCD[y]	287 AB	1056 DE
56	0.0	4.0 BCD	195 CDE	1074 DE
112	0.0	4.3 ABCD	239 ABCDE	1318 CDE
56	18.7	4.0 BCD	172 DE	1237 CDE
112	18.7	3.7 D	154 E	1261 CDE
56	37.4	4.6 AB	253 ABCD	1391 BCD
112	37.4	4.4 ABC	270 ABC	1412 BCD
56	56.1	4.6 AB	206 ABCDE	1391 BCD
112	56.1	4.6 AB	215 ABCDE	1741 AB
0	18.7	3.9 CD	194 CDE	993 E
0	37.4	4.6 AB	202 BCDE	1371 CDE
0	56.1	4.9 A	293 A	1493 BC
EDB 90 (l/ha)	18.7	4.9 A	202 BCDE	1953 A

[x]The subjective appearance index was based on a scale of 1-5 where a value of 1 represented plots with unthrifty chlorotic plants 70 days after planting and a value of 5 was ascribed to plots with plants showing excellent growth and no stunting.

[y]Means within the same column followed by a common letter were not statistically different ($P = 0.05$). Means are the average of eight replications.

Telone II at 18.7 l/ha. Factorial analysis of the data on larvae showed no significant interaction between Telone II and NH_3 applications. The analysis also indicated that NH_3 had no effect on larval numbers; however, a significant reduction in numbers of larvae was evident in response to Telone II applications at rates of 18.7 l/ha although higher rates of the fumigant did not reduce larval populations. The correlation coefficient for yield and number of larvae was not significant.

The only two treatments that resulted in significant improvements in subjective appearance were the one with EDB 90 and the highest rate of Telone II applied alone. Factorial analysis of the data on subjective appearance revealed no significant interaction between NH_3 and Telone II. The analysis indicated no significant effect of NH_3 on subjective appearance; however, when the effect of Telone II on subjective appearance was considered independently of that from NH_3 significant improvements in subjective appearance were evidenced in response to the two highest rates of Telone II.

DISCUSSION

Results from this study corroborate earlier findings on the activity of NH_3 against nematodes. Previous greenhouse and field experiments showed that NH_3 is a weak nematicide against *Meloidogyne* spp (12); dosages of 200 kg N/ha or higher were required before consistent reductions in larval populations of these nematodes could be obtained.

Our results suggest that when NH_3 and 1,3-D are used in combination treatments a synergistic effect occurs resulting in improved control of *Meloidogyne* spp and yield response over what is obtained with the use of 1,3-D alone. Other studies (12) have shown a similar synergistic effect for another halogenated hydrocarbon, ethylene dibromide (EDB). In soybean experiments, performance of EDB against *H. glycines* and *M. arenaria* significantly improved when the nematicide was applied in combinations with NH_3.

The data from the present study do not permit us to determine the mode of action of NH_3 with 1,3-D against nematodes. At present, two interpretations appear plausible: one based on a direct nutritional effect on soybeans, and the other possibly related to enhancement by the treatments of mycoflora antagonistic to nematodes. Application of NH_3 at planting time may supply N to the developing soybean plant during the first weeks of growth before significant N fixation from *Rhizobium* nodules occurs; previous work has shown that NH_3 applied to soybeans at the rates used in the present study did not inhibit development of *Rhizobium* nodules (12). It is also possible that the use of ammoniacal N in combination with halogenated hydrocarbons may stimulate fungal parasites of *H. glycines* and *Meloidogyne* spp. resulting in increased effectiveness of these nematicides. A number of fungal species capable of parasitizing eggs of *H. glycines* and of *Meloidogyne* spp have been isolated from Alabama fields in the vicinity of the test areas (9,10,11).

Our results showed a significant difference between responses obtained with *M. incognita* and with *M. arenaria* to the treatments of the study. Combinations of NH_3 and 1,3-D were generally more effective in improving yields in the field with *M. incognita* than with *M. arenaria.* It is possible that this difference in response may be due to the separate locations of the experiments. However, we believe that the damage caused by *M. incognita* on Ransom soybeans is not as severe as that caused by *M. arenaria.* If this is so, significant reductions in larval populations of *M. incognita* can be expected to result more consistently in yield increases; equivalent reductions in larval populations of *M. arenaria* may result in no significant yield increases or in more limited yield responses. We have no equations available at present to relate soybean yield losses to numbers of *M. incognita* larvae as we reported for *M. arenaria* (16), consequently our interpretation must remain speculative. However, our data show that four treatments in the *M. arenaria* experiment resulted in significant reductions in larval populations but no significant increases in yield. In contrast, two treatments in the *M. incognita* experiment reduced larval populations and resulted in significant yield increases and several treatments failed to reduce larval populations but increased yields.

In conclusion, the results from this study indicate that while the use of some combinations of 1,3-D with NH_3 result in yield increases of Ransom soybean in root-knot infested fields, the cost of these treatments offer no advantage over what is obtained with the currently recommended EDB-90 treatment of 18.7 l/ha for control of the parasites.

LITERATURE CITED

1. COLLINS, R. J. 1972. Relationship of fertilizer treatments and cropping sequence to populations of plant parasitic and free living nematodes. Ph.D. Thesis. Auburn University, Auburn. 202 pp.
2. GAZAWAY, W.S., F.A., GRAY, and R. ADAMS. 1977. Alabama plant disease and nematicide chemical control handbook. Ala. Coop. Ext. Serv. Cir. ANR-105, Auburn. 94 pp.
3. INGRAM, E.G., and R. RODRIGUEZ-KABANA. 1980. Nematodes parasitic on peanuts and evaluation of methods for detection and study of population dynamics. Nematropica 10(1):21-30.
4. KINLOCH, R.A. 1982. The relationship between soil populations of *Meloidogyne incognita* and yield reduction of soybean in the Coastal Plain. J. Nematol. 14:162-167.
5. KINLOCH, R.A. 1980. The control of nematodes injurious to soybeans. Nematropica 10:141-153.
6. KINLOCH, R.A. 1979. Response of a resistant soybean cultivar to fumigation at planting for the control of soybean cyst and root-knot nematodes. Nematropica 9(1):27-32.
7. MINTON, N.A., and M.B. PARKER. 1979. Effects on soybeans and nematode populations of three soil fumigants applied at several rates at time of planting. Nematropica 9(1):36-39.
8. MINTON, N.A., M.B. PARKER, O.L. BROOKS, and C.E. PERRY. 1976. Evaluation of nematicides for control of nematodes in soybeans. Georgia Agr. Expt. Sta. Res. Bull. 189:19 pp.
9. MORGAN-JONES, G., G. GODOY, and R. RODRIGUEZ-KABANA. 1981. *Verticillium chlamydosporium,* fungal parasite of *Meloidogyne arenaria* females. Nematropica 11(1):115-119.
10. MORGAN-JONES, G. and R. RODRIGUEZ-KABANA. 1981. Fungi associated with cysts of *Heterodera glycines* in an Alabama soil. Nematropica 11:69-74.

11. MORGAN-JONES, G., B.O. GINTIS, and R. RODRIGUEZ-KABANA. 1981. Fungal colonization of *Heterodera glycines* cysts in Arkansas, Florida, Mississippi and Missouri soils. Nematropica 11:155-164.
12. RODRIGUEZ-KABANA, 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.
13. RODRIGUEZ-KABANA, R., H. W. PENICK, P.S. KING, F.A. GRAY, E.L. CARDEN, N.R. McDANIEL, and F.B. SELMAN. 1979. Efficacy of planting-time applications of 1,3-dichloropropenes for control of nematodes on soybeans. Nematropica 9(1):66-71.
14. RODRIGUEZ-KABANA, R., and M.H. POPE. 1981. A simple incubation method for the extraction of nematodes from soil. Nematropica 11(1): 175-185.
15. RODRIGUEZ-KABANA, R., H.W. PENICK, and P.S. KING. 1979. Control of nematodes on soybeans with planting time applications of ethylene dibromide. Nematropica 9:61-66.
16. RODRIGUEZ-KABANA, R., and J.C. WILLIAMS. 1981. Assessment of soybean yield losses caused by *Meloidogyne arenaria*. Nematropica 11(2):105-113.
17. RODRIGUEZ-KABANA, R., and J.C. WILLIAMS. 1981. Soybean yield losses caused by *Meloidogyne arenaria* and *Heterodera glycines* in a field infested with the two parasites. Nematropica 11(2):93-104.
18. STEEL, R.G.D., and J.H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York. 481 pp.

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