

## Non-Traditional Legumes as Potential Soil Amendments for Nematode Control

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**Abstract:** Dried ground plant tissues from 20 leguminous species were mixed with *Meloidogyne incognita*-infested soil at 1, 2 or 2.5, and 5% (w/w) and incubated for 1 week at room temperature (21 to 27 °C). Tomato ('Rutgers') seedlings were transplanted into infested soil to determine nematode viability. Most tissues reduced gall numbers below the non-amended controls. The tissue amendments that were most effective include: *Canavalia ensiformis*, *Crotalaria retusa*, *Indigofera hirsuta*, *I. nummularifolia*, *I. spicata*, *I. suffruticosa*, *I. tinctoria*, and *Tephrosia adunca*. Although certain tissues reduced the tomato dry weights, particularly at the higher amendment rates (5%), some tissues resulted in greater dry weights. These non-traditional legumes, known to contain bioactive phytochemicals, may offer considerable promise as soil amendments for control of plant-parasitic nematodes. Not only do these legumes reduce root-knot nematodes but some of them also enhance plant height and dry weight.

**Key words:** bioactive, genetic resources, legumes, root-knot nematode, soil amendment.

The legume family (*Fabaceae*) contains taxa of considerable agricultural utility. They are desirable rotational and cover crops because they fix nitrogen, adding up to 500 kg of nitrogen to soil per ha per year (NAS/NRC, 1979). In addition, legumes are also used as human food and forage for animals. Several uncultivated non-traditional legumes have agronomic potential. Genetic variation in legume species and their wild relatives is essential to successful breeding of improved crop cultivars with new "added value" uses. The USDA/ARS Plant Genetic Resources Conservation Unit (PGRCU) is dedicated to acquiring, conserving, and evaluating the genetic resources of crops adapted to the southern United States. Seed of more than 4,000 accessions of non-traditional legumes are stored at the PGRCU in Griffin, Georgia.

Legume species can serve as soil amendments as well as rotational or green manure cover crops for reducing nematode populations (Johnson et al., 1992; Mojta-hedi, et al., 1991, 1993; Rhoades, 1976; Stirling, 1991) and other soil-borne pathogens (Blum and Rodríguez-Kábana, 1997; Hampson and Coombes, 1991; Hoitink et al. 1997; Khan and Reeleder, 1996; Lewis et al., 1992; McSorley and Gallaher, 1997). Neem (*Azadirachta indica*) (Riga and Lazarovits, 2001; Roosti and Deses, 1989) and other natural products from plants (Alphay and Robertson, 1988; Insuaza, 1988) have been found to reduce plant-parasitic nematode populations. American jointvetch and hairy indigo, as forage or green manure crops, have potential for managing soybean nematodes in Alabama (Rodríguez-Kábana et al., 1989). Some legumes may be useful forages (Morris, 1997); others contain pharmaceutical and nutraceutical chemicals (Beckstrom-Sternberg and Duke, 1994; Morris, 1997). Nutraceutical is defined as a food constituent

or phytochemical with human health promoting characteristics. Legumes produce economically important organic compounds such as lectins from *Canavalia ensiformis* and rotenone, tephrosin, and deguelin from *Tephrosia vogellii*, which are used as pesticides (Beckstrom-Sternberg and Duke, 1994; Gaskins et al., 1972; Minton and Adamson, 1979; Tyler et al., 1976). The use of legumes as organic amendments for pest control may not yet be a widespread practice except in developing countries, but the practice may receive greater emphasis in the future because of the phaseout of methyl bromide as a soil fumigant. Most legume species have just begun to be surveyed for nematocidal constituents (Walker et al., 1997), and new sources of nematocidal compounds in other plant families remain to be discovered (Ferris and Zheng, 1999). The incorporation of dried legume tissues as a pest control method can lead to various results depending on the type of legume amendment, soil moisture, and microbial flora and fauna (Campbell, 1989). Nematicidal activity of organic amendments in soil can be attributed to chemical mineralization with the ultimate release of ammonia, increasing nitrogen and carbon dioxide, lowered oxygen levels, release of toxic compounds from plant tissues, or growth of fungi and bacteria antagonistic to nematodes. Soil amendments may also provide an acceptable, economic way to increase yields in developing countries (Pyndji et al., 1997).

The objective of this study was to use dried tissue of non-traditional legume species (*Aeschynomene* spp., *Canavalia* spp., *Crotalaria* spp., *Indigofera* spp., *Leucaena* spp., *Rhynchosia* spp., *Senna* spp., *Sesbania* spp., and *Tephrosia* spp.) as soil amendments to determine their effects on root galling by the root-knot nematode *Meloidogyne incognita* (Koford & White) Chitwood.

### MATERIALS AND METHODS

Leaf, stem, and flower tissues from different leguminous plant species (Table 1) were harvested from field plots at the USDA, Plant Genetic Resources Conservation Unit, Griffin, Georgia, during fall 1998 for experiments conducted in 1999. The 1999 experiment was

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TABLE 1. Legume species investigated as amendments for their nematicidal effect on root-knot nematode (*Meloidogyne incognita*) of tomato.

Taxon	Common name	Accession (PI) number	Origin
<i>Aeschynomene indica</i> L.		225551	Zambia
<i>Canavalia ensiformis</i> (L.) DC.	Jackbean	164695	India
<i>Crotalaria juncea</i> L.	Sunn hemp	299567	South Africa
<i>Crotalaria retusa</i> L.		346294	India
<i>Indigofera cassioides</i> Rottler Ex DC.		200075	India
<i>Indigofera glandulosa</i> J. C. Wendl		213521	India
<i>Indigofera hirsuta</i> L.	Hairy indigo	213523	India
<i>Indigofera microcarpa</i> Desv.	Indigo	337538	Argentina
<i>Indigofera nummularifolia</i> (L.) Liv. Ex Alston	Indigo	189493	India
<i>Indigofera semitrijuga</i> Forsskal.	Indigo	199504	India
<i>Indigofera spicata</i> Forsskal.	Spicate indigo	365597	Tanzania
<i>Indigofera suffruticosa</i> Miller	Anil indigo	163159	Brazil
<i>Indigofera tinctoria</i> L.	Common indigo	260642	India
<i>Leucaena leucocephala</i> (Lam.) de Wit	Leadtrees	281609	Mexico
<i>Leucaean leucocephala</i> (Lam.) de Wit	Leadtrees	281775	New Guinea
<i>Leucaean leucocephala</i> (Lam.) de Wit	Leadtrees	443556	Mexico
<i>Rhynchosia minima</i> L. (DC.)	Snout bean	322540	Brazil
<i>Senna angulata</i> (J. Vogel) H. Irwin & Barneby		322312	Brazil
<i>Sesbania speciosa</i> Taubert	Brazilian lucerne	321747	Pakistan
<i>Tephrosia adunca</i> Benth.		308580	Venezuela
<i>Tephrosia candida</i> DC.	White tephrosia	304569	Madagascar
<i>Tephrosia oxygona</i> Welw. Ex Baker		185583	South Africa

repeated in 2000 with nine of the more promising plant species. Tissue of each species was dried in a forced-air oven for 1 month at 70 °C, ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) through a 4-mm screen, and stored dry until use. Dried legume tissue was chosen over fresh green materials for experiments because amendments would be approximately the same moisture content. Ground material was mixed at 1, 2.5, and 5% in 1999 and 1 and 2% (w/w) in 2000 with 1 kg of dry steam-pasteurized soil (piedmont-clay-loam; pH 5.96) in polyethylene clear bags. Nematode-infested soil without tissue served as controls. Approximately 6,000 eggs of *Meloidogyne incognita*, obtained from Rutgers tomatoes, were pipeted in 5 to 10 ml of water (depending on dilution factor) into each bag and mixed with soil. Soil moisture was not too wet, thus creating anaerobic conditions, but was moist enough for seed germination. The bag contents were mixed thoroughly by hand and then sealed. Each treatment included three replications.

After incubation at 21 to 27 °C for 1 week, the contents of each bag were distributed equally among three 320-cm<sup>3</sup> styrofoam cups (subreps) and one 2-week-old tomato [*Lycopersicon esculentum* Mill., prom. cons. var. *esculentum* (Rutgers)] seedling was transplanted into each cup. Cups containing tomato plants, amendments, and nematodes were arranged in a randomized split-plot design on greenhouse benches. Whole plots were legume amendments and sub-plots were amendment rates (1, 2 or 2.5, and 5% w/w) arranged in randomized complete blocks. Each replicate consisted of three tomato plants per legume and amendment rate

combination. Greenhouse temperatures varied both years from 11 to 35 °C. Plants were fertilized 10 days after transplanting and at weekly intervals during the experiment with Peters water-soluble 20N-20P-20K (Grace-Sierra, Milpitas, CA) at the rate of 20 ml of solution containing 2.6 g/liter. After 2 months, tomato roots were washed free of soil, plant heights recorded, and the number of root-galls counted. Whole plant weights were recorded after drying tomato plants at 70 °C for 2 weeks. The means of the three subsamples were analyzed by Analysis of Variance, and differences in means for each dependent variable were determined by Fisher's protected LSD test at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

Dried tissues of non-traditional leguminous plants reduced ( $P = 0.0001$ ) the number of root galls on tomato when added to nematode-infested soil. In both the 1999 and 2000 experiments, the legume species ( $P \leq 0.05$ ) and rate of incorporation ( $P \leq 0.05$ ) affected the number of root galls per tomato plant. There was an interaction between legume species and amendment rate on root galling in the 1999 experiment ( $P \leq 0.05$ ) but not in the 2000 experiment. Except for the pine straw, there was a decline in root galling with increasing rates of incorporation. All legumes ( $P \leq 0.05$ ) reduced nematode galls when compared with the non-amended control. In the first experiment, tissues of *Canavalia ensiformis*, *Crotalaria retusa*, *Indigofera hirsuta*, *I. nummularifolia*, *I. spicata*, *I. suffruticosa*, *I. tinctoria*, and *Tephrosia adunca* were effective in reduc-

TABLE 2. Mean number of root-knot nematode galls, mean plant heights, and dry weights of tomato transplants grown in soil infested with 6000 *Meloidogyne incognita* eggs/kg and amended with different leguminous tissues and pine straw in 1999.

Amendment tissue (Taxon)	Number galls/plant <sup>a</sup> amendment rate (% w/w)			Mean	Plant height <sup>a</sup> (cm)	Dry weight <sup>a</sup> (g)
	1.0	2.5	5.0			
<i>Canavalia ensiformis</i>	18.1 a <sup>b</sup>	3.4 b	1.8 b	7.8 f <sup>c</sup>	18.8 bc <sup>c</sup>	3.28 cde <sup>c</sup>
<i>Indigofera nummularifolia</i>	16.7 a	3.3 b	2.4 b	7.5 f	20.9 abc	4.94 abc
<i>Indigofera spicata</i>	18.8 a	3.8 b	0.4 b	7.7 f	18.6 bc	3.09 de
<i>Tephrosia adunca</i>	18.6 a	9.2 ab	3.2 b	10.3 ef	20.5 abc	3.91 bcd
<i>Crotalaria retusa</i>	25.4 a	7.1 ab	1.6 b	11.4 def	21.2 abc	4.07 bcd
<i>Indigofera suffruticosa</i>	25.4 a	7.9 b	2.4 b	11.9 def	21.6 ab	5.27 ab
<i>Indigofera tinctoria</i>	22.1 a	11.9 a	2.2 a	12.1 def	20.3 abc	4.07 bcd
<i>Indigofera hirsuta</i>	20.9 a	16.2 a	2.2 b	13.1 def	21.1 abc	4.07 bcd
<i>Aeschynomene indica</i>	26.7 a	16.6 ab	4.1 b	15.8 cdef	22.2 ab	4.46 abcd
<i>Crotalaria juncea</i>	26.6 a	16.3 ab	5.7 b	16.2 cdef	21.5 ab	4.69 abcd
<i>Senna angulata</i>	31.4 a	13.0 b	6.4 b	17.0 cdef	20.9 abc	3.71 bcde
<i>Tephrosia oxygona</i>	35.0 a	13.2 b	5.6 b	17.9 cdef	20.1 abc	4.94 abc
<i>Rhynchosia minima</i>	31.2 a	18.6 b	11.6 c	20.4 cde	22.7 a	5.96 a
<i>Indigofera glandulosa</i>	47.9 a	9.4 b	5.4 b	20.9 cde	21.7 ab	4.57 abcd
<i>Indigofera semitrijuga</i>	31.3 a	22.3 ab	10.0 b	21.2 cd	22.7 a	4.91 abc
<i>Tephrosia candida</i>	42.8 a	20.9 b	2.7 c	22.1 cd	23.1 a	5.25 ab
<i>Indigofera cassioides</i>	49.1 a	18.1 b	5.1 b	24.1 c	21.7 ab	4.22 bcd
<i>Indigofera microcarpa</i>	48.7 a	13.7 b	10.6 b	24.3 c	20.8 abc	4.64 abcd
Pine Straw	55.9 a	29.3 b	23.4 b	36.2 b	17.7 c	2.15 e
Control (no tissue)				71.9 a	22.9 a	4.26 bcd

<sup>a</sup> Mean number based on three replications with three sub-samples in each. Percentage reduction for plant height, and weight data based on three rates combined.

<sup>b</sup> Means followed by the same letter across columns for the different amendment rates indicate no differences at  $P = 0.05$  by Fisher's protected LSD test.

<sup>c</sup> Means followed by the same letter within columns indicate no differences at  $P = 0.05$  by Fisher's protected LSD test.

ing galling on tomato by 80 to 90% (Table 2). In the second experiment, nematode galls per plant were reduced in soil amended with *I. nummularifolia* and *C. ensiformis* (Table 3).

Plant dry weights were higher from the *Rhynchosia minima* and *Tephrosia candida* in the first experiment, while *C. ensiformis* and the *Leucaena leucocephala* treatments were higher in the second experiment than other legume amendments. In both experiments, *I. suf-*

*fruticosa* was consistent for having high tomato dry weights when compared with other legumes. In a previous study (Walker et al., 1997), tomato plants in soil amended with *Desmodium gangeticum* had reduced plant heights and dry weights. Examination of these roots revealed necrotic tips that were not present on control plants, suggesting a phytotoxic effect of the amendment to tomato. In this study, these symptoms were not observed with any of the soil amendments, and none of the legumes reduced the dry weight of tomato plants.

Pine straw, which is widely used as mulch in landscapes, was included for comparison with the leguminous tissues in 1999. Pine straw reduced ( $P = 0.0001$ ) root galling when compared to the non-amended soil. However, there was more root galling in soil amended with pine straw than in soil with most legume species (Table 2). The pine straw amendment decreased height and dry weight of the tomato transplants.

Legumes in the genera *Canavalia* and *Indigofera*, particularly *C. ensiformis* and *I. nummularifolia*, suppressed root galling by *M. incognita* when incorporated into steam-heated soil as dry tissue. There is widespread experimental evidence that various organic plant residues reduce numbers of plant-parasitic nematodes (Johnson, 1985; Ritzinger and McSorley, 1998; Rodríguez-Kábana et al., 1989) by ammonification, secondary phytochemicals, or perhaps their breakdown products. Phytochemicals such as canavanine from *C. ensiformis*, indigotin from *I. tinctoria*, and rotenone or tephrosin from *T. candida* could be nematicidal. Based

TABLE 3. Mean number of root galls and plant weights of tomato grown in legume-amended soils infested with *Meloidogyne incognita* in 2000.

Tissue	Mean <sup>a</sup> number of galls/plant	Gall reduction (%)	Mean dry weight <sup>c</sup> (g)
<i>Indigofera nummularifolia</i>	21.8 de <sup>b</sup>	82	2.09 cd <sup>b</sup>
<i>Canavalia ensiformis</i>	29.4 d	75	7.15 a
<i>Tephrosia adunca</i>	54.4 c	54	3.24 cd
<i>Sesbania speciosa</i>	56.0 c	53	5.68 ab
<i>Indigofera suffruticosa</i>	62.4 c	47	7.59 a
<i>Indigofera cassioides</i>	62.1 c	47	1.29 d
<i>Indigofera semitrijuga</i>	73.6 bc	38	3.75 bc
<i>Leucaena leucocephala</i>	75.8 bc	36	6.24 a
<i>Crotalaria retusa</i>	87.6 b	26	2.58 cd
Control with nematodes	118.2 a		2.00 cd
Control without nematodes	0.0 e		1.13 d

<sup>a</sup> Based on three replications with three sub-samples for each tissue rate (1% and 2%).

<sup>b</sup> Means followed by different letters within columns are significant at  $P = 0.05$  by Fisher's LSD test.

<sup>c</sup> Mean number based on three replications with three sub-samples in each. Plant weight data based on two rates combined in this experiment.

on these results, leguminous species vary in their effectiveness for controlling nematodes. However, this is an initial study and the activity of these plants during growth and following incorporation into soil needs to be determined under field conditions.

Two potential uses for these legumes are as dried organic amendments and incorporation as a green manure following plant growth to reduce plant-parasitic nematodes and growth and yield of the following crop. Stimulating soil microorganisms by the addition of soil amendments may be more realistic than introducing biological control microbes (Bridge, 1996). However, the quantities of dried tissue used in our studies, if commercially available, may not be practical for large-scale crop production. One percent (w/w) of certain leguminous tissues generally suppressed the number of galls on the tomato assays, which is similar to the amendment level found to be effective with castor bean and velvetbean (Ritzinger and McSorley, 1998). As additional information becomes available on the active mechanism(s) in legume amendments (Marban-Mendoza et al., 1992), smaller quantities of tissues could be efficacious. In large-scale cropping systems, it may be more practical to grow the legumes and incorporate them as green manures. Several legumes were identified with plant-enhancing capabilities, especially for plant height and dry weight. These include *Aeschynomene indica*, *C. ensiformis*, *Indigofera semitrijuga*, *I. suffruticosa*, *R. minima*, and *Tephrosia candida*. The added income to producers of legumes that have dual purposes may increase utilization of these crops in large- and small-scale agricultural systems. For example, a crop may be harvested for valuable phytochemicals and the remaining plant material incorporated into soil as a green manure for nematode control.

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