COMBINATIONS OF ANHYDROUS AMMONIA AND 1,3-DICHLORO-PROPENES 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.

Accepted: 18.IV.1982
Aceptado:

ABSTRACT


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


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 los 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) así 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 preceding 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 significance 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.

<table>
<thead>
<tr>
<th>NH$_3$ (Kg N/ha)</th>
<th>Telone II (l/ha)</th>
<th>Subjective$^x$ Appearance Index</th>
<th>Larvae per 100 cm$^3$ Soil</th>
<th>Yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>3.9 C$^y$</td>
<td>200 ABC</td>
<td>799 H</td>
</tr>
<tr>
<td>56</td>
<td>0.0</td>
<td>3.9 C</td>
<td>233 AB</td>
<td>785 H</td>
</tr>
<tr>
<td>112</td>
<td>0.0</td>
<td>4.1 BC</td>
<td>263 A</td>
<td>927 FGH</td>
</tr>
<tr>
<td>56</td>
<td>18.7</td>
<td>4.5 AB</td>
<td>151 ABCD</td>
<td>924 GH</td>
</tr>
<tr>
<td>112</td>
<td>18.7</td>
<td>4.6 A</td>
<td>152 ABCD</td>
<td>1119 EFG</td>
</tr>
<tr>
<td>56</td>
<td>37.4</td>
<td>4.5 AB</td>
<td>169 ABCD</td>
<td>1310 CDE</td>
</tr>
<tr>
<td>112</td>
<td>37.4</td>
<td>4.7 A</td>
<td>110 CDE</td>
<td>1241 DE</td>
</tr>
<tr>
<td>56</td>
<td>56.1</td>
<td>4.6 A</td>
<td>61 DE</td>
<td>1778 B</td>
</tr>
<tr>
<td>112</td>
<td>56.1</td>
<td>4.7 A</td>
<td>170 ABCD</td>
<td>1513 C</td>
</tr>
<tr>
<td>0</td>
<td>18.7</td>
<td>4.5 AB</td>
<td>140 BCDE</td>
<td>785 H</td>
</tr>
<tr>
<td>0</td>
<td>37.4</td>
<td>4.7 A</td>
<td>136 BCDE</td>
<td>1143 EF</td>
</tr>
<tr>
<td>0</td>
<td>56.1</td>
<td>4.5 AB</td>
<td>125 BCDE</td>
<td>1424 CD</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>NH$_3$ (Kg N/ha)</th>
<th>Telone II (1/ha)</th>
<th>Subjective$^x$ Appearance Index</th>
<th>Larvae per 100 cm$^3$ Soil</th>
<th>Yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>4.0 BCD$^y$</td>
<td>287 AB</td>
<td>1056 DE</td>
</tr>
<tr>
<td>56</td>
<td>0.0</td>
<td>4.0 BCD</td>
<td>195 CDE</td>
<td>1074 DE</td>
</tr>
<tr>
<td>112</td>
<td>0.0</td>
<td>4.3 ABCD</td>
<td>239 ABCDE</td>
<td>1318 CDE</td>
</tr>
<tr>
<td>56</td>
<td>18.7</td>
<td>4.0 BCD</td>
<td>172 DE</td>
<td>1237 CDE</td>
</tr>
<tr>
<td>112</td>
<td>18.7</td>
<td>3.7 D</td>
<td>154 E</td>
<td>1261 CDE</td>
</tr>
<tr>
<td>56</td>
<td>37.4</td>
<td>4.6 AB</td>
<td>253 ABCD</td>
<td>1391 BCD</td>
</tr>
<tr>
<td>112</td>
<td>37.4</td>
<td>4.4 ABC</td>
<td>270 ABC</td>
<td>1412 BCD</td>
</tr>
<tr>
<td>56</td>
<td>56.1</td>
<td>4.6 AB</td>
<td>206 ABCDE</td>
<td>1391 BCD</td>
</tr>
<tr>
<td>112</td>
<td>56.1</td>
<td>4.6 AB</td>
<td>215 ABCDE</td>
<td>1741 AB</td>
</tr>
<tr>
<td>0</td>
<td>18.7</td>
<td>3.9 CD</td>
<td>194 CDE</td>
<td>993 E</td>
</tr>
<tr>
<td>0</td>
<td>37.4</td>
<td>4.6 AB</td>
<td>202 BCDE</td>
<td>1371 CDE</td>
</tr>
<tr>
<td>0</td>
<td>56.1</td>
<td>4.9 A</td>
<td>293 A</td>
<td>1493 BC</td>
</tr>
</tbody>
</table>

EDB 90 (1/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 specula-
tive. However, our data show that four treatments in the *M. arenaria* exper-
iment 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 NH₃ 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
Ext. Serv. Cir. ANR-105, Auburn. 94 pp.
parasitic on peanuts and evaluation of methods for detection and study
4. KINLOCH, R.A. 1982. The relationship between soil populations of
*Meloidogyne incognita* and yield reduction of soybean in the Coastal
5. KINLOCH, R.A. 1980. The control of nematodes injurious to soy-
6. KINLOCH, R.A. 1979. Response of a resistant soybean cultivar to
fumigation at planting for the control of soybean cyst and root-knot
7. MINTON, N.A., and M.B. PARKER. 1979. Effects on soybeans and
nematode populations of three soil fumigants applied at several rates at
8. MINTON, N.A., M.B. PARKER, O.L. BROOKS, and C.E. PERRY.
9. MORGAN-JONES, G., G. GODOY, and R. RODRIGUEZ-KABANA.
1981. *Verticillium chlamydosporium*, fungal parasite of *Meloidogyne
associated with cysts of *Heteroderagenicines* in an Alabama soil. Nema-


Received for publication: 9.III.1982

Recibido para publicar: