

Rotylenchulus reniformis below Plow Depth Suppresses Cotton Yield and Root Growth

A. F. ROBINSON,¹ C. G. COOK,² A. WESTPHAL,³ AND J. M. BRADFORD⁴

Abstract: Damage to cotton by *Rotylenchulus reniformis* below plow depth was evaluated in a sandy clay loam soil at Weslaco, Texas. In December 1999, 14 holes on 51-cm centers were dug 91 cm deep along the planting bed and adjacent furrow and 2 ml of 1,3-dichloropropene was placed 91, 61, and 30 cm deep as each hole was refilled and packed. This technique eliminated 96%, 81%, and 74% of *R. reniformis* down to 107 cm at distances 0, 25, and 51 cm laterally from the point of application ($P \leq 0.05$), whereas chisel fumigation at 168 liters/ha 43 cm deep reduced nematode numbers only in the top 61 cm ($P \leq 0.001$). Manual placement of fumigant increased yield 92%; chisel fumigation increased yield 88% ($P \leq 0.005$). A second experiment in February 2001 placed fumigant 43 or 81 cm deep, or at both 43 and 81 cm. Holes alone had no significant effect on nematode density at planting, midseason or harvest, on root length density at midseason, or on cotton lint yield. Fumigant at 43 cm reduced nematode numbers above fumigant application depth at planting 94% ($P \leq 0.02$), at midseason 37% ($P \leq 0.09$), and at harvest 0%, increasing yield 57% ($P \leq 0.002$). Fumigant at 81 cm reduced nematode numbers above fumigant application depth at planting 86% ($P \leq 0.02$), at midseason 74% ($P \leq 0.02$), and at harvest 48% ($P \leq 0.01$), increasing yield 53% ($P \leq 0.002$). Fumigating at both 43 and 81 cm reduced nematode numbers above 90 cm 94% at planting and 79% at midseason, increased midseason root-length density 14-fold below 76 cm, and doubled yield ($P \leq 0.02$ in all cases).

Key words: cotton, fumigation, *Gossypium hirsutum*, nematode, reniform, *Rotylenchulus reniformis*, vertical distribution, yield.

Upland cotton (*Gossypium hirsutum* L.) is a deeply rooting annual crop developed originally from primitive racestocks that generally occur naturally in semi-arid environments of the tropics and subtropics of Mexico and the Caribbean basin. Ninety-eight percent of all cotton grown in the United States is upland. *Rotylenchulus reniformis* Linford & Oliveira is commonly encountered in cotton fields in the southeastern United States and is considered an important yield-limiting factor in cotton production (Blasingame and Patel, 2004; Overstreet and McGawley, 1997). Its major impact is in Alabama, Arkansas, Georgia, Louisiana, Mississippi, and the Texas Lower Rio Grande Valley (LRGV). Resistant cultivars are not available.

Most studies of *R. reniformis* on cotton have examined nematodes only from the top 30 cm of soil, i.e. above plow depth. However, Heald and Thames (1980) noted *R. reniformis* to occur 1.75 m deep in an LRGV field. The field was maintained in cotton monoculture and resampled in 1998 (Robinson and Cook, 2001). In 1998, the highest population density (20 nematodes/gram of soil = 15 nematodes/cm³ at 1.3 soil bulk density) of *R. reniformis* was noted 100 cm below the surface. This was in striking contrast with co-occurring *Pratylenchus agilis* Thorne & Malek, which was not detected deeper than 75 cm and was at its greatest density in the top 15 cm. The population density of *R. reniformis* ob-

served would be considered damaging if found within the plow layer. The Cooperative State Agricultural Extension Service of Georgia, Louisiana, Mississippi, and North Carolina recommends nematicide application in cotton if *R. reniformis* densities in the top 30 cm are 2 to 20 nematodes/cm³ soil, depending on the state and time of year (Koenning, 2002; Komar et al., 2003; Overstreet, 2001; Sciumbato et al., 2004).

During 1999–2002, 16 additional cotton fields infested with *R. reniformis* were sampled 105–135 cm deep by the senior author, including two more fields in the central LRGV, two in southern and two in northern Louisiana, six in western Mississippi, one in central and one in southern Alabama, and two in central Georgia (Robinson et al., 2000; Robinson, unpubl. data). In 10 fields, more than half of the *R. reniformis* were below 36 cm whereas roots were concentrated near the surface, primarily in the top 24 cm. Subsequent studies confirmed occurrence of high population densities of *R. reniformis* below plow depth in additional cotton fields in Mississippi (Lee et al., 2003), Tennessee (Newman and Stebbins, 2002), and Texas (Westphal et al., 2004). Records of other species occurring in notably high concentrations deep in the soil are noted by Westphal and Smart (2003).

Standard soil fumigation practices for managing *R. reniformis* in cotton involve chisel placement of fumigant, usually 1,3-dichloropropene at a rate of 14 to 47 liters/ha, 20 to 42 cm deep (Gazaway, 1996; Lawrence and McLean, 2000; Overstreet and Erwin, 2003). Fumigating deeper than this can decrease root-knot nematode damage in cotton (Lembright et al., 1968). In California, chiseling 1,3-dichloropropene 46 to 56 cm deep at 84 liters/ha in cotton fields infested with *Meloidogyne incognita* (Kofoid & White) Chitwood increased yields 9% to 13% beyond those obtained in plots also chiseled 46 to 56 cm deep but with fumigant applied only 20 to 25 cm deep (Lembright et al., 1968).

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¹ Nematologist, Agricultural Research Service, U.S. Department of Agriculture, College Station, TX 77845.

² Global Coordinator, Cotton Breeding and Germplasm, New Traits Development, Syngenta Seeds, Inc., Victoria, TX 77905.

³ Assistant Professor, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907-2054.

⁴ Supervisory Soil Scientist, Agricultural Research Service, U.S. Department of Agriculture, Weslaco, TX 78596.

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E-mail: frobinson@cpru.usda.gov

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The objective of this research was to employ soil fumigant to assess the impact of *R. reniformis* below plow depth on cotton. This study is the first investigation of the impact of *R. reniformis* below the plow layer on cotton development and yield, as well as the first investigation of fumigating deeper than 75 cm in cotton. Our approach differed from that in the Westphal et al. (2004) study in that the present study site had been in continuous cotton rather than a soybean or sorghum rotation and had a siltier soil that was more typical of cotton fields where *R. reniformis* occurs in the LRGV (Robinson et al., 1987). Moreover, treatments were tested in 2 consecutive years with effects on yield, nematode populations, and root growth throughout the soil profile measured both years. A preliminary report has been published (Robinson et al., 2001).

MATERIALS AND METHODS

Soil collection and analysis: Soil was collected with a 14-cm-diam. posthole digger or a 3.3-cm-diam. 122-cm-long Environmentalists Subsoil Probe Plus (Clements Associates Inc., Newton, IA 50208) and separated into 15.2-cm vertical increments, each of which was thoroughly mixed and divided into 100-g or 40-g subsamples for analysis. Nematodes were extracted by the Baermann funnel technique (Robinson and Heald, 1991). Soil texture determinations were made by the Bouyoucos method (Piper, 1944). Soil moisture determinations were based on weight loss overnight at 105 °C divided by the soil dry weight. Roots were extracted by suspending a 40-g subsample in 8 liters water and decanting into nested sieves with sequential openings of 425, 180, and 150 µm. Root fragments were transferred with forceps from the 150-µm sieve to 2% formaldehyde solution and stored at room temperature. Total root length per sample was measured with a Win/Mac Rhizo root scanner (Regents Instruments, Ltd., Quebec, Canada).

Fumigant: The fumigant used in all experiments was 1,3-dichloropropene (Telone II).

1999–2000 test: All tests were conducted at the U.S. Department of Agriculture (USDA) North Farm, Weslaco, Texas. The field had a sandy clay loam soil with a uniform A horizon that extended below 1 m throughout the field. The precise textural composition changed slightly with depth from 56.9% sand, 21.9% silt, 21.2% clay at the surface to 50.9% sand, 30.2% silt, 18.9% clay at 1 m (mean of two cores). The field had been planted continuously to cotton with a winter fallow each year for more than 5 years and was considered uniformly infested with *R. reniformis* because 100% of 424 samples taken arbitrarily from the field at midseason and harvest in 1998 and 1999 were positive for *R. reniformis*, with no differences in population density across the field. Deep fumigation of a small area was achieved on 8 December 1999 by digging 14-cm-diam.

postholes 90 cm deep and placing 2 ml of fumigant at depths of 90, 60, and 30 cm as each hole was refilled and the soil compacted to simulate original soil structure. Seven holes were dug on 51-cm centers along the top of the bed the same day that beds were formed and another seven along the adjacent furrow 51 cm to one side, for a distance of 3 m (Fig. 1). Beds were spaced on 101.6-cm centers; hence, fumigant was applied at 232 liters/ha. A control plot 1.5 m down the row from the fumigated area was treated similarly but no fumigant was applied (Fig. 1).

On the same day and in the same part of the field, a chisel fumigation experiment with three replications in a randomized complete block design was established to compare results obtained by manual deep fumigation

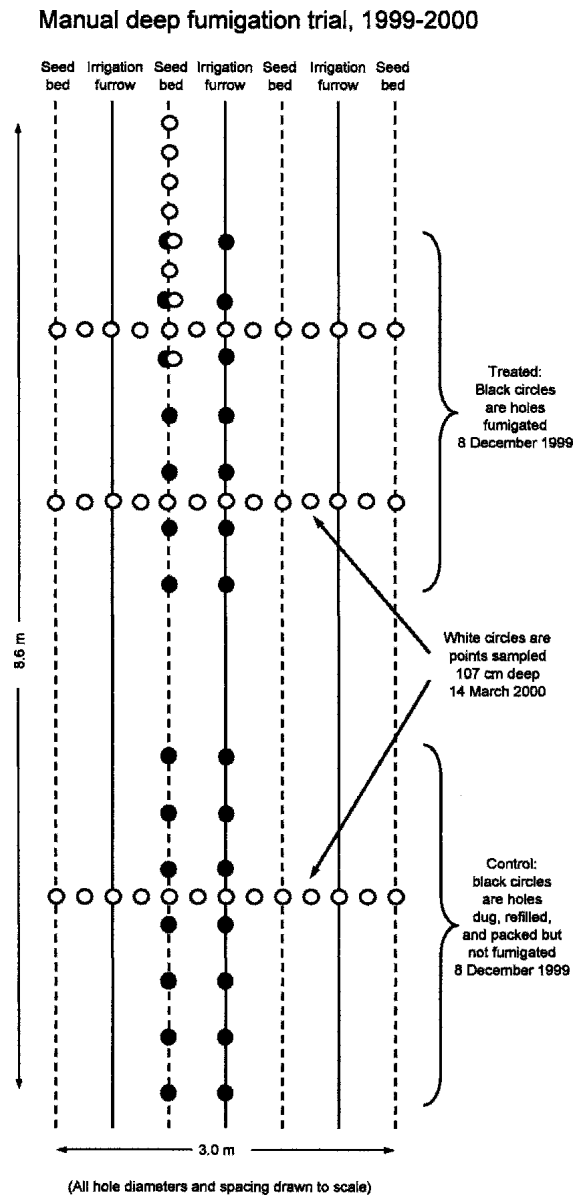


FIG. 1. Diagram of soil fumigation and sampling scheme for first trial examining effects of 90-deep fumigation on *Rotylenchulus reniformis* and cotton growth and yield during 1999–2000.

to those achievable with tractor-drawn chisel fumigation. Plots were 5 m long and four rows wide. Fumigant (168 liters/ha, 43 cm deep) was applied before bedding to three randomly selected plots with parabolic shanks spaced on 51-cm centers, parallel to the row direction. Controls included three plots that were chiseled but not fumigated and three plots that were neither chiseled nor fumigated.

Soil samples for nematode analysis were collected at cotton planting on 24 March 2000 at depths of 0 to 15, 15 to 30, 30 to 46, 46 to 61, 61 to 76, 76 to 91, and 91 to 107 cm (Fig. 1). Points where fumigant had been placed were identifiable as slight depressions in the surface. Samples from the manually fumigated area consisted of two series of 13 and one series of seven holes, all on 25-cm centers along the top of the fumigated bed or perpendicular to it. A fourth series of 13 samples 107 cm deep on 25-cm centers was taken perpendicular to the row direction in the control plot (Fig. 1). Thus, within the manually fumigated plot, samples could be pooled based on distance from fumigant placement. There were eight samples within the lines where fumigant had been placed; two samples halfway between the lines; five samples each that were 25, 51, and 76 cm from either line; and nine samples 1 m or more from either line. Data from samples at each distance were pooled for analysis. Within the non-fumigated posthole control plot, all 13 samples were more than 3 m from the nearest point where fumigant had been placed and were pooled. Graduated vertical samples to 107 cm were collected also in each of the nine plots of the chisel fumigation experiment, and at six additional untreated points spaced across the field.

The cotton cultivar Delta and Pine Land 50 was planted 24 March 2000. Seed cotton was harvested from 1.84 m in each plot on 20 July with lint yield calculated based on bulked percentage lint turnout.

At harvest, incremented vertical soil samples were taken randomly along the top of the bed of the same row where fumigant had been placed. Three points were sampled from the manually fumigated plot, three from the corresponding control plot, and one from each of the nine plots of the chisel fumigation experiment.

2001 test: An experiment with three replications in a randomized complete block design was established in an adjacent area of the same field where the 1999–2000 test was conducted. Plots were 4.6 m long and two 1.1-m rows wide with one or two border rows of cotton between plots. Soil was fumigated on 8 February 2001 as follows. In each fumigated plot, seven holes were dug 43 or 81 cm deep on 51-cm centers along two adjacent beds to provide 14 holes/plot. In total, 18 sets of holes were dug to permit three replications of six treatments in a randomized complete block design. Treatments included: (i) 2 ml fumigant placed at 43 cm (39 liters/ha), (ii) 2 ml fumigant at 81 cm (39 liters/ha), (iii) 2 ml

fumigant at both 43 and 81 cm in the same hole (77 liters/ha), (iv) 43-cm holes with no fumigant, (v) no holes planted to susceptible cotton cv. Fibermax 832, and (vi) no holes planted to resistant soybean cv. Padre (Robbins et al., 2001). Treatments 1 to 4 were planted to Fibermax 832. Plots were planted on 8 March and harvested on 27 July.

Two 122-cm-deep vertical soil cores were collected from each plot at planting, at midseason (21 May), and at harvest. Data collected from each soil layer of each core included soil moisture (controls only), nematode numbers on all three dates, and total root length at midseason. Plant heights at harvest were recorded and cotton yield was measured as in 2000, except that 15.7 m of row was harvested per plot, with seed cotton ginned and lint weighed separately for each row of each plot. Fifty additional 122-cm-deep vertical soil samples, graduated in 15.2-cm increments, were collected for nematode and root density analysis from the surrounding field.

Statistical analyses: Data were subjected to analysis of variance and treatment means for cotton lint yield, plant height, total nematodes 0 to 122 cm deep, and total root length 0 to 122 cm deep and were separated from control values by Dunnett's test when F values were significant. The depth-wise LSD also was calculated for each treatment to facilitate data interpretation and Student's *t* test was used to compare nematode or root densities above and below plow depth, or above and below point of fumigant placement as appropriate.

RESULTS

1999–2000 test: In March 2000, nematode density in the top 107 cm of soil indicated that manual fumigation killed 96% ($P = 0.001$) of nematodes along and between the two lines where fumigant had been placed (Fig. 1) when compared with nematodes more than 1 m away from fumigant placement. Eighty-one percent ($P = 0.001$) of nematodes 25 cm away from either line were killed, and 74% ($P = 0.001$) of the nematodes were killed 51 cm away. Less than 3% of the nematodes 76 cm away from the fumigant placement point were killed. When the average nematode population density at each depth for samples 1 m or more away from fumigant placement was taken as the expected value (i.e. 100%) at that depth, nematode population densities at distances of 25, 51, and 76 cm from fumigant placement indicated lateral movement of effective dosages of fumigant from the point of placement to be greater ($P = 0.01$) in the 46-to-91 than in the 0-to-46-cm layer. For example, at lateral distances of 25 and 51 cm from fumigant placement, 97% and 73% population reductions were indicated in the 46-to-91-cm layer, compared with 0% and 38% reductions in the 0-to-46-cm layer.

March samples indicated that chisel fumigation killed 86% of the nematodes above 61 cm but had no

effect below 61 cm (Fig. 2). Disrupting soil by digging holes or chiseling without applying fumigant had no effect on nematode population density.

At harvest, nematode population densities in manually fumigated and chisel-fumigated plots were 17% (different from 100% at $P = 0.001$) and 116% (not different from 100%), respectively, of the mean density in non-fumigated plots (Fig. 2). Root density in untreated plots was 38, 17, and 8 cm/100 g soil in the top three 15-cm layers of soil and 4 cm/100 g soil in the next three layers. In the 76-to-107-cm zone, chisel and manual fumigation treatments had 205% greater ($P = 0.01$) and 41% greater ($P = 0.05$) root length, respectively, than the untreated plots. Both fumigation treatments doubled yields ($P = 0.001$) (Fig. 1).

2001 test: Samples taken on 8 March indicated that disrupting soil in non-fumigated plots on 8 February by digging 14 postholes had no effect on nematode population density (Fig. 3). Fumigation consistently killed nematodes above the point of application. Plots fumigated at 43 cm had only 6% as many nematodes as control plots in the top 46 cm of soil ($P = 0.01$) but did not differ from controls below 61 cm. Plots fumigated at 81 cm and at both 81 and 43 cm, respectively, had 14% ($P = 0.08$) and 6% ($P = 0.07$) as many nematodes as

control plots 0 to 91 cm deep. Soil moisture varied little with depth, increasing from 15% to 16% between 15 and 107 cm deep.

At midseason, nematode population density 0 to 46 cm deep in plots fumigated only at 43 cm was 37% of that in control plots ($P = 0.09$) (Fig. 3). Population densities 0 to 91 cm deep in plots fumigated at 81 and at both 81 and 43 cm were 26% and 11% of those in controls ($P = 0.015$ and $P = 0.008$, respectively). Disruption of soil by digging holes without fumigating in January had no effect on root-length densities at midseason. Mean root-length densities for controls, in 15-cm increments from the surface downward, were 28, 25, 20, 8, 12, 4, 11, and 2 cm/100g soil. Fumigation at both 43 and 81 cm increased overall root-length density 0 to 122 cm deep by 27% ($P = 0.007$) and increased root-length density within the 76-to-122-cm layer by 14-fold ($P = 0.02$). Soil moisture varied little with depth, gradually increasing from 16% to 18% between 15 and 107 cm deep.

At harvest time, nematode population density 0 to 46 cm deep in plots fumigated 43 cm deep was not different from that of the control (Fig. 3). Population densities 0 to 91 cm deep for plots fumigated 81 cm or both 81 and 43 cm deep were 52% ($P = 0.06$) and 48% ($P =$

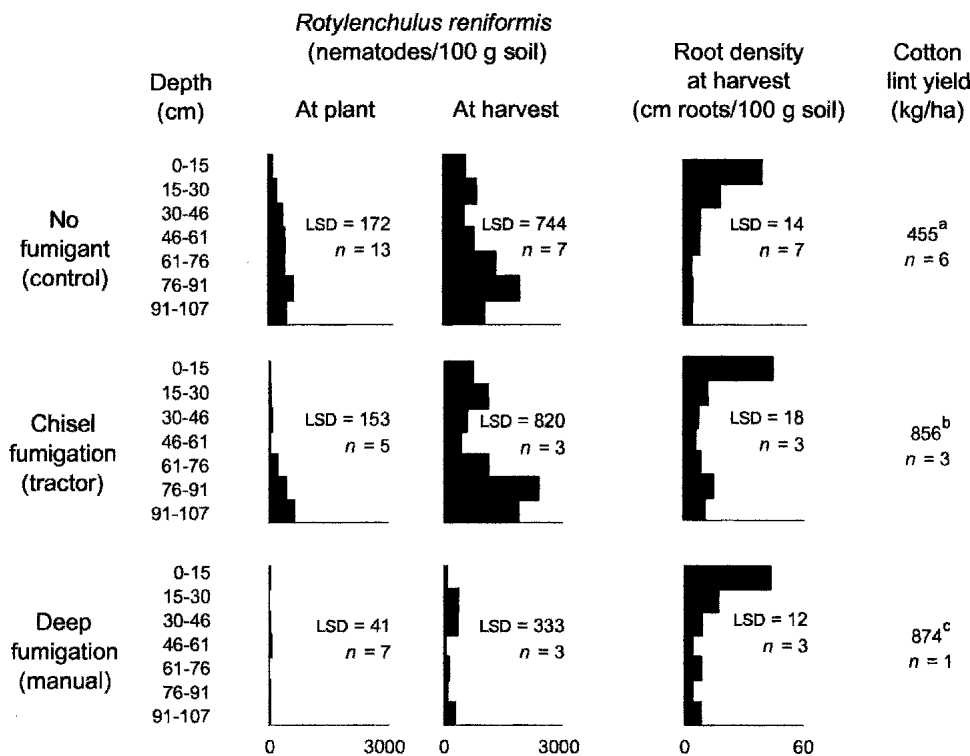


FIG. 2. Comparisons of *Rotylenchulus reniformis* population density, cotton root length density, and lint yield of cotton (cv. Delta and Pine Land 50) in 2000, following fumigation on 8 December 1999 with 1,3-dichloropropene. Chisel fumigation was 43 cm deep. Deep fumigation was manual placement of fumigant 30, 60, and 90-cm deep in holes spaced on 51-cm centers along the bed and the adjacent furrow 51 cm away. ^aYield data for undisturbed and disturbed non-fumigated control were not different and were pooled; ^bDifferent from control by Dunnett's test ($P < 0.01$); ^cYield for single replicate significant based on the following assumptions: no block effect ($P < 0.48$), control population mean equals pooled sample mean, and maximum σ ($\sigma_{\max} = 157$, $P < 0.05$) as estimated from the observed standard deviation ($s = 64$) at critical $\chi^2_{0.975} = 0.831$ (Mack, 1967) indicates probability ($P = 0.0038$) that $Z_{\max} = (Y - \mu) / \sigma_{\max} = 2.67$ exceeds predicted Z (Table A.4; Steel and Torrie, 1980).

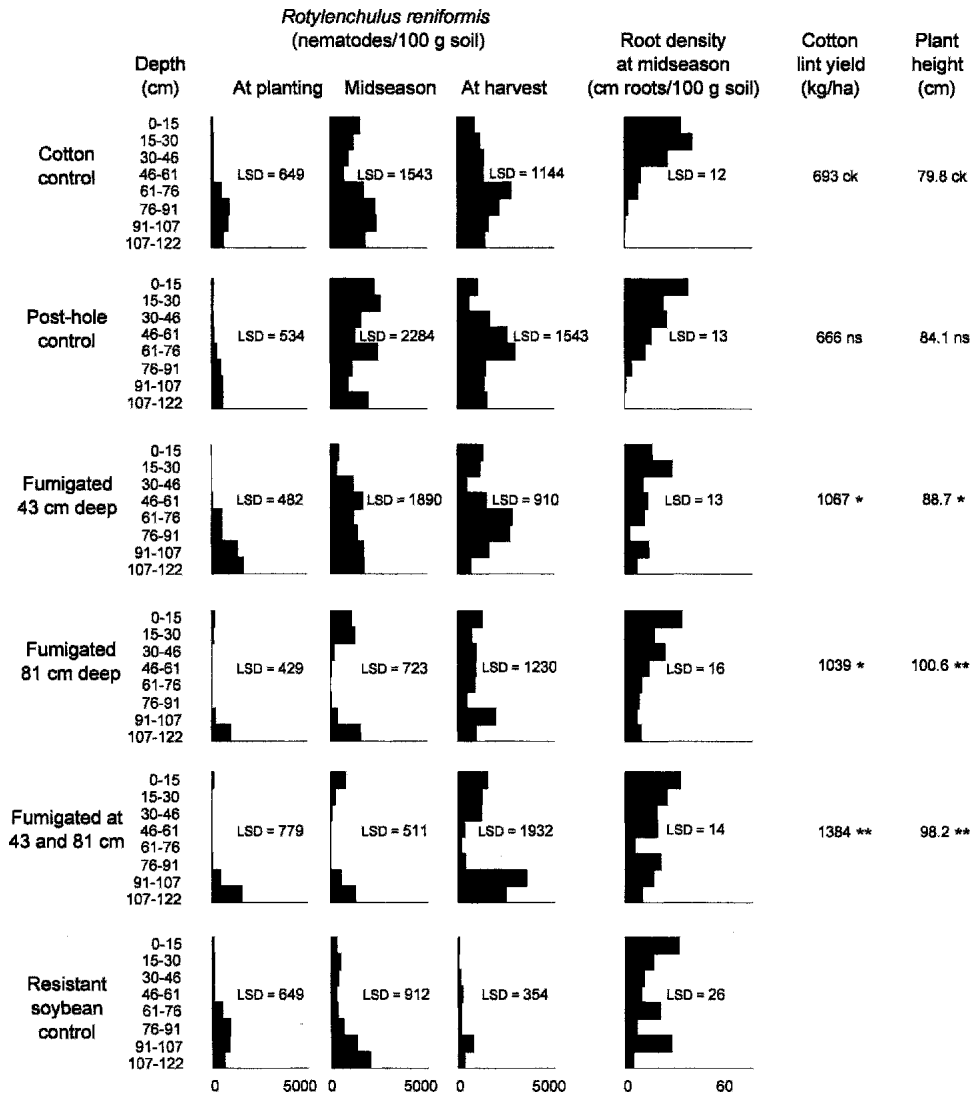


FIG. 3. Comparisons of effects of fumigating soil with 1,3-dichloropropene by manually placing 2 ml of fumigant at various depths on 51-cm intervals down the planting bed on 8 February 2001 on populations of *Rotylenchulus reniformis* in the soil profile during the season and vertical root distributions, fiber lint yield, and plant height of cotton cv. Fibermax 832. * $P < 0.05$; ** $P < 0.01$; ns = not significant; ck = statistical control.

0.008) of those in control plots, respectively. Fumigation at 43, 81, and 43 + 81 cm increased plant height by 11%, 26%, and 23% ($P = 0.0002$) and increased yield by 54%, 50%, and 99% ($P = 0.002$), respectively (Fig. 3).

Soybean cv. Padre suppressed the density of vermiform *R. reniformis* 0 to 122 cm deep at harvest by 86% ($P = 0.0001$) when compared with susceptible cotton (Fig. 3).

Soil moisture at harvest was notably lower throughout the soil profile than at earlier dates, increasing from 7% to 11% between 15 and 107 cm deep.

DISCUSSION

In cotton, economic returns from application of nematicides to manage *R. reniformis* often are marginal or inconsistent due to high fumigant prices, soil-dependent variability in fumigant efficacy, relatively low efficacy of non-fumigant nematicides, and low lint

prices (Overstreet et al., 2002; Zimet et al., 1999). Anecdotal reports based on observations across hundreds of infested fields (Gazaway, 1996; Hollis, 2003) consider cotton stunting and growth responses to fumigation in fields infested with *R. reniformis* to be stress-dependent, emphasizing the need to identify interactive factors contributing to yield loss when *R. reniformis* is present in the soil. In microplots, cotton yield suppression was dependent on strong interactions between soil texture and year (Koenning et al., 1996). Knowledge only of the population level in conventional samples from a field does not appear to be sufficient information for a reliable management decision.

Cotton commonly produces roots that penetrate soil and extract water and nutrients more than 1 m deep (Hons and McMichael, 1986; McMichael and Quisenberry, 1993; Oosterhuis and Bourland, 2001). Because only a small portion of the root system of a plant may be responsible for a large part of the total water uptake,

deep roots can be critical to crop water status even when they comprise a small portion of the total root volume (Stone et al., 1976). The rate of root growth at any depth, however, is critically dependent on soil moisture. Root length density of cotton increased deep in the soil and decreased in upper soil layers in several commercial cotton varieties when soil was allowed to dry (McMichael, 1986). Root length increase of cotton at a given soil layer was observed to stop when water content in that layer fell below $0.06 \text{ cm}^3/\text{cm}^3$, equivalent to -0.1 MPa water potential (Taylor and Klepper, 1974). Thus, sufficiently dry soil can inhibit the production of roots needed to access water in deep as well as shallow layers. In both of our experiments, penetration of cotton roots into soil layers below 75 cm was enhanced by fumigation. Yields were about twice as high as the untreated control and also increased substantially relative to shallow fumigation treatment. In 2001, plants were taller and more vigorous after fumigation. Our results confirm that high population densities of *R. reniformis* below plow depth retard the penetration of cotton roots into deep soil layers, contributing to stress that suppresses plant vigor and yield when moisture and nutrients in the upper soil layers become limiting.

Results from our first fumigation experiment in 1999 stimulated an experiment in 2001 at Monte Alto, Texas (Westphal et al., 2004), where an ongoing crop rotation study provided an opportunity to contrast effects from fumigation with those from a previous *R. reniformis*-resistant soybean rotation. At Monte Alto, soil was fumigated with 1,3-dichloropropene by pressing 120-cm-long injection rods into the soil at 51-cm intervals along the planting bed and fumigating at two shallow (15 and 45 cm) or two deep (75 and 105 cm) points. The 15 + 45-cm and 75 + 105-cm treatments eliminated most nematodes above the point of fumigant placement and provided 28% and 68% yield increases, respectively. Because the deeper treatment also killed many nematodes in the upper layer, it is not possible to separate its effects precisely; however, 40% or more yield gain beyond that obtained with the shallow treatment was measured. In crop rotation comparisons, the average *R. reniformis* population density in the top 120 cm of soil at harvest after the resistant soybean cv. Hyperformer HY798 was suppressed 85%, based on the average of midseason and harvest samples, when compared with the susceptible soybean cv. D6880RR. The soil at that study site averaged 69% sand and thus contained more sand than most of the deep alluvial soils of Texas where *R. reniformis* is problematic (Robinson et al., 1987; Starr et al., 1993).

The 86% suppression of *R. reniformis* we observed 0 to 122 cm deep with resistant soybean was comparable to that observed by Westphal et al. (2004) in the LRGV and to reductions observed in July and November by Davis et al. (2003) in Georgia, supporting the potential of resistant soybean as a rotational crop for managing

R. reniformis in cotton. Our nematode population density data in 1999 indicated greater lateral movement of fumigant in deep than in shallow soil layers. This effect could offset the obvious economic challenge of applying fumigants deeply enough to manage *R. reniformis* deep in the soil in cotton. In Tennessee, Newman and Stebbins (2002) observed ca. 50% mortality of *R. reniformis* 46 to 91 cm deep and significant yield increases after side-dressing cotton with aldicarb, indicating that non-fumigant nematicides might be more cost-effective against deeply occurring *R. reniformis* if application methods and timing were optimized.

Our study was not designed to discover more effective ways to control deeply occurring *R. reniformis* in cotton. Rather, this study was conducted to test whether those nematodes have an economically significant impact on the crop. Lint yields in our control treatments were 436 kg/ha in 2000 and 716 kg/ha in 2001, and were profitable at market prices those years. Any additional yield could then be considered profit after paying nematicide application costs. In 2000, although lint yield was doubled by fumigation regardless of application depth, the chisel fumigation rate (168 liters/ha) was six times the rate most commonly used in cotton. In 2001, when lower rates were used, yield also was doubled by fumigating at both 43 and 81 cm (77 liters/ha when combined), and a 50% yield improvement was obtained with 39 liters/ha at either 43 cm or 81 cm. Apparently, fumigation rates were too high in 2000 to distinguish between placement depths, whereas in 2001, as in the Westphal et al. (2004) experiment, the yield increase that could be attributed to deeply occurring nematodes was in the range of 33% to 50%. Both years, the impact of *R. reniformis* on yield appeared important and comparable to year effects from weather.

Assuming prices for 1,3-dichloropropene (US\$2.87/liter) and cotton lint (US\$1.32/kg) from Zimet et al. (1999), the relative cost of 1,3-dichloropropene can be taken at 2.2 kg of cotton lint/liter of fumigant applied. The relative economic returns that we observed in 1999 were 2.4 and 1.8 kg lint/liter fumigant applied for chisel and manual fumigation, respectively. In 2001, the relative returns for the 43-cm, 81-cm, and 43 + 81 cm fumigation treatments were 9.6, 8.9, and 9.0 kg lint/liter fumigant. Yield increases, therefore, offset nematicide cost in four of five cases. However, we still know almost nothing about variability in the impact of deeply occurring *R. reniformis* in other cotton-growing regions, or about the extent to which nutrient and water stress exacerbate disease symptoms. We conclude that the impact of deeply occurring *R. reniformis* in cotton merits further study.

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