



Suppressing sting nematodes using botanical extracts

The natural defense mechanisms present in poinsettia and wild mustard may protect turfgrass from sting nematodes.



Golf courses often provide an ideal environment for elevated populations of sting nematodes (*Belonolaimus longicaudatus* Rau): sandy soil types, warm temperatures, ample soil moisture and susceptible plant cultivars. Even so, sting nematodes typically are able to damage turfgrass when population levels are relatively low, at 10-20 nematodes per 6 cubic inches (100 cubic centimeters) of soil (4).

Looking for allelochemicals

In anticipation of the restricted availability of commercial products for selective nematode suppression, researchers are investigating alternative means of nematode control. Botanical extracts may provide a “natural” way to suppress nematodes. Plant compounds may elicit nematode behavior such as attraction or repulsion from plant roots, making allelopathic interactions (in which one plant will introduce compounds into the soil to limit the growth of other plant species) a fundamental component of nematode investigations (2).

The use of naturally occurring allelochemicals for nematode control has been attempted in farming systems through crop rotation, intercropping or the use of green manures. Unfortunately, because turfgrass is a monoculture perennial crop, traditional means of providing allelochemicals for nematode suppression cannot be used on golf courses.

Field observations have led to the assumption that the presence of certain plants in thinned turf areas is an indication of sting nematode infestation. These plants include various species of spurge

(*Chamaesyce* species), which are members of the Euphorbiaceae (Spurge) plant family. An obvious question might be, what allows these plants to thrive in a site heavily infested with nematodes while turfgrass continues to decline? It is theorized that chemicals produced within certain plants are antagonistic to nematodes. A common characteristic of the Euphorbiaceae family is the production of milky, sticky, latex-type sap. Certain members of the Compositae plant family, such as tall lettuce (*Lactuca canadensis* L.), also produce a similar sap. Is this sap toxic to nematodes, and if it is, can it be extracted, stabilized and eventually used as a nematicidal agent?

Our research had two objectives: investigate whether sting nematode can be controlled by applying selected plant extracts applied to soil, and determine whether irrigation would enhance the effectiveness of sting nematodes control by plant extracts.

Materials and methods

Preliminary laboratory study

Mature specimens of spotted spurge (*Chamaesyce maculata* L. Small), poinsettia (*Euphorbia pulcherrima* Willd. Freedom Red), lantana (*Lantana camara* v. *Hybrida*), tall lettuce (*Lactuca canadensis* L.) and goldenrod (*Solidago altissima* L. v. *scabra*), plus a seed meal extract from wild mustard (*Brassica juncea* v. Pacific Gold [BSM]) were used in a preliminary laboratory screening study to expose nematodes to extract of either shoot or roots of each plant



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species. Nematode mortality counts were made daily for four days. Poinsettia and spurge shoots and seed meal extract from wild mustard were the most successful treatments in these preliminary laboratory studies. However, because of the difficulty of growing large masses of the spurge plants, spotted spurge was deleted in subsequent greenhouse studies. Therefore, seed meal extract from wild mustard and poinsettia shoots were selected for further evaluation in the greenhouse because of their potential for suppressing nematode populations within a reasonable time period (96 hours).

Pot establishment and inoculation

Conetainer-style pots were used throughout the greenhouse studies for individual replications. Common bermudagrass seed, 35 seeds per conetainer, was planted on top of the growth media of topdressing sand.

Following germination and growth, seedlings were mowed at 0.75 inch (1.91 centimeters). The bermudagrass was fertilized to maintain desirable growth and color before the investigation. Fertilizer applications ended when the trials began to prevent interactions with the treatments.

Nematode populations used in the greenhouse studies were established and maintained by Robin Giblin-Davis, Ph.D., at the University of Florida (REC, Fort Lauderdale, Fla.). From these samples, nematodes were extracted by the sugar flotation method (3). Following extraction, nematodes were individually selected, 50 adult and advanced stage juveniles at a time, and placed into microcentrifuge tubes containing 0.04 ounce (1.2 milliliters) deionized water. Once 50 nematodes were stored in the microcentrifuge tubes (usually within one hour), they were immediately transferred to individual pots in the greenhouse. Pots were inoculated with the nematodes using a 0.17-ounce (5-milliliter) glass syringe, five days before treatment application.

Inoculations were conducted in advance of treatment applications to help balance nematode populations. The transfer process from the host soil to individual pots typically has a physical impact on the fitness of the nematodes. We expected the transfer process to cause some mortality, and a five-day period was used to help stabilize and balance the study populations before treatment.

Treatment preparation and application

Treatment preparation via plant extract maceration and filtration was performed according to standard methods, where the solutions were filtered to the 0.2-micrometer level. Treatments for the greenhouse studies are listed in Table 1.

Individual pots were treated with 0.5 ounce (15



milliliters) of plant extract solution. Control pots received 0.5 ounce (15 milliliters) of water at the same time extracts were applied to treated pots. A plastic 0.34-ounce (10-milliliter) syringe was used to apply the treatments, 0.17 ounce (5 milliliters) at a time, until the root zone of the individual conetainers was saturated with plant extract solution. Each pot represented an individual treatment replication. Following injection, selected pots were immediately watered (irrigated) with 0.34 ounce (10 milliliters) of tap water to ensure proper infiltration and distribution of the plant extracts into the root zone. Each plant source represented a treatment, as did use of water following treatment application.

Extraction and treatment determination

Following a five-day exposure period, nematodes were individually extracted from the pots in the lab using the sugar-flotation method (3). In all of the studies, physical stimulation of individual nematodes included using a dental pick to lift them from the bottom of the petri dish and out of the water for one to two seconds. This technique of physically stimulating the nematodes was applied to all studies and was a successful measure to determine the effect of the treatment, especially when death was apparent.

The sandy soils, warm temperatures, ample soil moisture and susceptible turfgrasses found on golf courses provide ideal habitat for sting nematodes. Photo by B. McCarty



Nematodes were transferred from host material to research pot by injection. Photos by C. Cox

Treatments

Treatment [†]	Irrigation	No irrigation
Wild mustard seed extract	15 milliliters extract + 10 milliliters water	15 milliliters extract
Poinsettia	15 milliliters extract + 10 milliliters water	15 milliliters extract
Control	15 milliliters water	15 milliliters water

[†]Treatments were replicated eight times.

Table 1. Extracts from listed plants were applied to soil environments containing 50 sting nematodes, followed by irrigation or without irrigation to investigate their nematicidal characteristics.



Study 1: Nematode control

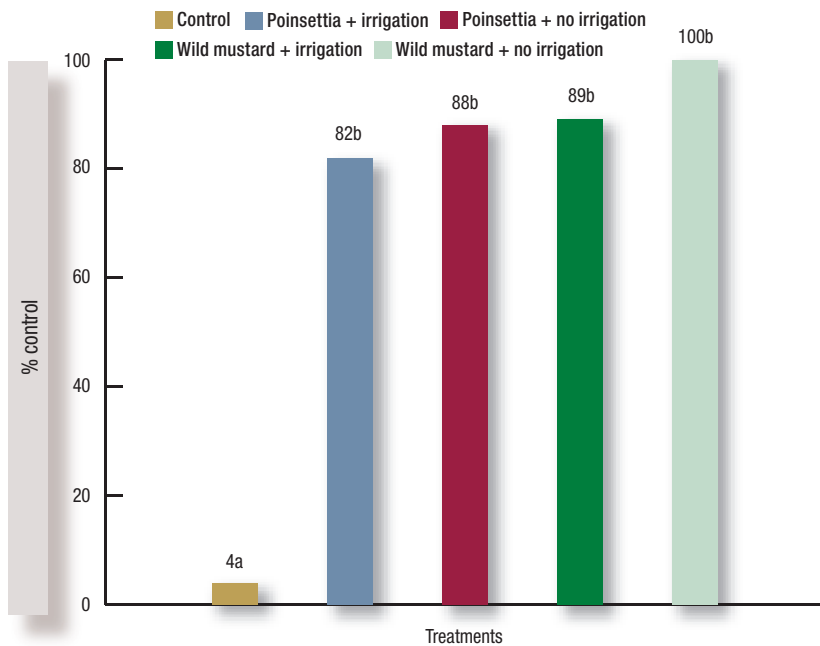


Figure 1. Values for control of sting nematodes in untreated turf and turf treated with selected plant extracts. Pots were inoculated with 50 nematodes and treated with plant bioextracts. Note that 4% of the nematodes died in the untreated control pots.

Study 2: Nematode control

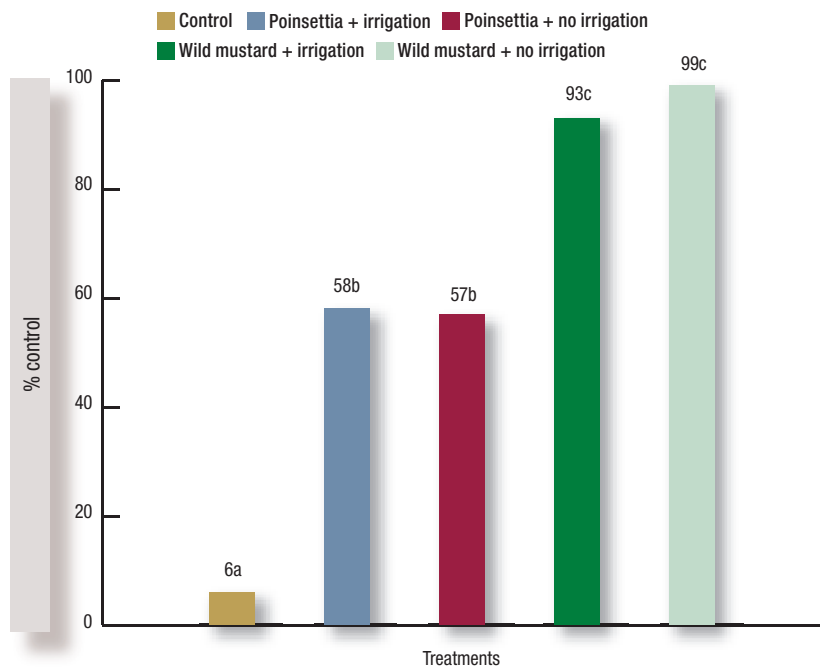


Figure 2. Values for control of sting nematodes in untreated turf and turf treated with selected plant extracts. Pots were inoculated with 50 nematodes and treated with plant bioextracts. The efficacy of the plant extracts was then evaluated for their nematocidal properties. Note that 6% of the nematodes died in the untreated control pots.

Final nematode populations were reduced in the control pots as expected, and we were never able to extract 100% of nematodes added to the pots (Figures 1, 2). Treatment efficacy was based on the mean extraction efficiency of the control pots. Extraction efficiency in these studies was defined as the total number of nematodes retrieved from the pots, regardless of whether the nematode recovered was dead or alive. Mortality counts of the treated pots, therefore, were based on the expected mean final population recovered from the control pots.

Statistical analysis

The individual greenhouse studies were analyzed separately, and treatment means were compared (1).

Results

Study 1

Extraction efficiency, as described earlier, was the number of nematodes recovered from each pot, dead or alive. The values were used to help calculate final mortality rates of the individual treatments (data not shown).

All treatments increased the mortality of sting nematode populations following a five-day exposure to the selected plant extracts (Figure 1). Poinsettia treatments with irrigation provided 82% control compared to untreated pots, and nonirrigated poinsettia treatments provided 88% control compared to untreated pots. Seed meal extract from wild mustard provided 89% control with irrigation and 100% control without irrigation compared to untreated pots.

These results agree with the laboratory studies, where treatments with poinsettia extract and seed meal extract from wild mustard caused significantly higher mortality ($\geq 90\%$) to nematode populations than the control ($\sim 5\%$) after 96 hours.

Study 2

A small portion of the untreated nematode population (6%) died. The remaining untreated population (94%) was alive and appeared healthy. All treatments significantly reduced the population after the five-day treatment period (Figure 2). Irrigated poinsettia treatments provided 58% control, and nonirrigated poinsettia provided 57% control compared to the untreated pots. Irrigated seed meal extract from wild mustard provided 93% control, and nonirrigated seed meal extract from wild mustard provided 99% control compared to the untreated pots. Overall, the trends observed in the laboratory study continued through the greenhouse trials. Both the selected plant species provided a certain level of suppression in a sterilized soil environment.



Extract from wild mustard seed was applied to both pots. The pot on the right was irrigated after the extract was applied, and the pot on the left was not.



The root structures of a bermudagrass plant treated with extract from wild mustard seed and irrigation (23), plants treated with the same extract and no irrigation (21 and 22), and an inoculated control treatment (24). Slight root burn occurred in the plant that was irrigated after the extract treatment.

Discussion and conclusions

Greenhouse studies indicate plant species producing isothiocyanate-derived compounds (for example, glucosinolates from wild mustard seed meal extract) had a strong initial effect against sting nematodes. Plants in the Euphorbiaceae family (for example, spurge and poinsettia) also showed the potential to cause nematode death over time, but these effects were delayed up to four days. In nature, this delay is important because the concentration of the *Euphorbia* species plant extract may not be strong enough after four days, reducing their efficacy.

These studies help support existing theories that certain plants in the Euphorbiaceae family (for example, spotted spurge) may produce a compound that protects their growth in soil infested with sting nematode. Although this nematicidal compound may not be concentrated in the physical soil environment around the plants, the compounds within the plant prevent or reduce nematode feeding. The ability of poinsettia extracts to suppress nematode populations in the field is uncertain because we do not know how much continuous exposure to poinsettia extracts is necessary to cause nematode mortality.

Of the plant extracts evaluated in this study, wild mustard extracts appear to have greater potential to reduce sting nematode populations because of the immediacy with which these compounds affect this sting nematode species.

Data were not collected on the effect of the plant extracts on turf health and growth. However, visual observations indicated shoots may be highly susceptible to burn. Root systems remained healthy following five-day saturation with wild mustard extracts, but irrigation practices following treatment appeared to influence root health.

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The research says

→ Plant extracts from members of the *Euphorbiaceae* and seed meal extract from wild mustard families can be a potential alternative for nematode suppression if the environment in which they are applied will sustain the compounds.

→ Plants producing isothiocyanate compounds (seed meal extract from wild mustard) appear more likely to reduce a plant parasitic nematode population in a naturally infested soil than the members of the *Euphorbiaceae* family selected for investigation in this study.

→ Irrigation appears important in distributing the nematicidal extract and in protecting plants from severe shoot and root phytotoxicity.