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RESEARCH ARTICLE / *ARTIGO*

Cover crops for reniform nematode suppression in cotton: greenhouse and field evaluations

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ABSTRACT

Two greenhouse and one field experiment were carried out to evaluate the reaction of cover crops to reniform nematode, *Rotylenchulus reniformis,* and their effect on nematode populations in a naturally infested soil (2,359 nematodes/200cm^3) and on cotton yield. Oil radish (*Raphanus sativus*), Mulato grass (*Brachiaria ruziziensis* x *B. brizantha*), forage sorghum (*Sorghum bicolor*), tef (*Eragrostis tef*), foxtail millet (*Setaria italica*), Algerian (*Avena byzantina*) and black (*A. strigosa*) oats, pearl millet (*Pennisetum glaucum*), and finger millet (*Eleusine coracana*) were determined to be poor hosts for *R. reniformis* in greenhouse experiments. Grain amaranth (*Amaranthus cruentus*) and quinoa (*Chenopodium quinoa*) were good hosts to *R. reniformis*. In the field, lower nematode densities were observed after Mulato grass, oil radish and forage sorghum. Higher cotton fiber yields were obtained from plots cultivated with Mulato grass or sorghum during the winter compared to clean fallow. Cotton yield was inversely correlated with both reproduction factor ($p < 0.05$) of the nematode on the winter cover crops and population of *R. reniformis* at cotton planting ($p < 0.01$).
Keywords: *Gossypium hirsutum*, nematode management, no-till cropping system, *Rotylenchulus reniformis.*

RESUMO

Culturas de cobertura para o manejo do nematóide reniforme em algodoeiro: avaliações em casa de vegetação e campo

Foram realizados dois experimentos em casa de vegetação e um experimento em campo, com o objetivo de avaliar a reação de culturas de cobertura ao nematóide reniforme, *Rotylenchulus reniformis,* e seu efeito sobre a população do nematóide em uma área naturalmente infestada (2.359 nematóides/200cm^3) e sobre a produção de algodão. As culturas de nabo forrageiro (*Raphanus sativus*), capim-mulato (*Brachiaria ruziziensis* x *B. brizantha*), sorgo forrageiro (*Sorghum bicolor*), tef (*Eragrostis tef*), capim-moa (*Setaria italica*), aveias amarela (*Avena byzantina*) e preta (*A. strigosa*), milheto (*Pennisetum glaucum*) e capim-pé-de-galinha (*Eleusine coracana*) comportaram-se como maus hospedeiros de *R. reniformis* nos experimentos em casa de vegetação. Amaranto (*Amaranthus cruentus*) e quinoa (*Chenopodium quinoa*) foram bons hospedeiros de *R. reniformis*. No campo, as menores densidades dos nematóides foram observadas nas parcelas onde foram cultivados capim-mulato, nabo forrageiro e sorgo forrageiro. As maiores produtividades de fibra de algodão foram obtidas nas parcelas cultivadas durante o outono e inverno com capim-mulato ou sorgo forrageiro, quando comparadas com aquelas que permaneceram em alqueive. A produtividade de algodão correlacionou-se negativamente com o fator de reprodução do nematóide nas culturas de cobertura ($P < 0,05$) e com a população de *R. reniformis* no plantio de algodão ($P < 0,01$).
Palavras-chave: *Gossypium hirsutum*, manejo de nematóides, sistema plantio direto, *Rotylenchulus reniformis.*

INTRODUCTION

The reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira, 1940) is a major plant-parasitic nematode of cotton worldwide (Starr, 1998), especially in the USA, where it is widely distributed. Reniform nematode is also becoming a very important pathogen in Brazil, being found in almost all important cotton growing areas in the central region of the country (Asmus, 2004). Due to the limited knowledge about non-host plants for crop rotation and resistant cotton cultivars (Robinson *et al*., 1997; Robinson, 2002), its management has been restricted to the use of nematicides, whose detrimental effects on the environment are well known. The use of cotton cultivars more tolerant to *R. reniformis*, and spring or autumn crop rotations with non-hosts to this nematode have minimum impact on the environment, and should be encouraged.

In the absence of host plants and/or under adverse climatic conditions, nematode populations in soil tend to decrease (McSorley, 1998). Therefore, overwinter fallow with weed control (clean fallow) should contribute to nematode management. However, in a no-tillage cropping system, the soil is kept covered by one or more crops during autumn and winter seasons, a condition that is particularly important in tropical areas with high temperature and low rainfall in winter, such as in the Brazilian Central Region

(Salton *et al*., 2001). Depending on the susceptibility of the cover crops used locally, *R. reniformis* populations can increase and reach damaging levels to crops planted the following season (Gallaher *et al*., 1988; Jones & McLean, 2004). Approximately 13.5 million hectares have been cultivated under the no-tillage system in Brazil (Oliveira & Veiga Filho, 2002), and pearl millet represents the main cover crop used (Zancanaro & Tessaro, 2006). Other plant species including amaranth (*Amaranthus cruentus*), quinoa (*Chenopodium quinoa*), forage sorghum (*Sorghum bicolor* and *S. bicolor* x *S. sudanense)* and several annual or perennial grasses have also been tested as cover crops (Spehar & Santos, 2002; Spehar *et al*., 2003; Lamas, 2007). However, their susceptibility to *R. reniformis* was not known. The objectives of this work were: 1) to evaluate the ability of selected plant species potentially suitable for cover crops for suppression of reniform nematode under greenhouse conditions and, 2) to evaluate the effect of these cover crops on soil populations of *R. reniformis* and on cotton yield under field conditions.

MATERIAL AND METHODS

Two greenhouse and one field experiment were carried out from September 2003 to April 2005. The greenhouse experiments were located at Embrapa Agropecuária Oeste, in Dourados, Mato Grosso do Sul State, Brazil. The field experiment was conducted in a cotton field naturally infested with *R. reniformis* (2,359 nematodes/200 cm^3 soil), in Aral Moreira, Mato Grosso do Sul State, Brazil.

Greenhouse Experiments

Host status for *R. reniformis* of 12 cover crop species was evaluated in Experiment 1. The tested plants were grain amaranth (*Amaranthus cruentus* 'BRS Alegria'), two cultivars of black oat (*Avena strigosa* 'Embrapa 140' and 'Common'), finger millet [*Eleusine coracana* 'Agronorte'], two cultivars of forage sorghum [*Sorghum bicolor* 'Santa Elisa 38' and 'IPA 7301011'], foxtail millet (*Setaria italica*), oil radish (*Raphanus sativus* var. *oleiferus* 'Siletina'), pearl millet (*Pennisetum glaucum* 'BRS 1501'), two cultivars of quinoa (*Chenopodium quinoa* 'BRS Piabiru' and 'Common'), and tef (*Eragrostis tef*). French marigold (*Tagetes patula*) was included as a resistant control, according to Caswell *et al*. (1991). Three seeds of each plant species were sown in 500-cm^3 pots containing 400 cm^3 of sterilized substrate (58.5% sand, 7% silt and 34.5% clay) and the seedlings were thinned to one per pot prior to nematode inoculation.

R. reniformis inoculum used in experiment 1 was originally collected from soybean roots (*Glycine max*) and multiplied for 90 days on castor roots (*Ricinus communis*) in the greenhouse. Eggs and larvae of *R. reniformis* were extracted from castor roots using the blender-sieving method followed by sucrose centrifugation (Coolen & D'Herde, 1972) one day before nematode inoculation. Inoculum of 1,216 eggs and larvae was distributed into two holes about 3.0 cm deep and 1.0 cm from the plant stem. Final population (Pf) of *R. reniformis* was estimated 60 days after inoculation. Nematodes were extracted from the roots (Coolen & D'Herde, 1972) and from the substrate (Jenkins, 1964), and the reproductive factor (RF = Pf/Pi) calculated.

In a second experiment (Experiment 2), the tested plants were black oat (*A. strigosa* 'Embrapa 29'), Mulato grass (*Brachiaria ruziziensis* x *B. brizantha*), Algerian oat (*A. byzantina* 'São Carlos'), oil radish 'Siletina', pear millet 'BRS 1501' and forage sorghum 'Santa Elisa 38'. Soybean 'BR 96-25619' and French marigold were included as standard good and poor hosts, respectively. Approximately 1,000 eggs + larvae of *R. reniformis* were inoculated per pot. Sixty days after inoculation, nematodes were extracted from roots and soil using the same procedures as in Experiment 1.

Both experiments were arranged in a completely randomized design with eight (experiment 1) or six (experiment 2) replications. Each experimental unit consisted of a plastic pot with one plant. Data on nematode reproductive factor were log transformed [log (x+1)] prior to analysis of variance, and treatments were compared using LSD test.

Field Experiment

As forage sorghum 'Santa Elisa 38', Mulato grass, pearl millet 'BRS 1501', and oil radish 'Siletina' responded as poor hosts of *R. reniformis* in greenhouse experiments and are species very well adapted for the Central Region of Brazil, they were also included in a subsequent field experiment designed to determine their influence on *R. reniformis* population density and on cotton production. Clean fallow (with manual weed control) was included as *R. reniformis* negative control treatment.

The experiment was carried out between April 2004 and April 2005 at a commercial farm located in Aral Moreira, Mato Grosso do Sul State, Brazil (S22º54'54" W055º31'39", annual average temperature of 25ºC and 1,687 mm annual average rainfall). The experimental design was a completely randomized block with five replications. Each experimental plot was 50 m^2 in size. Cover crops were established immediately after mechanical destruction of crop residues following cotton harvest. On 29 April 2004, cover crops were sown manually in rows 0.40 m apart. The amount of seeds planted was 10 $kg.ha^{-1}$ (forage sorghum and Mulato grass), 15 $kg.ha^{-1}$ (pearl millet), and 3 $kg.ha^{-1}$ (oil radish). Cover crops were grown for six months, and precipitation was recorded. Weeds were managed manually. On 22 October 2004, cover crops were sprayed with glyphosate (3.0 kg $a.i.ha^{-1}$), and on 9 November 2004 cotton 'Delta Opal' was sown directly over the crop residues in eight rows, 0.80 m apart, in each plot. Cultural practices followed local recommendations for no-till practice. On 4 April 2005, all bolls of the two 5.0 m-long central rows were harvested. Lint percentage and boll weight were estimated based on 20 bolls from each plot.

Soil population of *R. reniformis* was determined from a pooled sample consisting of eight soil cores (20-cm depth) taken at random from each plot. Soil samples were collected on 29 April 2004 (just prior to cover crops sowing; initial population = P1), and on 9 November 2004 (at cotton sowing; population after cover crops = P2). A 200-cm[3] subsample from each pooled sample was processed for nematode extraction using sieving and centrifugal flotation (Jenkins, 1964). Roots were sampled from four cotton plants in each plot at flowering (11 January 2005; P3) and the nematodes extracted (Coolen & D´Herde, 1972). After counting, the number of nematodes (eggs and vermiform stages) per gram of root was calculated. Nematodes extracted were fixed by heating the suspension to 55ºC for five minutes and were then kept in 2% formalin. Numbers of nematodes were estimated in Peters' counting slide under microscope. Changes in *R. reniformis* population after cover crops (RFC) were estimated by RFC = P2/P1. Nematode counts were log-transformed (log (x+1)) prior to analysis of variance (ANOVA). Means of treatments were compared using LSD test. RFC, P2, and the number of nematodes/ g of roots were correlated with seed cotton yield using Pearson's correlation analysis.

RESULTS AND DISCUSSION

Greenhouse Experiments

All cover crops tested were poor hosts for *R. reniformis*, except grain amaranth and quinoa, which resulted in RF > 1 (Table 1). The highest RF was observed on grain amaranth 'BRS Alegria', followed by the two cultivars of quinoa. Thus, these plant species should not be used as cover crops in areas infested with the reniform nematode. Host status of these plant species for *R. reniformis* had not been evaluated before. Previously, Lal *et al*. (1976) evaluated the host status of species of *Amaranthus* and *Chenopodium* and rated *Chenopodium murale* as good host, *Amaranthus spinosus* as poor host, and *A. viridis* and *C. album* as non-hosts to *R. reniformis* based on the number of egg-masses and young females per plant. Therefore, host status of *Amaranthus* and *Chenopodium* for *R. reniform* is species-dependent.

Robinson *et al*. (1997) listed host status of *R. reniformis* on 364 plant species tested, and 314 of them showed contradictory host status, such as that shown by *Sorghum bicolor*. Our results showed that both forage sorghum cultivars tested are poor hosts for the nematode. Thus, the host status of sorghum cultivars should be characterized prior to their use as cover crops in *R. reniformis*-infested fields. Our results confirm previous findings that oats and oil radish are poor hosts of *R. reniformis* (Birchfield & Brister 1962; Hutchinson *et al*., 2003; Jones & McLean, 2004). Interestingly, all tested monocots rated as poor host species in our study. This is in agreement with the findings of Ferraz (1985) and the previously mentioned paper. Monocots, especially gramineous species, should be the main crops to be used as rotational or cover crops in *R. reniformis*-infested areas.

TABLE 1 - Reproduction factor (RF)[1] of *Rotylenchulus reniformis* on cover crops under greenhouse conditions

Cover crop	Experiment 1	Experiment 2
Soybean 'BR 96-25619	-	16.50 a
Grain amaranth 'BRS Alegria'	6.68 a	-
Quinoa 'BRS Piabiru '	4.59 b	-
Quinoa 'Common'	3.89 c	-
French marigold	0.79 d	0.31 b
Mulato grass	-	0.09 c
Tef	0.67 d	-
Oil radish 'Siletina'	0.21 e	0.13 bc
Forage sorghum 'Santa Elisa 38'	0.14 e	0.08 c
Forage sorghum 'IPA 7301011'	0.12 e	-
Foxtail millet	0.12 e	-
Black oat 'Embrapa 29'	-	0.04 c
Black oat 'Embrapa 140'	0.12 e	-
Black oat 'Common'	0.10 e	-
Algerian oat 'São Carlos'	-	0.04 c
Pearl millet 'BRS 1501'	0.09 e	0.02 c
Finger millet 'Agronorte'	0.08 e	-
CV(%)	33,31	30,68

[1]RF = Final (Pf)/Initial (Pi) population; Pi = 1,216 nematodes/plant (experiment 1) and 1,000 nematodes/plant (experiment 2)
Means are average of eight (experiment 1) or six (experiment 2) replications. Data followed by the same letter were not different according to LSD test at P = 0.05 based on log (x+1) transformed values.

Field Experiment

Precipitation during the six months that cover crops were grown was 863 mm. All cover crop species established very well with sufficient plant residues for no-till practice. The emergence of cotton was markedly reduced in plots where oil radish had been grown during the winter. For this reason we did not use data from those plots for yield assessment. Allelopathic effects of several Brassicaceae on both cotton germination and vigor were reported by Haramoto & Gallandt (2005). As oil radish proved to have beneficial effects in reducing *R. reniformis* soil populations during winter, further studies to investigate the time needed between burning down oil radish and sowing cotton are urgently needed. In general, there were significant (P < 0.05) differences among treatments after cover crops (P2) and in the middle season of cotton roots (P3) (Table 2). Since there were significant differences in initial populations (P1), the effect of treatments on *R. reniformis* populations was better characterized by the RFC.

Due to the absence of hosts, clean fallow led to a natural decline of *R. reniformis* during winter (Table 2), as was also observed by Sharma *et al*. (1996). However, in plots where Mulato grass, oil radish and forage sorghum were grown, a decrease in nematode populations during winter was greater (P < 0.05) than under clean fallow. Caswell *et al*. (1991) observe that the use of *Crotalaria juncea*, *Chloris gayana* and *Tagetes patula* as inter-cycle cover crops in pineapple fields caused a reduction in the population of *R.*

reniformis that could eventually be compared to a fallow situation. Adverse effects on nematode populations were also observed with oat (*Avena sativa*) cv. Hazek, sorghum (*S. bicolor*) cv. CSH 6, rye (*Secale cereale*) cv. Wren's Abruzzi and hairy vetch (*Vicia sativa*) cv. Chaba White (Ko & Schmitt, 1996; Sharma *et al*., 1996; Gazaway *et al*., 2000). Our results suggest that all tested plants can be considered as good winter cover crops for *R. reniformis* suppression.

The cultivation of cotton after Mulato grass and forage sorghum yielded higher amounts of seed cotton and fiber than cotton cultivation after clean fallow (Table 3). The high fiber percentage and boll weight of cotton after Mulato grass and forage sorghum gave higher cotton fiber production after these cover crops. The number of nematodes at cotton sowing (P2) and in cotton roots [P3 (Rr/ g roots)], and the reproduction factor after cover crops (RFC) were negatively correlated with seed cotton yield (Table 4). From this it can be suggested that cover crops can have a significant effect on cotton yield in infested areas by decreasing *R. reniformis* population.

TABLE 2 - Soil and cotton root populations[1] of *Rotylenchulus reniformis* (Rr) before and after cultivation of selected cover crops during the winter

Cover crop	P1 Rr/200 cm^3	P2 Rr/200 cm^3	RFC (P2/P1)	P3 Rr/g root[z]
Forage sorghum	2,462 ab	960 ab	0.39 b	266 ab
Pearl millet	1,800 b	1,422 ab	0.79 ab	678 a
Oil radish	2,330 ab	766 b	0.33 b	-
Mulato grass	3,068 a	1,182 ab	0.38 b	166 b
Clean fallow	2,114 b	1,818 a	0.86 a	771 a
C.V. (%)	5.19	13.48	71.35	13.81

[1]P1 = 29 April 2004 (cover crops sowing); P2 = 9 November 2004 (cotton sowing); P3 = 11 January 2005 (cotton flowering); RFC = reproduction factor of the nematode after cover crops (P2/P1).
Means are average of five replications. Data followed by the same letter were not different according to LSD test at P = 0.05 based on log (x+1) transformed values.

TABLE 3 - Seed and fiber cotton yields, fiber percentage and cotton boll weight after selected cover cropping in a site naturally infested by *Rotylenchulus reniformis*

Cover crop	Seed cotton ($kg.ha^{-1}$)	Cotton fiber ($kg.ha^{-1}$)	Fiber (%)	Boll weight (g)
Forage sorghum	2,031[z] ab	1,076 ab	52.72 a	5.49 a
Pearl millet	1,931 bc	924 bc	47.87 bc	5.23 bc
Mulato grass	2,294 a	1,178 a	51.18 ab	5.40 ab
Clean fallow	1,657 c	751 c	44.96 c	5.12 c
C.V. (%)	18.43	24.28	10.91	4.93

Means are average of five replications. Data followed by the same letter were not different according to LSD test at P = 0.05.

TABLE 4 - Pearson´s correlation analysis between seed cotton yield and *Rotylenchulus reniformis* (Rr) soil and root populations

Nematode population measured	R	P
P2 (Rr/ 200 cm^3)	- 0.48	0.0075**
RFC	- 0.34	0.0480*
P3 (Rr/ g roots)	- 0.37	0.0339*

P2 = 5 November 2004 (cotton sowing); P3 = 11 January 2005 (cotton flowering); RFC = reproduction factor of the nematode after cover crop (P2/P1); R = Pearson's correlation coefficient; P = probability; * and ** = significant at 5 and 1%, respectively.

Although the main benefit of the cover crops tested was reduction of soil population of *R. reniformis*, maintenance of soil moisture in no-till cover cropping practice may be another positive effect of cover crops on cotton yield. It is not unusual to observe that, even without significant suppression on *R. reniformis* populations, some cover crops may still lead to high cotton production of subsequent crops, as was observed by Gazaway *et al*. (2000) and Jones & McLean (2004), using hairy vetch (*Vicia villosa*) and Crimson clover (*Trifolium incarnatum*), respectively. In these studies, nitrogen left after leguminous crops could have been the reason for high cotton yield.

Although the greenhouse experiments have demonstrated that various cover crops can reduce soil population of *R. reniformis*, field experiments are more important to evaluate the effect of cover crops on cotton yield. Mulato grass and forage sorghum in particular, used as autumn or winter cover crops in fields infested by *R. reniformis*, proved to reduce the nematode population and thereafter increase the yield of seed and cotton fiber. Different cover crops should be considered if fields have been infested by another important cotton nematode, the root-knot nematode, *Meloidogyne incognita.* In this case, Dias-Arieira *et al*. (2003) suggested that *Brachiaria* species should be grown as cover crop. We concluded that the use of cover crops that are non-hosts or poor hosts is an effective strategy against *R. reniformis*. This is especially adoptable in cotton fields that practice no-tillage.

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