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Effect of Mowing Cotton Stalks and Preventing Plant Re-Growth on Post-Harvest Reproduction of *Meloidogyne incognita*

Ping Lu,[1] Richard F. Davis,[2] Robert C. Kemerait[1]

Abstract: The southern root-knot nematode (*Meloidogyne incognita*) is a major parasite of cotton in the U.S., and management tactics for this nematode attempt to minimize population levels. We compared three post-harvest practices for their ability to reduce nematode population levels in the field, thereby reducing initial nematode population for the next year's crop. The three practices tested were: 1) chemical defoliation before harvest plus cutting cotton stalks after harvest, 2) chemical defoliation plus applying a herbicide to kill plants prior to cutting the stalks, and 3) chemical defoliation without cutting stalks. Experiments were conducted in both the greenhouse and in the field. The greenhouse experiments demonstrated that *M. incognita* reproduction (measured as egg counts and root gall rating indices) was significantly greater when stalks were not cut. Cutting stalks plus applying herbicide to kill cotton roots did not significantly reduce nematode reproduction compared to cutting stalks alone. In field experiments, cutting stalks reduced egg populations and root galling compared to defoliation without stalk cutting. In a greenhouse bioassay which used soil from the field plots, plants grown in soil from the defoliation only treatment had greater root gall ratings and egg counts than in the stalk cutting plus herbicide treatment. Therefore, we conclude that cutting cotton stalks immediately after harvest effectively reduces *M. incognita* reproduction, and may lead to a lower initial population density of this nematode in the following year.

Key words: Cotton, cultural control, defoliation, *Gossypium hirsutum*, herbicide, *Meloidogyne incognita*, nematode management, post-harvest, reproduction, roots, southern root-knot nematode.

Cotton (*Gossypium hirsutum*) is the most important fiber crop to the textile industry. Cotton production in the U.S. has increased 66% in the past 40 years (Mitchell and Robinson, 2009), and in recent years, the U.S. has produced about 20% of the world's annual supply (Mitchell and Robinson, 2009). The southern root-knot nematode (*Meloidogyne incognita* [Kofoid & White] Chitwood) is a very damaging parasite of cotton and causes significant economic loss. The estimated yield loss of cotton caused by *M. incognita* in the U.S. was 2.4% in 2007, which was greater than any other cotton disease and resulted in a loss of more than 106,000,000 kg of lint (Cotton Disease Loss Estimate Committee, 2008). In Georgia in 2007, *M. incognita* caused a 6% reduction in yield resulting in a loss of 25,000,000 kg of lint (Cotton Disease Loss Estimate Committee, 2008).

Meloidogyne spp. are obligate sedentary endoparasites. The second-stage juvenile (J2) of *M. incognita* can penetrate cotton root tips and migrate through cortical cells at temperatures above 18°C (Roberts et al., 1981). Once the J2 establish feeding sites in the differentiated region of the vascular tissue, they lose the ability to move and will complete their life cycle inside the root (Bridge and Starr, 2007). The development and reproduction of *M. incognita* requires both a temperature above 10°C (Vrain et al., 1978) and a living host. When a host plant dies, a *M. incognita* feeding site will deteriorate and the nematodes will not be able to survive.

Cotton harvest in the southeastern U.S. often occurs in October when soil temperatures are still above the threshold of *M. incognita* development, therefore, *M. incognita* that have already entered roots and established feeding sites prior to harvest should be able to continue development and reproduction, thereby leading to an increase in overwintering *M. incognita* populations. Sugar beet cyst nematode (*Heterodera schachtii* Schmidt) development on post-harvest sugar beet (*Beta vulgaris*) plants has been documented (Steele, 1972), but post-harvest development of *M. incognita* on cotton has not. It is not known whether post-harvest nematode reproduction in cotton can significantly increase nematode population levels. If the nematode population level significantly increases following harvest, the likelihood of having greater initial inoculum for the next year's crop increases.

Defoliation is a preparation for harvesting cotton that is accomplished by applying chemicals which induce plants to form abscission layers causing the petiole to detach from the stem (Ayala and Silvertooth, 2001). The defoliation chemicals do not kill the plants, and regrowth of the plants commonly occurs until cold weather stops plant growth (Showler et al., 2006). If the plants remain alive and temperatures are above the nematode developmental threshold, *M. incognita* may continue to produce eggs.

Cotton is a perennial shrub in its native habitat. In Georgia, intact cotton stalks following harvest may shelter boll weevils (*Anthonomus grandis*) (Lemon et al., 2003). Therefore, cotton stalks are mowed after harvest, in part to help control the cotton boll weevil. But growers, at least in the southeastern U.S., usually will not mow cotton stalks until all or most of their fields are harvested. It often can be one to two months after harvest before the cotton stalks are mowed.

The purpose of this study was to determine whether *M. incognita* reproduction continues on cotton roots following harvest and to evaluate whether post-harvest

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[1]University of Georgia, Department of Plant Pathology, P.O. Box 748, Tifton, GA 31793, USA.
[2]USDA Agricultural Research Service, Crop Protection and Management Research Unit, P.O. Box 748, Tifton, GA 31793, USA.
Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the University of Georgia or the United States Department of Agriculture.
E-mail: plu758@gmail.com
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reproduction was influenced by simple cultural practices. The specific objectives of this study were: 1) to document the extent of *M. incognita* reproduction following defoliation of cotton plants, and 2) to determine whether mowing cotton stalks or herbicide application influenced the post-harvest reproduction of *M. incognita*.

Materials and Methods

The experiment was conducted at two field sites at the University of Georgia Coastal Plain Station, Tifton, GA in 2007 and in two greenhouse trials using sterilized soil inoculated with *M. incognita* second-stage juveniles (J2). Greenhouse bioassays were also conducted using soil collected from plots at the end of the field experiments in 2007.

The two greenhouse trials were conducted in 2008 using steam pasteurized soil (Tifton Loamy Sand; 83% sand, 9% silt, 7% clay, and <1% organic matter). The cotton variety used in greenhouse trials was the non-Roundup Ready cv. DP 5415. The first trial was planted in 15-cm-diam. pots on 14 July 2008 and the second trial was planted on 28 July 2008. Both trials used a randomized complete block design with ten replications. Thirty days after planting, 4,000 *M. incognita* J2 were added to each pot. Nematode inoculum was collected by mist extraction for 72 hr from eggplant (*Solanum melongena*) roots cultured in the greenhouse. Inoculum was placed into two holes approximately 2.5 cm deep near the base of the plant, the holes were covered with soil, and the pots were watered. Seven d after inoculation, all plants were sprayed with chemical defoliants. The three treatments tested were: 1) defoliate plants, cut stalks 7 d later and allow re-growth to occur after cutting the stalks; 2) defoliate plus kill plants with a systemic herbicide to prevent re-growth and cut stalks 7 d after defoliation; and 3) defoliate plants but do not cut the stalks and allow re-growth to occur after defoliation. With all three treatments, the plants were defoliated with Thidiazuron (Dropp SC at 234 ml/ha; Bayer CropScience, Research Triangle Park, NC) plus Tribufos (Folex 6 EC at 1.17 l/ha; Amvac Chemical Corporation, Los Angeles, CA). Glyphosate herbicide (Roundup; Monsanto, St. Louis, MO) was applied to treatment 2 at 7.9 ml/liter. On 25 September (for test 1; on 9 October for test 2), 30 d after cutting stalks, cotton roots were removed from the soil and rinsed to remove adhering soil. The roots were weighed, rated for galling on a 0 to 5 scale with 0 = no galling, 1 = trace infection with a few small galls, 2 = galling evident on <25% of the roots, 3 = 25-50%, 4 = 50-75% and 5 = >75% of the roots galled (Kinloch, 1990). Nematode eggs were extracted from roots with 1.25% sodium hypochlorite (NaOCl) for 4 min (Hussey and Barker, 1973) and J2 were extracted from 100 cm^3 soil from each pot using centrifugal-flotation (Jenkins, 1964) and counted. Data from the two tests were pooled for analysis of variance and means were separated by Fisher's least significant differences (LSD; $P \leq 0.05$). Egg count data was log transformed prior to analysis.

Treatments at the two field sites were arranged in a randomized complete block design with six replications in trial 1 and eight replications in trial 2. Plots were 12.2 m wide in trial 1 and 9.1 m long in trial 2, and all plots were 2 rows wide. The three treatments applied in the field were: 1) defoliate plants and harvest cotton, cut stalks after harvest but allow re-growth to occur; 2) defoliate plants and harvest cotton, kill plants with a systemic herbicide prior to cutting stalks; and 3) defoliate plants and harvest cotton but do not cut the stalks to allow any re-growth to occur. Cotton cv. DP 555BR was planted in one field and cv. DP 458BR in the other. Cotton plants were defoliated on 1 October 2007. The harvesting date was 19 October 2007 for the field with DP 458BR, and was 31 October 2007 for the field with DP 555BR. The defoliation chemicals and rates used were Ethephon (Boll'D at 2.3 liter/ha; Winfield Solutions, LLC, St. Paul, MN), Thidiazuron (Dropp SC 3.2 at 234 ml/ha) and Tribufos (Folex 6 EC at 1.2 liter/ha). On 29 October 2007, approximately 4 to 6 wk after defoliation, cotton stalks were mowed in treatment 1 and soil samples were collected from all treatments to assess *M. incognita* J2 population levels. Flumioxazin (Valor® at 0.4 liter/ha; Valent, Walnut Creek, CA), a systemic herbicide, was applied to treatment 2 on 30 October 2007 to kill the cotton roots. Seven d later the stalks were cut in treatment 2. On 27 November 2007, which was approximately two mon after defoliation, roots and samples were collected to assess root mass, galling (on a 0 to 10 scale based on percentage of the root system galled; 0 = no galling, 1 = 10% galled, 2 = 20% galled, etc., and 10 = 100% galled), and *M. incognita* egg and J2 populations. Gall ratings were made on 10 plants per plot. Five root systems and approximately 500 cm^3 of soil were collected from each plot for egg and J2 extraction, using the methods described above. Soil temperatures (20 cm deep) were recorded by a weather station on a nearby farm from 1 October 2007 (defoliation) until 27 November 2007 (root and soil collection), and degree-days above the base developmental temperature of 10°C for *M. incognita* were calculated (daily mean temperature minus base temperature) (Vrain et al., 1978; Ploeg and Maris, 1999). Data from the two tests were pooled for analysis of variance and means were separated by Fisher's LSD ($P \leq 0.10$). A $\log_{10}$ transformation was used prior to analysis of egg count data.

Two greenhouse bioassay trials using soil collected from each field plot at the end of the field experiments (28 November 2007) were conducted to provide an additional measure of nematode population levels. As in the field, there were six replicates of each treatment in trial 1 and eight replicates in trial 2. Eggplant cv. Florida Market seedlings, approximately 6 wk old, were planted (one per pot) in pots containing soil collected

from the different field plots. Sixty-four d after transplanting, the roots of eggplants were separated from soil, weighed, and rated for galling on a 0 to 10 scale as described above. *Meloidogyne incognita* eggs were extracted from the roots with 1.25% NaOCl for 4 min and counted. Data from the two tests were pooled for analysis of variance and means were separated by Fisher's LSD ($P \leq 0.05$). Egg count data was log transformed prior to analysis.

Results

In the greenhouse trials, there was no interaction between trial and treatment for egg populations or root weights, so data were pooled for analysis. The plants with the defoliation only treatment had lower root weights (data not shown) but higher egg populations than plants where the stalks were cut (Table 1). There was a significant interaction ($P = 0.029$) between trial and treatment for the gall rating index data. In trial 1, the defoliation only treatment had significantly higher gall ratings than the other two treatments, but in trial 2 there were no significant differences among treatments. The difference between cutting stalks and cutting stalks plus herbicide treatment was not significant for root weights, gall ratings, and egg populations. There were no differences in *M. incognita* J2 populations among the three treatments in the pooled analysis of variance, but J2 populations (data not shown) were very low with treatment means ranging from 1.6 to 3.2 J2/ 200 cm^3 soil.

In the field experiments (Table 2), the stalk cutting only treatment had significantly higher J2 populations than the other two treatments immediately after cutting (28 d after defoliation). However, 1 mon after the treatments were applied (57 d after defoliation), gall ratings and egg populations in the stalk cutting only treatment were significantly lower than the other treatments. At the end of the field experiments, there were no differences in J2 populations among the treatments. No difference was observed between the defoliation only and the stalk cutting plus herbicide treatments for any parameter. The number of accumulated degree-days (base 10°C) between defoliation and collection of root and soil samples at the end of the field experiments was 603 degree-days.

The results of the bioassay trials (Table 3), which used soil collected from each field plot at the end of the field experiments, were similar to the results of the greenhouse trials in that plants grown in soil from the defoliation only treatment had greater gall ratings and egg populations than in the stalk cutting plus herbicide treatment. Although gall ratings did not differ between the stalk cutting and stalk cutting plus herbicide treatments, egg populations were lower in the stalk cutting plus herbicide treatment. Neither gall ratings nor egg populations differed between the stalk cutting without herbicide and the defoliation only treatment.

Table 1. Effect of post-harvest cotton treatments on *Meloidogyne incognita* egg populations and root galling of cotton (*Gossypium hirsutum*) in two greenhouse trials.

Treatment[a]	# eggs/root system[b]	Gall rating[c] (Trial 1)	Gall rating (Trial 2)[d]
Cutting stalks	2,580 b[e]	0.3 b	2.8 a
Cutting stalks + herbicides	2,436 b	0.3 b	1.8 a
Defoliation only	15,720 a	3.5 a	3.0 a
P-value	0.0004	<0.0001	0.4532

[a]See text for details of treatments.
[b]Data for the two tests were pooled for analysis and data was $\log_{10}$-transformed prior to $LSD_{(0.05)}$ comparison among treatments.
[c]Gall rating on a 0 to 5 scale: 0 = no galling, 1 = trace infection with a few small galls, 2 = galling evident on <25% of the roots, 3 = 25-50%, 4 = 50-75% and 5 = >75% of the roots galled (Kinloch, 1990).
[d]Gall rating results from both trials are shown, because there was a significant interaction between trials and treatments ($P = 0.029$).
[e]Means in a column followed by the same letter are not significantly different.

Discussion

This study demonstrates that post-harvest cultural practices can reduce post-harvest *M. incognita* reproduction in cotton. The defoliation only treatment consistently had the greatest egg populations and root galling. It is less clear whether applying a herbicide in addition to cutting the stalks is necessary. The greenhouse and field tests did not suggest an additional benefit from herbicide application, but the greenhouse bioassay trials did. This discrepancy could have been caused by nematode reproduction on weeds in the field plots since galling and egg population data from the field trial was collected only from cotton roots but the bioassay data was derived from soil from those plots and would also have included any nematodes produced on weeds. *Meloidogyne incognita* has a very wide host range, and many weeds in agricultural fields may serve as hosts (Tedford and Fortnum, 1988). Although most weeds are not better hosts for *M. incognita* than cotton, some can still lead to a significant amount of egg production (Davis and Webster, 2005). Applying herbicide may not decrease nematode reproduction on cotton roots, but killing weed hosts may contribute to nematode suppression. However, because applying a herbicide would require significant addition, labor and expense, more evidence of its effectiveness is needed before it can be recommended.

The greenhouse and bioassay experiments provided greater precision than the field experiments over data collection, in part because it is difficult to recover smaller lateral roots when removing cotton root systems from soil. Most of the post-harvest reproduction of *M. incognita* would be expected to be on smaller roots because the nematodes penetrate primarily at the root tips (Hussey, 1985). Additionally, root galling is accumulated throughout the season, therefore, the cotton roots in the field experiments already had significant galling before harvest, which may have masked the relatively small additional root galling after harvest. In the greenhouse and bioassay trials,

TABLE 2. Effect of post-harvest cotton treatments on of *Meloidogyne incognita* eggs and juveniles (J2) populations and root galling of cotton (*Gossypium hirsutum*) in two field experiments in Tifton, GA.

Treatment[a]	Eggs/root system[b]	Gall rating[c]	J2/100 cm^3 soil at stalk cutting 10/29/07	J2/100 cm^3 soil after stalk cutting 11/27/07
Cutting stalks	3,429 b[d]	4.4 b	752 a	409 a
Cutting stalks + herbicides	5,700 a	5.3 a	542 b	480 a
Defoliation only	5,786 a	5.3 a	480 b	539 a
P-value	0.0967	0.0473	0.0273	0.4289

[a]See text for details of treatments.
[b]Data for the two tests were pooled for analysis and data was $\log_{10}$-transformed prior to $LSD_{(0.10)}$ comparison among treatments.
[c]Gall rating on a 0 to 10 scale based on percentage of the root system with galls (0 = no galling, 1 = 10% galled, 2 = 20% galled, etc., and 10 = 100% galled).
[d]Means in a column followed by the same letter are not significantly different.

plants were grown in *M. incognita*-infested soil for a limited period of time and with no preexisting galling, thereby making it easier to detect differences among treatments.

Cutting stalks immediately after cotton harvest appears to be a useful tool in minimizing carryover *M. incognita* population levels. We can speculate that the increase in *M. incognita* populations when stalks are not cut would result in increased damage to a subsequent crop. Approximately 400 to 410 degree-days (base 10°C) are needed for *M. incognita* to complete its life cycle (Vrain et al., 1978; Ploeg and Maris, 1999), so there were more than enough degree-days (603) accumulated in our field experiments to complete a generation following defoliation. There were probably enough degree-days for some nematodes that were already well into the developmental process at the time of defoliation to produce eggs which could then complete an entire life cycle of their own. When winter cover crops that are susceptible to *M. incognita* are grown, they can support additional nematode reproduction following cotton harvest leading to greater subsequent crop damage (Timper et al., 2006). Cutting cotton stalks following harvest prevents further nematode reproduction compared to leaving the stalks intact and should help reduce *M. incognita* on the following crop.

Root-destruction following harvest is a recommended practice for *M. incognita* in tobacco (Johnson, 1989). Although the principle of root destruction for nematode control is the same in cotton, a significant difference is that tobacco in the southern U.S. typically is harvested two or more months before cotton thereby providing a longer period with warmer temperatures for continued nematode reproduction. Root destruction for control of *M. incognita* in cotton has not been reported. However, in a two-year study where cotton roots were physically destroyed by pulling them out of the ground following harvest, at-plant population levels of *Hoplolaimus columbus* the following spring were reduced in one year but not in the other and subsequent yields were not affected (Davis et. al., 2000).

Preventing a nematode population from increasing is preferable to trying to reduce it after it has increased. In this study, cutting stalks immediately after harvest was an effective practice to suppress *M. incognita* reproduction post-harvest. However, the effect was relatively small and additional research is needed to determine whether cutting cotton stalks affects the initial *M. incognita* population or cotton yield in the following growing season.

TABLE 3. Effect of post-harvest cotton treatments on *Meloidogyne incognita* egg populations and root galling of cotton (*Gossypium hirsutum*) in greenhouse bioassays using soil from plots treated in the field.

Treatment[a]	Eggs/root system[b]	Gall rating[c]
Cutting stalks	22,114 a[d]	3.4 a b
Cutting stalks + herbicides	7,329 b	2.2 b
Defoliation only	27,043 a	4.4 a
P-value	0.0274	0.0285

[a]See text for details of treatments.
[b]Data for the two tests were pooled for analysis (N = 14) and data was $\log_{10}$-transformed prior to $LSD_{(0.05)}$ comparison among treatments.
[c]Gall rating based on a 0 to 10 scale based on percentage of the root system covered with galls (0 = no galling, 1 = 10% galled, 2 = 20% galled, etc., and 10 = 100% galled). Data for the two tests were pooled for analysis (N = 14)
[d]Means in a column followed by the same letter are not significantly different.

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