EVALUATION OF CROTALARIA JUNCEA POPULATIONS AS HOSTS AND ANTAGONISTIC CROPS TO MANAGE MELOIDOGYNE INCOGNITA AND ROTYLENCHULUS RENIFORMIS

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ABSTRACT

Marla, S. R., R. N. Huettel, and J. Mosjidis. 2008. Evaluation of *Crotalaria juncea* Populations as Hosts and Antagonistic Crops to Manage *Meloidogyne incognitia* and *Rotylenchulus reniformis*. Nematropica 38: 155-162.

Crotalaria juncea populations collected from different countries (PI 207657 from Sri Lanka, PI 314239 from Russia, PI 322377 from Brazil, PI 391567 from South Africa and PI 426626 from Pakistan) were obtained from the National Plant Germplasm System. These *C. juncea* populations were evaluated for their ability to suppress southern root-knot nematode, *Meloidogyne incognita,* and reniform nematode, *Rotylenchulus reniformis* in greenhouse conditions. All *C. juncea* populations were able to significantly suppress ($P \leq 0.05$) *M. incognita* and *R. reniformis* in greenhouse tests. *Crotalaria juncea* roots stained with McCormick Schilling® red food color were found to contain all juvenile stages, low numbers of mature females of *M. incognita* with egg masses and 1-2 female reniform nematodes per 10 gm of roots, indicating that these nematodes were able to infest and reproduce on *C. juncea* populations. However, the reproduction on *C. juncea* was very low when compared to the tomato or cotton controls. Freeze-dried *C. juncea* root exudates tested against both *M. incognita* and *R. reniformis* demonstrated that concentrated root exudates could kill both nematodes.

Key words: root-knot nematode, reniform nematode, sunn hemp, root exudates.

RESUMEN

Marla, S. R., R. N. Huettel, and J. Mosjidis. 2008. Evalvacion de poblaciones de *Crotalaria juncea*, como cultivo de cobertura para el manejo de *Meloidogyne incognita* y *Rotylenchus reniformis*. Nematropica 38: 155-162.

Poblaciones de *Crotalaria juncea*, colectadas en varios países (PI 207657 en Sri Lanka, PI 314239 en Rusia, PI 322377 en Brasil, Pi 391567 en Sur África y PI 426626 en Pakistán), fueron obtenidas del National Plant Germoplam System (Sistema Nacional de Germoplasma Vegetal). Estas poblaciones de *C. juncea* se evaluaron de acuerdo con su capacidad para suprimir el nematodo del nudo radical, *Meloidogyne incognita*, y el nematodo reniforme *Rotylenchus reniformis*, bajo condiciones de invernadero. Todas las poblaciones de *C. juncea* fueron capaces de suprimir significativamente ($P \leq 0.05$) *M. incognita* y *R. reniformis* en las pruebas de invernadero. Raíces de *Crotalaria juncea* teñidas con rojo McCormick Schilling® presentaron todos los estados juveniles, un bajo número de hembras maduras de *M. incognita* con masas de huevos y 1-2 hembras del nemátodo reniforme por cada 10 gramos de raíces. Estos resultados indican que los nematodos fueron capaces de infestar y reproducirse en las poblaciones de *C. juncea*. Sin embargo, la reproducción en *C. juncea* fue mucho mas baja en comparación con la encontrada en tomate y algodón usados como control. Exudados de la raíz de *C. juncea* secos y congelados fueron evaluados contra *M. incognita* y *R. reniformis*, demostrando que los exudados radicales de esta planta pueden matar ambos nemátodos.

Palabras clave: *Crotalaria juncea,* nematode, del nvdo radical, nematodo reniform, crotalaria, exudados radicals.

INTRODUCTION

Crotalaria juncea L. (sunn hemp) is a legume crop that has received attention due to its green manure properties, its potential to reduce soil erosion and improve nutrient levels in soils with low organic matter content (Mansoer *et al.*, 1997). In addition, *C. juncea* is a poor host to many plant-parasitic nematodes and is reported to increase the number of free-living nematodes in the soil (McSorley, 1999). *Crotalaria juncea* can increase nitrogen in the soil especially in organic production systems (Wang *et al.*, 2004b). This combination of nematode and nitrogen management could be especially useful in sustainable and organic production systems where neither nematicides nor synthetic nitrogen fertilizers can be used (McSorley, 1999).

The major constraint affecting the extensive use of *C. juncea* in the continental United States is limited reproduction and non-availability of seeds for large-scale cultivation. *Crotalaria juncea* cannot reproduce except under tropic conditions. In the United States, 'Tropic Sun' is the most common *C. juncea* cultivar available; however, most of the 'Tropic Sun' evaluations and breeding programs have been limited to Hawaii (Rotar and Joy, 1983). To overcome the problem of seed production, a breeding program conducted at Auburn University evaluated *C. juncea* populations collected from different countries. These populations are being evaluated for agronomic characteristics and for their ability to produce seeds under the Southeastern U.S. climatic conditions. This research described herein focuses on evaluating the ability of *C. juncea* populations to suppress the southern root-knot nematode, *Meloidogyne incognita,* and the reniform nematode, *Rotylenchulus reniformis.*

MATERIALS AND METHODS

Crotalaria juncea populations

Populations collected from different countries were obtained from the National Plant Germplasm System. Seed of these populations were increased under the same environmental conditions in 2003 at the E. V. Smith Research Center, Tallassee, AL and under greenhouse conditions. The populations were PI 207657 from Sri Lanka, PI 314239 from Russia, PI 322377 from Brazil, PI 391567 from South Africa and PI 426626 from Pakistan.

Nematode extraction from roots

Meloidogyne incognita and *R. reniformis* populations were maintained on the host plants of tomato (*Solanum lycopersici* cv. Rutgers) and cotton (*Gossypium hirsutum* cv. DP 555 BG/RR), respectively. Nematode eggs on the roots were extracted using 0.6% NaOCl (Hussey and Barker, 1973). The hypochlorite solution containing nematode eggs was poured through a series of sieves with 75-µm sieve on top and 25-µm at the base. Nematode eggs were collected on the 25-µm sieve and were gently washed with water. The solution containing nematode eggs was standardized and quantified under a Nikon-T 100® inverted microscope for greenhouse inoculations.

Greenhouse evaluations of C. juncea populations

Experiments were conducted to evaluate the effect of *C. juncea* populations on plant-parasitic nematodes. *Crotalaria juncea* populations were grown in 500 cm^3 polystyrene cups filled with autoclaved loamy sand soil (72.5%, 25%, 2.5%, S-S-C, pH 6.4) and fine sand in 3:1 ratio. One *C. juncea* seed from each population was hand-sown in each cup. Tomato (*S. lycopersici* cv. Rutgers) and cotton (*G. hirsutum* cv. DP 555 BG/RR) were used as the controls for *M. incognita* and *R. reniformis* evaluations, respectively. The experiments were arranged in a completely randomized block design, replicated eight times and repeated twice. One week after the *C. juncea* germination, individual seedlings were inoculated with ca. 4000 eggs of *M. incognita* or ca. 2000 eggs of *R. reniformis*. After 50 days the *C. juncea* roots were gently washed, fresh weight of the aerial parts and roots were recorded. Nematodes eggs on the roots of *C. juncea* populations were extracted as described above and counted. The number of eggs present per gram of root was calculated.

Staining of C. juncea roots

Crotalaria juncea roots from inoculated plants were gently washed with water and blotted dry. The roots were weighed and 10-gm subsample were cut in to 2-cm-pieces which were suspended for 15 sec in a 500-ml beaker containing 10% (v/v) solution of McCormick Schilling® red food color (Thies *et al.*, 2002). The roots were observed for *M.incognita* and *R. reniformis* egg masses and counted under a Nikon T-100® inverted microscope (10x). A second 10-gm subsample of stained roots was chopped in an Oster Blender® for 20 sec in water. The stained roots were rinsed in tap water, blotted dry and then suspended into acidified glycerin (40 ml glycerin and 5 drops of 5N HCL). The roots were mounted between glass microscopic slides to observe the life stages present in roots. The chopped suspension was observed under a Nikon® inverted microscope and juvenile stages were counted.

Nematicidal activity of freeze-dried C. juncea root exudates

To evaluate the nematicidal activity of root exudates, the entire root system of the *C. juncea* plants were submerged in a beaker containing 250 ml distilled water. The beakers were wrapped with aluminum foil to prevent photolysis of the exudates. The beakers were agitated using an Environ® automatic shaker at 150 rpm for 12 hours. *Crotalaria juncea* root exudates collected in the beakers were decanted into brown plastic bottles and stored at 0°C. These exudates were freeze dried.

Freeze-dried root exudates were reconstituted by adding 15 ml of distilled water. Nematode isolates maintained on susceptible hosts of tomato and cotton were extracted using 0.6% NaOCl (Hussey and Barker, 1973). Nematode eggs were allowed to hatch by incubating in 1 ml distilled water at 25°C for one day. Approximately 25 second-stage juveniles (J2) were handpicked and added to glass vials containing 5 grams of sand (Halbrendt *et al.*, 2007). The treatments included three different concentrations of reconstituted root exudates, 50-µl, 100-µl, and 250-µl in a final volume of 1 ml distilled water. The control was in 1 ml distilled water. The experiment was arranged in a complete randomized block design with five replications. After 24 hours, the nematodes were washed into a series of sieves with 350-µm on top and 25-µm at the base. The suspended solution on 25-µm sieve was transferred to a Petri plate and the number of live juveniles were counted under Nikon® inverted microscope. The nematodes that were moving and burst open when punctured using a needle were considered alive. In contrast, nematodes that were straight in shape, paralyzed and did not burst open when punctured were considered dead.

Data analysis

All nematode counts were log transformed and the transformed data were analyzed using Proc GLM (SAS institute, Inc., Cary, NC). The effects of *C. juncea* treatments was determined by the probability of the F-value (P less than or equal to 0.05). Treatment means were separated by Fisher's protected Least Significant Differences.

RESULTS

Greenhouse evaluations of C. juncea populations

Crotalaria juncea populations supported very low numbers of *M. incognita*. There was a significant difference in *M. incognita* numbers ($P \leq 0.05$) between all the *C. juncea* populations and the tomato control (Table 1). There were no differences among the populations of *C. juncea*. The decreasing order of *M. incognita* reproduction on *C. juncea* populations was tomato, PI 207657, PI 314239, PI 322377, PI 391567, and PI 426626 (Table 1). Reproduction of *R. reniformis* was also low on the *C. juncea* populations ($P \leq 0.05$) to the cotton (Table 1). Among the *C. juncea* populations, PI 322377 supported the lowest *R. reniformis* nematode reproduction while PI 314239 supported the highest reproduction. There were no differences among *C. juncea* on *R. reniformis* reproduction but there was a significant difference between all populations and the control. The numerically relative decreasing susceptibility of *C. juncea* for *R. reniformis* was cotton, PI 314239, PI 391567, PI 426626, PI 207657, and PI 322377.

Staining of C. juncea roots

All of the *C. juncea* populations were found to contain a few *M. incognita* juveniles and adults within the root tissues. All the juvenile stages of *M. incognita* were

Table 1. Effect of *Crotalaria juncea* populations on southern root-knot nematode, *Meloidogyne incognita* and reniform nematode, *Rotylenchulus reniformis* under controlled conditions at the Plant Science Research Center, located on campus of Auburn University, Auburn, AL.

	M. incognita evaluations			*R. reniformis* evaluations		
Cultivar	Root weight	Nematode eggs[a]	Nematode eggs[b]	Root weight	Nematode eggs[a]	Nematode eggs[b]
PI 207657	8.7	220 b	1.31	16.5	26.1 cd	0.49
PI 314239	12.4	151 b	1.17	15.4	282.4 b	1.15
PI 322377	14.0	76 b	1.03	20.2	13.9 d	0.16
PI 391567	13.6	101 b	0.73	16.9	135.2 bc	0.89
PI 426626	11.8	37 b	0.52	17.4	80.1 bcd	0.68
Control	12.3	11171 a	3.73	9.5	2122.8 a	3.24
LSD ($P \leq 0.05$)	NS	0.86	NS	NS	0.75	NS

[a]Number of nematodes present per gram of root weight.
[b]Log (x + 1) of the number of nematode eggs present per gram of root weight. The analysis was done on the log transformed data.
Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test ($P \leq 0.05$).
Tomato was the control for southern root-knot nematode evaluations and cotton was the control for reniform nematode evaluations.

observed within the roots. In case of *R. reniformis* nematodes, only 1-2 adult females were present on the *C. juncea* root tissues.

Nematicidal activity of freeze-dried C. juncea root exudates

Freeze-dried root exudates of *C. juncea* killed most of the *M. incognita* (Table 2) and *R. reniformis* juveniles (Table 3). There were significant differences ($P \leq 0.05$) in the nematode survival rates between the *C. juncea* root exudates and the control. Nematodes were most susceptible to the highest concentration of exudates when exposed whereas the survival rate was highest in the water control (Tables 2 and 3).

DISCUSSION

All *C. juncea* populations tested suppressed *M. incognita* and *R. reniformis* population densities when compared to the control. Between populations of *C. juncea*, there were differences in both *M. incognita* and *R. reniformis* reproduction levels which could be due to the different genetic constitution of the plants. Overall however, all populations significantly reduced the nematodes thus making any one a good candidate for breeding purposes.

All juvenile stages of *M. incognita* and a few adults were present within the root system, indicating there was penetration and reproduction on all the *C. juncea* populations tested but in limited numbers. Adult females of the *R. reniformis* nematodes were present on roots, indicating that *R. reniformis* can infest and reproduce on the *C. juncea* roots, also. *Crotalaria juncea* freeze-dried root exudates were able to kill the both *M. incognita* and *R. reniformis* juveniles. The mechanism responsible for nematode mortality is not clearly known but might be due to some nematotoxic compounds released from the roots in to the water. Root exudates may serve as an alternative to chemical nematicides in organic production systems. Jourand *et al.*, (2004) demonstrated that both shoot and root aqueous crude extracts of *C. juncea* paralyzed J2 of *M. incognita*, *M. javanica* and *M. mayaguensis*. Further biochemical studies need to be

Table 2. Evaluation of nematicidal activities of root exudates from *Crotalaria juncea* populations on the root-knot nematode, *Meloidogyne incognita*.

Population	Root weight gm	Nematodes alive in different concentration of C. juncea root exudates		
		50-µl[a]	100-µl[a]	250-µl[a]
PI 207657	38.1	0.6 de	0.2 c	0.0 c
PI 314239	23.9	3.8 b	2.6 b	1.6 b
PI 322377	33.3	2.2 bc	1.4 bc	1.0 b
PI 391567	28.6	1.2 cd	0.6 c	0.2 c
PI 426626	33.7	0.4 e	0.4 c	0.0 c
Control	—	16.8 a	16.6 a	17.4 a
LSD ($P \leq 0.05$)		1.39	1.82	1.10

[a]Average of the number of nematodes alive in different concentrations of *Crotalaria juncea* root-exudates. The analysis was conducted on log transformed data.
Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test ($P \leq 0.05$).

Table 3. Evaluation of the nematicidal activities of root exudates from *Crotalaria juncea* populations on the reniform nematode, *Rotylenchulus reniformis.*

Population	Root weight gm	Nematodes alive in different concentration of *C. juncea* root exudates		
		50µl	100µl	250µl
PI 207657	38.1	1.6 c	0.6 c	0.0 d
PI 314239	23.9	4.0 b	2.6 b	1.0 bc
PI 322377	33.3	3.2 b	1.6 bc	1.4 b
PI 391567	28.6	1.6 c	1.2 bc	0.6 cd
PI 426626	33.7	1.0 c	0.6 c	0.0 d
Control	—	17.0 a	17.8 a	17.6 a
LSD (P ≤ 0.05)		1.43	1.96	1.29

[a]Log (x+1) of number of nematodes alive in different concentrations of *Crotalaria juncea* root-exudates. The analysis was conducted on log transformed data.
Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test (P ≤ 0.05).

conducted to determine the chemical nature of the compounds present in the *C. juncea* root exudates. It is possible that *C. juncea* is both a poor host for both root-knot and reniform nematodes while the root tissues when decomposing in soil may release toxic byproducts that kill the remaining juveniles. This would explain why these nematodes exhibited low reproduction rates on roots and high mortality when exposed to exudates.

All populations of sunn hemp plants tested in this study were poor hosts for *M. incognita* and *R. reniformis.* Similarly, McSorley (1999) described sunn hemp to be highly resistant to nematodes, but not immune to *Meloidogyne* spp. He observed significant differences in invasion and developmental rates of *M. incognita* and *M. javanica* on sunn hemp and on susceptible controls. These previously reported results were similar to this study with *M. incognita* and *R. reniformis* and the susceptible tomato and cotton controls. The results of this study reported herein indicate that the populations of *C. juncea* tested reduced nematode population densities and therefore any of these populations could be used to develop cultivars adapted to the continental U.S.A.

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