

## Crop Rotation and Nematicides for Management of Mixed Populations of *Meloidogyne* spp. on Tobacco<sup>1</sup>

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**Abstract:** The effects of crop rotation and the nematicides 1,3-dichloropropene (1,3-D), ethoprop, and fenamiphos on the relative frequency of *Meloidogyne incognita* race 3, *M. arenaria* race 2, and *M. javanica* and tobacco yields on a sandy loam soil were determined. Cropping sequences altered the species composition and population densities of *Meloidogyne* spp. *Meloidogyne arenaria* and *M. incognita* predominated when cotton, corn, sorghum, or rye-fallow preceded tobacco. *Meloidogyne javanica* and *M. arenaria* predominated when tobacco preceded tobacco. Sorghum, cotton, corn, or rye-fallow preceding tobacco enhanced yields compared to tobacco preceding tobacco in plots containing mixtures of *Meloidogyne* species. Sorghum supported minimal reproduction of any *Meloidogyne* spp. Application of 1,3-D increased tobacco yields and reduced root galling when compared to untreated controls. Both fenamiphos and ethoprop treatments were less effective than 1,3-D in controlling *Meloidogyne* spp. or increasing yields. A rotation crop x nematicide interaction was not observed. In continuous tobacco, use of the *M. incognita*-resistant tobacco cv. Coker 176 increased tobacco yields when compared to the *M. incognita*-susceptible cv. Coker 319 when 1,3-D was not applied.

**Key words:** corn, cotton, *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, nematicide, root-knot nematode, rotation, rye, sorghum, tobacco.

Root-knot nematodes (*Meloidogyne* spp.) are commonly associated with field crops, particularly flue-cured tobacco (*Nicotiana tabacum* L.) in the southeastern United States (Johnson, 1982; Lucas, 1975). Approximately 90% of the tobacco acreage in South Carolina is treated annually with a nematicide, but root-knot still decreases South Carolina's total tobacco production by 0.5% to 1.0% (Gooden et al., 1999). Moderate populations of root-knot nematodes rarely kill a plant but will reduce leaf thickness (Fortnum et al., 1991) and, subsequently, leaf yield (Fortnum and Currin, 1993). Yield reductions of 5% to 10% may go undetected, resulting in an underestimation of root-knot nematode-induced yield losses. Losses on tobacco caused by root-knot nematodes worldwide are estimated to be 15% (Shew, 1991) and reflect a lower use of soil-applied nematicides than in the United States.

*Meloidogyne incognita* (Kofoid and White) Chitwood, races 1 and 3, is the most common species of *Meloidogyne* in North and South Carolina tobacco fields; however, *M. arenaria* (Neal) Chitwood, *M. javanica* (Treub) Chitwood, and *M. incognita* races 2 and 4 are increasing in importance (Barker, 1989; Fortnum et al., 1984; Rich et al., 1989). This complicates traditional crop-rotation schemes because reproduction of different species of *Meloidogyne* varies with crop and cultivar (Johnson, 1982; Johnson and Motsinger, 1989). Species of *Meloidogyne* that are more aggressive than *M. incognita*, such as *M. arenaria* and *M. javanica*, appear to be increasing in frequency in most flue-cured tobacco-

producing states, possibly due to widespread use of *M. incognita*-resistant cultivars (Barker, 1989; Fortnum et al., 1984) or selection of rotation crops that favor more aggressive species. Soybean when grown in rotation with tobacco will enhance the development of *M. arenaria* race 2 in mixed *M. incognita* and *M. arenaria* populations (Fortnum and Currin, 1993). In areas where a dramatic increase in cotton production has occurred, the predominance of *M. incognita* may increase (Fortnum and Currin, 1993). With dynamic changes in species composition, the design of a nematode management system is more complex in fields containing mixtures of *Meloidogyne* spp.

Plant resistance to *M. incognita* races 1 and 3 and nematicides have been key components in the management of root-knot nematode on tobacco. *Meloidogyne arenaria* is more difficult to control with nematicides than *M. incognita* (Melton et al., 1995), and no commercial tobacco cultivars are resistant to *M. arenaria* (Barker et al., 1981; Johnson, 1989). Commonly used nematicides such as ethoprop are not labeled for use on the more aggressive species such as *M. arenaria* or *M. javanica* but are still used for control of soil insects, *M. incognita* and *Pratylenchus* spp. With the increasing occurrence of mixed *Meloidogyne* spp. in South Carolina, nematode resistance in tobacco may play a diminished role in root-knot nematode control unless commercial cultivars with resistance to *M. arenaria* and *M. javanica* are developed.

Crop rotation has been used successfully to reduce nematode population densities and increase tobacco yields (Fortnum and Currin, 1993; Gaines, 1968). Because populations of the root-knot nematodes composed of multiple species are increasingly widespread, effects of crop rotation must be completely characterized. With wide variation in aggressiveness and host compatibility among *Meloidogyne* spp., the differential effects of crop rotation or nematicide use on *Meloidogyne* spp. may play a key role in the design of long-term control programs for root-knot nematodes. We report

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on field experiments to determine the value of selected rotation crops and the nematicides 1,3-dichloropropene (1,3-D), ethoprop, and fenamiphos for the management of mixed populations of *M. arenaria* race 2, *M. javanica*, and *M. incognita* race 3 on tobacco.

#### MATERIALS AND METHODS

**Field preparation:** The trial was located at the Pee Dee Research and Education Center, Florence County, South Carolina, on a Norfolk sandy loam soil (75% sand, 17% silt, 8% clay, 0.08% organic matter; pH 5.9). Tobacco had been planted on this site the previous summer, 1987. The site had been infested with a mixture of *M. incognita* race 3, *M. arenaria* race 2, and *M. javanica* (Fortnum et al., 1987) in equal proportions. Nematode species present were confirmed within the site during summer 1987 by differential host tests, perineal patterns, and the body length of second-stage juveniles (J2) (Taylor and Sasser, 1978). The test site was tilled with a moldboard plow and disc-harrowed twice in a perpendicular direction.

**Crop sequence, nematicide application, and *Meloidogyne* spp. populations:** Selected rotation crops were planted into the infested plots and alternated with tobacco in a 2-year rotation. Crop sequences and nematicide treatments are listed in Table 1. Rotation crops were selected based on their levels of resistance to *Meloidogyne* spp. and were classified susceptible, moderately resistant, or resistant based on the levels of reproduction supported. Corn was susceptible to *M. incognita* with

moderate resistance to *M. arenaria* and *M. javanica* (Windham and Williams, 1987); cotton was a nonhost to *M. arenaria* and *M. javanica* and was susceptible to *M. incognita* (Taylor and Sasser, 1978); sorghum was resistant to *M. incognita*, *M. javanica*, and *M. arenaria* (Fortnum and Currin, 1988). A rye winter cover crop followed by a weed-free fallow was a nonhost control (Johnson and Motsinger, 1989). Within each main block, selected plots were planted continuously with tobacco varieties either resistant or susceptible to *M. incognita* (Table 1). The selected crops and planting dates included: corn (*Zea mays* L. 'Pioneer 3320'), 28 April 1988 and 14 May 1990; sorghum (*Sorghum bicolor* (L.) Moench 'Coker 7723'), 18 May 1988 and 14 May 1990; rye (*Secale cereale* L. 'Abruzzi')-summer fallow, 10 November 1987 and 15 December 1989, respectively. Seeding rates for corn, cotton, and sorghum were 6, 13, and 20 seeds/m row, respectively. Rye seeds were broadcast (100 kg/ha). Rye plots were moved at maturity and disc-harrowed as needed to suppress weeds.

The selected crops were planted into subplots consisting of four rows (rows were spaced 1 m wide × 10.6 m long) centered within each 4.8-m wide subplot. All crops were nonirrigated and maintained by standard agronomic practices. Tobacco seedlings were transplanted into test subplots consisting of four rows (rows were spaced 1.2 m apart × 10.6-m long), with plants spaced 60 cm apart within the row. Tobacco seedlings, cultivar Coker 319 (*M. incognita*-susceptible) or Coker 176 (*M. incognita*-resistant), were transplanted on 5 May 1989 and 16 May 1991 into plots previously planted to

TABLE 1. Crop sequence, nematicide application, and data analysis information for a *Meloidogyne* spp. rotation trial, 1988–1991.

| Crop sequence <sup>a</sup> |             |             |             | Nematicide treatments<br>1989 and 1991        | Treatments included in<br>factorial analysis <sup>b</sup> |             |
|----------------------------|-------------|-------------|-------------|---|---|-------------|
| 1988                       | 1989        | 1990        | 1991        |   | Analysis 1  | Analysis 2  |
| Corn                       | tobacco (s) | corn        | tobacco (s) | nontreated<br>ethoprop<br>fenamiphos<br>1,3-D | +   | +<br>+<br>+ |
| Cotton                     | tobacco (s) | cotton      | tobacco (s) | nontreated<br>ethoprop<br>fenamiphos<br>1,3-D | +   | +<br>+<br>+ |
| Sorghum                    | tobacco (s) | sorghum     | tobacco (s) | nontreated<br>ethoprop<br>fenamiphos<br>1,3-D | +   | +<br>+<br>+ |
| Rye-fallow                 | tobacco (s) | rye-fallow  | tobacco (s) | nontreated<br>ethoprop<br>fenamiphos<br>1,3-D | +   | +<br>+<br>+ |
| Tobacco (s)                | tobacco (s) | tobacco (s) | tobacco (s) | nontreated                                    | +   |             |
| Tobacco (s)                | tobacco (s) | tobacco (s) | tobacco (s) | 1,3-D   | +   |             |
| Tobacco (r)                | tobacco (r) | tobacco (r) | tobacco (r) | nontreated                                    | +   |             |
| Tobacco (r)                | tobacco (r) | tobacco (r) | tobacco (r) | 1,3-D   | +   |             |

<sup>a</sup> (s) = *M. incognita*-susceptible cv. Coker 319; (r) = *M. incognita*-resistant cv. Coker 176.

<sup>b</sup> Analysis 1, treatments were analyzed in a 6-to-2 factorial design with crop sequence as main plots and 1,3-D application as subplots; analysis 2, treatments were analyzed in a 4-to-4 factorial design with crop sequence as main plots and nematicide treatments as subplots. Each analysis contained for replications. The less effective nematicides, ethoprop and fenamiphos, were not applied in continuous tobacco.

the rotation crops in 1988 and 1990. All crop and nematicide treatments were replicated four times.

A 1.2-m border separated all whole-plots and subplots within a block, and a 4.6-m border separated each block. Cultivation for bed preparation, fertilizer applications, and weed control were conducted parallel to the rows from block to adjacent block. This direction of cultivation provided a 4.6-m border between cultivated plots to minimize plot-to-plot contamination.

The fumigant nematicide was applied to a 4-row sub-subplot within each 16-row subplot (each rotation crop) and to two 4-row sub-subplots within the continuous tobacco (cv. Coker 319 and cv. Coker 176) on 29 March 1989 and 16 April 1991. A positive pressure pump was used to inject 6.7 ml 1,3-D/m row (56 liters/ha) 15 cm deep with a single chisel placed in the center of a 60-cm-wide bed. Bedding discs were used to seal the chisel opening and form a 36-cm-high bed with fumigant placement 40 cm from the top of the bed. The nonfumigant nematicides fenamiphos (6.7 kg a.i./ha) and ethoprop (13.4 kg a.i./ha) were applied on 3 May 1989 and 24 April 1991 as broadcast soil sprays in 280 liters water/ha and incorporated with a disc harrow. Nonfumigant nematicides were applied to plots rotated with corn, cotton, sorghum, or rye-fallow. Control plots planted to continuous tobacco within each whole block were untreated or fumigated with 1,3-D.

Soil samples consisting of a composite of 40 cores/plot, each 2-cm diam. × 20 cm deep, were removed from each subplot immediately preceding fumigant application and bioassayed for *Meloidogyne* spp. A tomato (*Lycopersicon lycopersicum* (L.) Karsten 'Rutgers') seedling was transplanted into a pot containing the soil and maintained in a greenhouse. After 50 days, roots were removed from the pots and washed free of soil. Each root system was stained in phloxine B (150 mg/liter) for 15 minutes, and egg masses were counted (Dickson and Struble, 1965). Plant roots were rated for root galling on a 0-to-10 scale, where 0=no galls, 1=1–10%, 2=11–20%, 3=21–30%, 4=31–40%, 5=41–50%, 6=51–60%, 7=61–70%, 8=71–80%, 9=81–90%, and 10=91–100% of the root tissue galled (Barker et al., 1986).

Soil samples consisting of a composite of 20 cores/plot, each 2-cm diam. × 20 cm deep, were removed from each subplot at 60 days following tobacco transplanting and immediately following the last harvest. A 500-g soil sample from each subplot was assayed for nematodes after extraction by semiautomatic elutriation and centrifugal-flotation (Byrd et al., 1976; Jenkins, 1964). Mature tobacco leaves were harvested three times from the center two rows in each plot. Yield was based on fresh leaf weight, assuming that cured leaf weight was 20% of fresh weight. After the last harvest, 10 plants from the center two rows in each plot were excavated at random and rated for root galling as previously described (Barker et al., 1986).

Following the last tobacco harvest (1991), a 1,000-g soil sample consisting of a composite of 40 cores/subplot was removed from each subplot (each nematicide treatment within each preceding crop) and bioassayed for *Meloidogyne* spp. A tomato seedling (cv. Rutgers) was transplanted into a pot containing the soil and maintained in a greenhouse for 50 days. Plant roots were removed from the pots and washed free of soil. Nematode-infected roots were immersed in pectinase to facilitate the removal of adult females. Individual adult females were identified to species using esterase isozyme analysis (Esbenshade and Triantaphyllou, 1990) using the Phastsystem automated electrophoresis unit (Pharmacia, LKB Biotechnology, Piscataway, NJ) with a modified protocol (Hussey, unpubl.).

Data were analyzed using analysis of variance (ANOVA); factorial analysis and means were compared with planned contrasts (Steel and Torrie, 1960). All calculations were performed with the Statistical Analysis System-JMP (SAS Institute, Cary, NC).

## RESULTS

*Previous crop and soil fumigation affected tobacco yields and Meloidogyne populations, analysis 1:* Cotton, sorghum, corn, and rye-fallow preceding tobacco resulted in higher tobacco yields ( $P \leq 0.001$ ) than tobacco preceding tobacco in plots containing mixtures of *M. incog-*

TABLE 2. Source of variation and *P* values for main effects and interactions of crop rotation or continuous tobacco and 1,3-D application on *Meloidogyne* spp. (Pi), tobacco yields, and root-gall index at harvest.

| Source of variation | <i>P</i> values                           |                    |                            |                    |                |     |      |     |
|---------------------|---|--------------------|----------------------------|--------------------|----------------|-----|------|-----|
|                     | Tomato bioassay of soil (Pi) <sup>a</sup> |                    |                            |                    | Yields (kg/ha) |     |      |     |
|                     | 1989                                      |                    | 1991                       |                    | 1989           |     | 1991 |     |
|                     | Egg masses/<br>root system                | Root-gall<br>index | Egg masses/<br>root system | Root-gall<br>index |                |     |      |     |
| Crop (C)            | *** <sup>b</sup>                          | ***                | ns                         | *                  | **             | *** | *    | *   |
| 1,3-D               | ns  | ns                 | ns                         | ns                 | ***            | *** | *    | *** |
| C × 1,3-D           | ns  | ns                 | ns                         | ns                 | ns             | ns  | ns   | ns  |

<sup>a</sup> Soil was collected for bioassay immediately preceding 1,3-D application. Treatments were analyzed in a 6-to-2 factorial design with crop sequence as main plots and 1,3-D application as subplots with 4 replications.

<sup>b</sup> \*\*\**P* = 0.001; \*\**P* = 0.01; \**P* = 0.05; ns = non significant.

TABLE 3. Yield of tobacco and root-galling index as affected by previous crop with (+) and without (-) 1,3-D applications.

| Crop sequence <sup>a</sup> |             |             |             | Yield (kg/ha) |       |       |       | Root galling   |     |      |     |
|----------------------------|-------------|-------------|-------------|---------------|-------|-------|-------|----------------|-----|------|-----|
|                            |             |             |             | 1989          |       | 1991  |       | 1989           |     | 1991 |     |
| 1988                       | 1989        | 1990        | 1991        | -             | +     | -     | +     | -              | +   | -    | +   |
| Corn                       | tobacco (s) | corn        | tobacco (s) | 2,428         | 3,311 | 2,033 | 2,634 | 5.5            | 3.0 | 5.0  | 2.6 |
| Cotton                     | tobacco (s) | cotton      | tobacco (s) | 2,554         | 3,023 | 2,268 | 2,181 | 3.9            | 3.4 | 3.5  | 2.7 |
| Sorghum                    | tobacco (s) | sorghum     | tobacco (s) | 2,496         | 3,202 | 2,043 | 2,420 | 4.0            | 4.0 | 4.1  | 3.8 |
| Rye-fallow                 | tobacco (s) | rye-fallow  | tobacco (s) | 2,683         | 3,292 | 2,043 | 2,335 | 6.3            | 4.9 | 3.5  | 2.5 |
| Tobacco (s)                | tobacco (s) | tobacco (s) | tobacco (s) | 939           | 2,320 | 688   | 1,603 | — <sup>b</sup> | 2.4 | —    | 1.7 |
| Tobacco (r)                | tobacco (r) | tobacco (r) | tobacco (r) | 1,912         | 2,165 | 1,240 | 1,538 | —              | 2.1 | —    | 1.4 |

  

| Contrasts                              |  |  |  |                  |     |         |     |
|--|--|--|--|------------------|-----|---------|-----|
| Continuous tobacco vs. crop rotation   |  |  |  | *** <sup>c</sup> | *** | ***     | **  |
| Rye-fallow vs. Corn + sorghum + cotton |  |  |  | ns               | ns  | (0.056) | ns  |
| Untreated vs. 1,3-D                    |  |  |  | ***              | *** | *       | *** |
| Continuous tobacco (s,-) vs. (r,-)     |  |  |  | *                | *   | —       | —   |
| Continuous tobacco (s,-,+) vs. (r,-,+) |  |  |  | ns               | ns  | —       | —   |
| Continuous tobacco (s,+) vs. (r,+)     |  |  |  | ns               | ns  | —       | —   |

<sup>a</sup> (s) = *M. incognita*-susceptible cv. Coker 319; (r) = *M. incognita*-resistant cv. Coker 176.

<sup>b</sup> Root-galling index could not be recorded in nonfumigated continuous tobacco due to plant mortality (50% or less survival at last harvest). Root-galling index was lower than expected in all continuous tobacco due to extensive root necrosis.

<sup>c</sup> \*\*\* $P = 0.001$ ; \*\* $P = 0.01$ ; \* $P = 0.05$ ; ns = non significant.

*nita*, *M. arenaria*, and *M. javanica* (Tables 1, 2, 3). When averaged across tobacco varieties (r + s) and nematicide application, continuous tobacco yielded less (1,551 kg/ha) than tobacco preceded by corn, cotton, sorghum, or rye fallow (2,959 kg/ha) ( $P < 0.001$ ). A rye winter crop followed by a clean summer fallow did not increase tobacco yields (2,588 kg/ha) or reduce root galling over plots rotated to corn, sorghum, or cotton (2,549 kg/ha) (Table 3). The number of egg masses and root-gall index on the tomato bioassays grown in soil collected from corn, sorghum, cotton, and rye-fallow plots immediately preceding tobacco was less than the number of egg masses or root-gall index from

plants grown in soil from continuous tobacco ( $P \leq 0.05$  and  $P \leq 0.001$ , respectively). Populations of *Meloidogyne* spp. J2 extracted from soil were greater in cotton and corn than continuous tobacco ( $P = 0.03$ ) (Table 4). Soil populations of J2 extracted from plots grown in sorghum were lower than in plots planted in cotton, corn, or rye fallow ( $P = 0.06$ ).

Application of 1,3-D increased tobacco yields from 1,946 to 2,504 kg/ha ( $P \leq 0.001$ ) and reduced the root-gall index from 3.85 to 2.86 ( $P \leq 0.05$ ) across rotation crops and continuous tobacco (Tables 2, 3). Because continuous tobacco without soil fumigation resulted in >50% plant mortality and severe root necrosis

TABLE 4. Population densities of *Meloidogyne* spp. as affected by corn, cotton, sorghum, and a rye-fallow grown between tobacco crops.

| Crop sequence <sup>a</sup> |             |             |             | Tobacco 89                              |                    |   |     | Tobacco 91                           |                    |
|----------------------------|-------------|-------------|-------------|---|--------------------|---|-----|--------------------------------------|--------------------|
|                            |             |             |             | 1989 (Pi)<br>Tomato bioassay of soil    |                    | Rotation 90<br>J2/100 cm <sup>3</sup> soil 1990 |     | 1991 (Pi)<br>Tomato bioassay of soil |                    |
| 1988                       | 1989        | 1990        | 1991        | Egg masses/<br>root system <sup>b</sup> | Root-gall<br>index | Pm  | Pf  | Egg masses/<br>root system           | Root-gall<br>index |
| Corn                       | tobacco (s) | corn        | tobacco (s) | 138                                     | 2.3                | 1.5   | 552 | 39                                   | 0.9                |
| Cotton                     | tobacco (s) | cotton      | tobacco (s) | 85                                      | 3.8                | 0.1   | 419 | 34                                   | 0.8                |
| Sorghum                    | tobacco (s) | sorghum     | tobacco (s) | 13                                      | 1.5                | 2.4   | 20  | 16                                   | 1.0                |
| Rye-fallow                 | tobacco (s) | rye-fallow  | tobacco (s) | 121                                     | 3.9                | 0.4   | 37  | 32                                   | 1.0                |
| Tobacco (s)                | tobacco (s) | tobacco (s) | tobacco (s) | 792                                     | 6.6                | 5.0   | 152 | 334                                  | 2.4                |
| Tobacco (r)                | tobacco (r) | tobacco (r) | tobacco (r) | 659                                     | 7.0                | 3.3   | 169 | 170                                  | 2.0                |

  

| Contrast                               |  |  |  |                  |        |    |        |    |     |
|--|--|--|--|------------------|--------|----|--------|----|-----|
| Continuous tobacco vs. crop rotation   |  |  |  | *** <sup>c</sup> | ***    | ns | ns     | *  | *** |
| Rye-fallow vs. corn + sorghum + cotton |  |  |  | ns               | ns     | ns | (0.08) | ns | ns  |
| Sorghum vs. corn + cotton + rye-fallow |  |  |  | ns               | (0.07) | ns | (0.06) | ns | ns  |

<sup>a</sup> (s) = *M. incognita*-susceptible cv. Coker 319; (r) = *M. incognita*-resistant cv. Coker 176.

<sup>b</sup> Soil was collected for bioassay immediately preceding nematicide application. Treatments were analyzed in a 6-to-2 factorial design with crop sequence as main plots and 1,3-D application as subplots with 4 replications. Because no nematicide or crop to ematicide interaction ( $P = 0.05$ ) were observed, data were pooled and examined with contrasts.

<sup>c</sup> \*\*\* $P = 0.001$ ; \*\* $P = 0.01$ ; \* $P = 0.05$ ; ns = non significant.

on surviving plants at final harvest, root galling was not recorded. Application of 1,3-D in continuous tobacco in 1989 and 1991 improved plant survivability, but severe root necrosis resulted in lower root-galling indices than would normally be expected. The tomato bioassay of soil prior to planting (egg masses/root system) showed populations of *Meloidogyne* spp. were greater ( $P \leq 0.05$ ; 1989 and 1991) in continuous tobacco than in rotated plots (Table 4). Analysis of variance indicated a crop  $\times$  nematicide interaction was not observed (Table 2). The use of a tobacco cultivar with resistance to *M. incognita* races 1 and 3 in nonfumigated plots increased tobacco yields ( $P \leq 0.05$ ) over a tobacco cultivar susceptible to all nematode species (Table 3). No yield response was observed between a resistant and susceptible tobacco cultivar when the plots were fumigated with 1,3-D (Table 3).

*Previous crop and nematicides affect tobacco yields and Meloidogyne populations, analysis 2:* Tobacco planted in plots previously grown in either corn, cotton, sorghum, or rye fallow in 1989 and 1991 did not differ ( $P = 0.05$ ) in yield or root-gall index when averaged over nematicide treatments (Table 5). The nematicides (ethoprop, fenamiphos, and 1,3-D) affected tobacco yields (1989) and root-gall indices (1989 and 1991) when averaged across rotation crops ( $P \leq 0.05$ ). Yields were generally lower in 1991 than in 1989. A significant nematicide  $\times$  crop interaction was observed for root galling in 1991 ( $P = 0.05$ ). Application of 1,3-D increased tobacco yields ( $P \leq 0.05$ ) and reduced the root-gall index ( $P \leq 0.01$ ) across rotation crops when compared to an unfumigated control (Table 6). Ethoprop and fenamiphos-treated plots had lower tobacco yields in 1989 ( $P \leq 0.01$ ) than tobacco fumigated with 1,3-D, but not in 1991. Yields did not differ between plots treated with ethoprop and fenamiphos, but root-gall indices were lower ( $P \leq 0.01$ ) in plots treated with fenamiphos (1991) than in plots treated with ethoprop (Table 6).

*Cropping sequences and relative frequency of M. incognita, M. arenaria, and M. javanica:* The relative frequency (percentage of population) of *M. incognita*, *M. arenaria*, and *M. javanica* were determined after the final harvest

using tomato bioassay of soil samples. *Meloidogyne arenaria* and *M. incognita* predominated when tobacco was preceded by cotton, corn, sorghum, or rye fallow. *Meloidogyne javanica* and *M. arenaria* predominated when tobacco preceded tobacco. Populations (percentage of total population) of *M. javanica* increased ( $P = 0.05$ ) and populations of *M. incognita* declined ( $P = 0.09$ ) in continuous tobacco when compared to plots rotated to corn, sorghum, cotton, or rye fallow (analysis 1; Tables 1,7). Populations of *M. incognita* were greater ( $P = 0.05$ ) following cotton and corn when compared to sorghum and rye-fallow (analysis 1; Tables 1,7). Monoculture of tobacco over the 4 years of the study resulted in few *M. incognita* being recovered from continuous tobacco and a dramatic increase in *M. javanica*. Application of ethoprop, fenamiphos, or 1,3-D did not alter the relative frequency of *Meloidogyne* spp. No crop rotation  $\times$  nematicide interactions were observed (Table 7).

## DISCUSSION

Crop rotation has been a traditional method of managing root-knot nematodes (*Meloidogyne* spp.) on tobacco. The usefulness of this control method has been challenged in recent years due to the increasing occurrence of fields containing two or more species of *Meloidogyne*. This complicates the selection of rotation crops because reproduction of different species of *Meloidogyne* varies with crop and cultivar (Johnson, 1982; Johnson, 1989). The total population of *Meloidogyne* spp. and the relative percentage of each species within the population would impact future losses to root-knot nematode on a high-value target crop such as tobacco. Commonly planted tobacco cultivars are resistant to *M. incognita* races 1 and 3 (Gooden et al., 1999).

Certain rotation crops are better hosts for one species of root-knot nematode than others and can shift root-knot nematode populations from more aggressive to less aggressive populations or vice versa (Fortnum and Currin, 1993; Hirunsalee et al., 1995a, 1995b). Thus, soybean grown in rotation with tobacco will enhance the development of the more aggressive species, *M. arenaria*, when mixtures of *M. incognita* and *M. are-*

TABLE 5. Source of variation and  $P$  values for main effects and interactions of crop rotation with corn, cotton, sorghum, or rye-fallow and nematicides 1,3-D, ethoprop, and fenamiphos application on *Meloidogyne* spp. (Pi), tobacco yields, and root-gall index.

| Source of variation | $P$ values                              |                                  |                            |                    |                |        |      |        |
|---------------------|---|----------------------------------|----------------------------|--------------------|----------------|--------|------|--------|
|                     | Greenhouse tomato bioassay of soil (Pi) |                                  |                            |                    | Yields (kg/ha) |        |      |        |
|                     | 1989                                    |                                  | 1991                       |                    | 1989           |        | 1991 |        |
|                     | Egg masses/<br>root system <sup>a</sup> | Root-gall/<br>index <sup>b</sup> | Egg masses/<br>root system | Root-gall<br>index |                |        |      |        |
| Crop (C)            | ns                                      | *                                | ns                         | ns                 | ns             | ns     | ns   | (0.10) |
| Nematicides (N)     | ns                                      | ns                               | ns                         | ns                 | ***            | (0.09) | *    | ***    |
| C X N               | ns                                      | ns                               | ns                         | ***                | ns             | ns     | ns   | *      |

<sup>a</sup> Soil was collected for bioassay immediately preceding nematicide application. Treatments were analyzed in a 4-to-4 factorial design with crop sequence as main plots and nematicide treatments as subplots. Each analysis contained 4 replications.

<sup>b</sup> \*\*\* $P = 0.001$ ; \*\* $P = 0.01$ ; \* $P = 0.05$ ; ns = non significant.

TABLE 6. Yield of *Meloidogyne incognita*-susceptible Coker 319 tobacco, and root-galling index as affected by previous crop with and without nematicide applications.

| Crop sequence                   |         |            |         | Nematicide | Yield (kg/ha) <sup>a</sup> |       | Root-gall index <sup>b</sup> |        |
|---------------------------------|---------|------------|---------|------------|----------------------------|-------|------------------------------|--------|
| 1988                            | 1989    | 1990       | 1991    |            | 1989                       | 1991  | 1989                         | 1991   |
| Corn                            | tobacco | corn       | tobacco | Nontreated | 2,428                      | 2,033 | 5.5                          | 5.0    |
|                                 |         |            |         | Ethoprop   | 3,018                      | 2,160 | 4.8                          | 4.8    |
|                                 |         |            |         | Fenamiphos | 3,151                      | 2,573 | 3.8                          | 3.3    |
|                                 |         |            |         | 1,3-D      | 3,312                      | 2,634 | 3.0                          | 2.6    |
| Cotton                          | tobacco | cotton     | tobacco | Nontreated | 2,555                      | 2,268 | 3.9                          | 3.5    |
|                                 |         |            |         | Ethoprop   | 2,801                      | 2,019 | 3.0                          | 2.7    |
|                                 |         |            |         | Fenamiphos | 3,017                      | 2,244 | 4.2                          | 2.7    |
|                                 |         |            |         | 1,3-D      | 3,023                      | 2,181 | 3.4                          | 2.7    |
| Sorghum                         | tobacco | sorghum    | tobacco | Nontreated | 2,496                      | 2,043 | 4.0                          | 4.1    |
|                                 |         |            |         | Ethoprop   | 2,939                      | 2,470 | 2.8                          | 3.1    |
|                                 |         |            |         | Fenamiphos | 2,728                      | 2,537 | 2.9                          | 2.5    |
|                                 |         |            |         | 1,3D       | 3,202                      | 2,421 | 4.0                          | 3      |
| Rye-fallow                      | tobacco | rye-fallow | tobacco | Nontreated | 2,684                      | 2,043 | 6.3                          | 3.5    |
|                                 |         |            |         | Ethoprop   | 3,126                      | 2,033 | 4.7                          | 4.6    |
|                                 |         |            |         | Fenamiphos | 2,910                      | 1,989 | 4.1                          | 2.8    |
|                                 |         |            |         | 1,3-D      | 3,292                      | 2,335 | 4.9                          | 2.5    |
| Contrasts                       |         |            |         |            |                            |       |                              |        |
| Nontreated vs. 1,3-D            |         |            |         | ***c       | *                          | **    |                              | ***    |
| Ethoprop + fenamiphos vs. 1,3-D |         |            |         | **         | ns                         | ns    |                              | (0.08) |
| Ethoprop vs. fenamiphos         |         |            |         | ns         | ns                         | ns    |                              | **     |

<sup>a</sup> Data are the means of four replications.

<sup>b</sup> Root-gall index based on a 0-to-10 scale: 0=no root galling and 10=100% of the root surface galled.

<sup>c</sup> \*\*\**P* = 0.001; \*\**P* = 0.01; \**P* = 0.05; ns = non significant.

*naria* occur within the same field (Fortnum and Currin, 1993). In contrast, cotton and corn would favor the development of *M. incognita*, a species that is less aggressive than *M. arenaria* on tobacco. Rotation with corn, cotton, sorghum, or a rye-summer fallow maintained populations of *M. incognita* and substantially reduced losses due to root-knot nematodes in this trial containing mixed populations of *M. incognita*, *M. are-*

*naria*, and *M. javanica*. These rotation crops were selected based on host resistance to the more aggressive species and their ability to shift populations from more aggressive to less aggressive species on tobacco. In contrast, plots planted continuously to tobacco shifted the populations to the more aggressive species such as *M. javanica* and *M. arenaria*, and populations of *M. incognita* were reduced below detectable levels.

TABLE 7. Effect of previous crop and nematicide application on percentages of *Meloidogyne* spp. within a mixed population of *M. arenaria* race 2, *M. incognita* race 3, and *M. javanica*.

| Crop sequence <sup>a</sup>             |         |            |         | Alternate crop resistance ratings <sup>a</sup> |     |      | Tomato bioassay of soil (%) (1992) <sup>b</sup> |                     |                    |    |
|--|---------|------------|---------|--|-----|------|---|---------------------|--------------------|----|
| 1988                                   | 1989    | 1990       | 1991    | MI   | MA  | MJ   | <i>M. arenaria</i>                              | <i>M. incognita</i> | <i>M. javanica</i> |    |
| <i>Previous crop</i>                   |         |            |         |  |     |      |   |                     |                    |    |
| Corn                                   | tobacco | corn       | tobacco | S  | MR  | S/MR | 265   | 32                  | 3                  |    |
| Cotton                                 | tobacco | cotton     | tobacco | S  | R   | R    | 47  | 40                  | 13                 |    |
| Sorghum                                | tobacco | sorghum    | tobacco | R  | R   | R    | 94  | 6                   | 0                  |    |
| Rye-fallow                             | tobacco | rye-fallow | tobacco | R  | R   | R    | 82  | 15                  | 3                  |    |
| Tobacco                                | tobacco | tobacco    | tobacco | (s)  | (s) | (s)  | 65  | 0                   | 35                 |    |
| Tobacco                                | tobacco | tobacco    | tobacco | (r)  | (s) | (s)  | 56  | 9                   | 35                 |    |
| Contrasts                              |         |            |         |  |     |      |   |                     |                    |    |
| Continuous tobacco vs. crop rotation   |         |            |         | ns <sup>c</sup>                                |     |      | ns  |                     |                    | *  |
| Rye-fallow vs. corn + sorghum + cotton |         |            |         | ns   |     |      | ns  |                     |                    | ns |
| Corn + cotton vs. sorghum + rye-fallow |         |            |         | ns (0.09)                                      |     |      | *   |                     |                    | ns |

<sup>a</sup> MI = *M. incognita*; MA = *M. arenaria*; MJ = *M. javanica*. S = susceptible; MR = moderate resistance; R = resistant. Tobacco (s) = *M. incognita*-susceptible cultivar Coker 319; tobacco (r) = *M. incognita*-resistant cultivar Coker 176.

<sup>b</sup> Data are the means of four replications. Treatments were analyzed in a 6-to-2 factorial design with crop sequence as main plots and 1,3-D application as subplots. Because no nematicide or crop to nematicide effects (*P* = 0.05) were observed, data were pooled and compared with contrasts. Species determinations were conducted by examining esterase phenotypes with a Phast gel electrophoresis system.

<sup>c</sup> \**P* = 0.05; ns = non significant.

The predominance of a particular species of *Meloidogyne* in a multi-species population following a rotation crop could critically impact yields. Although J2 populations were lower ( $P = 0.06$ ) in sorghum at harvest than in either corn or cotton, this did not result in lower galling indices on tobacco following sorghum than tobacco following either corn, cotton, or rye-summer fallow the previous year. In addition, many weeds are excellent hosts for *Meloidogyne* spp. and could be hosts for both aggressive and nonaggressive species (Tedford and Fortnum, 1988). Some weed growth was present within the plots.

Application of 1,3-D increased tobacco yields and reduced root galling across rotation crops. A 1-year rotation to any of the evaluated crops was insufficient to completely suppress root-knot nematode populations. The observed increases in tobacco yields following a 1,3-D application suggest that population densities of *Meloidogyne* spp. were sufficient following a 1-year rotation to reduce yields. Abundant root galling was observed in all plots that did not receive a fumigant nematicide in 1989 and 1991. Nonfumigant nematicides increased yields, but yield responses were lower than those in fumigated plots. Nonfumigant nematicides, such as ethoprop or fenamiphos, also control some soil and foliar insect populations (Gooden et al., 1999). However, nematicidal rates of ethoprop or fenamiphos are higher than rates routinely used to control insects. Crop rotation in concert with soil fumigation resulted in the highest yields but failed to eliminate late-season development of *Meloidogyne* spp.

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