

# Velvetbean (*Mucuna pruriens*) extracts: impact on *Meloidogyne incognita* survival and on *Lycopersicon esculentum* and *Lactuca sativa* germination and growth†

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**Abstract:** Velvetbean (*Mucuna* spp.) is a summer annual that has been used as a cover crop to reduce erosion, fix nitrogen and suppress weeds and plant-parasitic nematodes. Crude aqueous extracts (1:15 dry weight plant/volume water) were made from velvetbean plant parts, and various concentrations of the extracts were evaluated *in vitro* for toxicities to different stages of *Meloidogyne incognita* (Kofoid and White) Chitwood and for suppression of hypocotyl and root growth and inhibition of germination of tomato (*Lycopersicon esculentum* L.) and lettuce (*Lactuca sativa* L.). Germination was only affected by the full-strength extract from leaf blades. Lettuce root growth was the most sensitive indicator of allelopathic activity of the plant part extracts. Lettuce and tomato root growth was more sensitive to the extract from main roots than to extracts of other plant parts, with lethal concentration (LC<sub>50</sub>) values of 1.2 and 1.1% respectively. *Meloidogyne incognita* egg hatch was less sensitive to extracts from velvetbean than the juvenile (J2) stage. There was no difference among LC<sub>50</sub> values of the extracts from different plant parts against the egg stage. Based on LC<sub>50</sub> values, the extract from fine roots was the least toxic to J2 (LC<sub>50</sub> 39.9%), and the extract from vines the most toxic (LC<sub>50</sub> 7.8%). The effects of the extracts were nematocidal because LC<sub>50</sub> values did not change when the extracts were removed and replaced with water.

Published in 2006 by John Wiley & Sons, Ltd

**Keywords:** cover crop; allelopathy; nematocidal effect; lethal concentration; *Mucuna* spp.

## 1 INTRODUCTION

In the early twentieth century, velvetbean (*Mucuna* spp.) was used extensively as a forage and green manure cover crop in the USA and elsewhere. However, with the advent of modern agricultural practices, velvetbean cultivation sharply declined and its cultivation presently is minimal. Velvetbean is a vigorous annual legume that possesses many positive agronomic attributes. The plant provides ground cover and thereby reduces erosion, fixes nitrogen and so reduces the need for nitrogen fertilizers, suppresses weeds through competition and production of allelopathic compounds and reduces populations of plant-parasitic nematodes.<sup>1,2</sup> In south Florida, velvetbean can be grown in the rainy summer months in rotation with vegetable crops grown in the dry winter months.

Velvetbean has been shown to suppress some weed species in tropical production systems. The smothering effect of velvetbean was equivalent to

that of herbicides for cogongrass (*Imperata cylindrica* (L.) Beauv.) control in corn.<sup>3</sup> Competition for light and nutrients, as well as allelopathic compounds, play an important role in the ability of velvetbean to suppress weeds.<sup>1</sup> Velvetbean was the most effective tropical cover crop at controlling weeds, providing 95–100% cover of the soil at the end of the cropping cycle. It provided good control of spiny amaranth (*Amaranthus spinosus* L.), smooth pigweed (*Amaranthus hybridus* L.), field sandbur (*Cenchrus insertus* MA Curtis) and bitterweed (*Parthenium hysterophorus* L.).<sup>4</sup>

Plant-parasitic nematodes are also of concern in tropical production systems. Velvetbean has been investigated as a plant-parasitic nematode management practice. Results have suggested that complex modes of action operating in velvetbean-amended soils were responsible for nematode suppression.<sup>5</sup> These modes of action include: non-host status of velvetbean,<sup>2,6</sup> improved plant nutrition,<sup>7</sup> enhanced

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(Received 22 July 2005; revised version received 12 December 2005; accepted 29 January 2006)

Published online 4 September 2006; DOI: 10.1002/ps.1281

growth of antagonistic organisms<sup>5</sup> and release of toxic compounds.<sup>8</sup>

An effective pest management alternative is needed to support the production of summer vegetables during the winter in south Florida. For velvetbean to be implemented by growers, they must be convinced that the plant has some potential use in suppressing weeds and plant-parasitic nematodes. While it appears promising to implement velvetbean into pest management systems,<sup>9</sup> the mechanism of pest suppression by this plant is not fully understood. The objective of this study was to determine the *in vitro* toxicity of extracts from velvetbean plant parts to the eggs and second-stage juveniles (J2) of *Meloidogyne incognita* (Kofoid and White) Chitwood and to hypocotyl and root growth and germination of tomato (*Lycopersicon esculentum* L.) and lettuce (*Lactuca sativa* L.).

## 2 MATERIALS AND METHODS

### 2.1 Extract preparation

Velvetbean (*Mucuna pruriens* DC var. *utilis* (Wight) Burck PI365315 01 SD origin Mozambique) was grown out-of-doors in pots containing 38 L of Krome gravelly loam soil at the Tropical Research and Education Center, University of Florida, Homestead, FL. Two months after planting, the main stem of each plant was cut at ground level, and the soil was washed gently from the roots. The plants were partitioned into main and fine roots, leaf blades, petioles and vines. Collected plant material was dried at 70 °C for 1 week, ground in a Wiley mill (Model No. A75-A; Arthur Thomas Co., Philadelphia, PA) to pass through a 40-mesh screen and stored at 4 °C until use.

Dry, ground material (8 g) was incubated in 120 mL deionized water (1:15 w/v) in 500 mL Erlenmeyer flasks.<sup>10</sup> The flasks were sealed with a parafilm layer, placed on a shaker and incubated for 24 h in the dark at 4 °C with constant shaking at 100 rpm. The slurry was filtered through six layers of cheesecloth and centrifuged for 10 min at 3046 × *g*. The supernatant solution that represented the crude aqueous extract was kept at 4 °C until use. For *M. incognita* assays the crude extract was syringe filtered through 1.0 and 0.45 and sterile 0.2 µm filters to remove any microbial contaminants prior to use.

### 2.2 Tomato and lettuce seed assays

The undiluted extract and aqueous dilutions of 1:1, 1:3 and 1:7 (by volume) and a water control were used in assays to determine the suppressive activity of velvetbean on germination and hypocotyl and root growth of lettuce 'Great Lakes' and tomato 'Rutgers'.<sup>11</sup> Seeds of lettuce and tomato (50 per dish) were germinated on Whatman No. 1 filter paper in 100 × 15 mm petri dishes using 2.5 mL of extract per dish. The dishes were sealed with parafilm, placed on trays and held in the dark in a constant-temperature growth chamber at 26 °C. The trays

were positioned at a 45° angle to encourage geotropic growth to facilitate hypocotyl and root measurements after 3 days.<sup>2</sup> Percentage germination and hypocotyl and root lengths were determined after 3 days for lettuce and after 5 days for tomato. All experiments were conducted twice using five replicate dishes for each seed type and dilution.

### 2.3 Nematode assay

*Meloidogyne incognita* race 1, originally isolated from a field near Salisbury, MD, and cultured on greenhouse-grown pepper (*Capsicum annuum* L.) 'PA-136' was used. Individual egg masses were picked from the roots, placed in water for 30 min and rinsed with sterile deionized water (DI) 3 times. Egg masses were transferred to a 25 mL scintillation vial, 0.5% sodium hypochlorite was added and the eggs were agitated for 3 min and allowed to settle for 30 s. Sterilized eggs were retained on a 500-mesh sieve, collected in sterile DI water, refrigerated overnight and used 24 h later. To obtain J2 inoculum, sterilized eggs were placed in an autoclaved Pyrex storage dish (Corning 3250; Corning, NY) on an 85 mm circle of 30 µm Speta/Mesh nylon filter (Spectrum Laboratories Inc., Rancho Dominguez, CA) sandwiched between two 70 mm polypropylene funnels trimmed to 20 mm deep and held together with a small binder clip. Enough sterile DI water was added to bring the water level up to the filter; 72 h later, J2 were collected in water and used.

*Meloidogyne incognita* eggs and J2 were exposed to the undiluted extract, to aqueous dilutions of 1:0.3, 1:1, 1:3 and 1:7 (by volume) and to a water control in polystyrene 24-well cell culture plates (Costar, Corning, NY).<sup>12</sup> To each well, 900 µL of extracts and then approximately 200 eggs or 50 J2, each in 100 µL sterile DI water, were added. Therefore, the final concentration of the extract to which nematodes were exposed was less than that for the seeds. The experiment was conducted twice; treatments were replicated 5 times per trial. The plates were covered and incubated at 24 °C. Exposure periods were 1 week for eggs and 48 h for J2. Percentage egg hatch of *M. incognita* was determined by counting the number of J2 present in wells that had originally received eggs. Juvenile survival was assessed by counting moving *versus* inactive nematodes. To determine the effects of the compounds on J2 survival, the extracts were removed after the 48-h exposure period and replaced with sterile DI water, and, 24 h later, moving *versus* inactive nematodes were counted again.

### 2.4 Statistical analysis

Results from repeated assays were similar and therefore combined for analysis. All nematode survival data were expressed as a percentage decrease in the number of nematodes surviving in the water controls. Seed germination and hypocotyl and root length data were expressed as the ratio of the treatment value divided by the control in which seeds

were germinated in water. For chemical–organism dose–response curves, PROC NLIN (SAS Institute, Cary, NC) was used to fit Gompertz and log-linear regression models to percentage hypocotyl or root growth, germination or nematode survival relative to concentrations. The appropriate nonlinear regressions for each dataset were used to estimate the concentration that caused 50% reduction in nematode survival and germination or hypocotyl and root growth ( $LC_{50}$ ;  $\pm SE$ ). Significant differences among  $LC_{50}$  values between treatments and organisms were determined using PROC MIXED. Means were compared with Bon *p*-value adjustments ( $P < 0.05$ ).

### 3 RESULTS

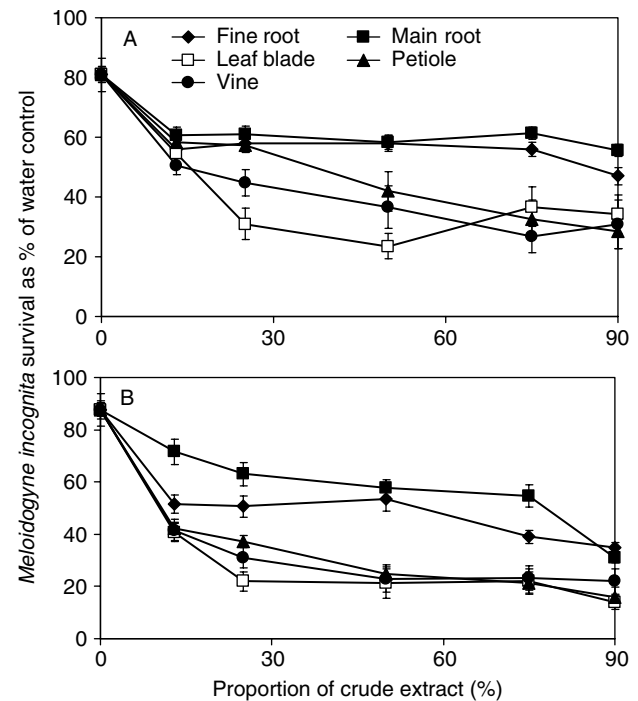
#### 3.1 Nematode survival

Based upon  $LC_{50}$  values, the extracts from velvetbean affected *M. incognita* J2 survival more than egg hatch (Table 1). The  $LC_{50}$  values for *M. incognita* egg survival were higher than those for the J2 stage, but only significantly different for extract from fine roots. Results for the egg stage were much more variable than for the J2 stage, and there were no statistically significant differences between the extract  $LC_{50}$  values against the egg stage. *Meloidogyne incognita* egg response to the extract from main roots was insufficient to estimate the proportion of extract needed to reduce survival by 50%.

After a 48-h exposure, all extracts decreased J2 survival to varying degrees (Fig. 1A). There was 29–56% survival in the undiluted crude extracts compared with 81% in the water control. The relative order of toxicity of the extracts from plant parts was vine > leaf > petiole > fine root > main root (Table 1); only the  $LC_{50}$  value for extract from fine roots was significantly different from that

of the extract from vines. An  $LC_{50}$  value could not be calculated immediately after the 48-h exposure to the extract from main roots because J2 survival was <50%.

The effects of the extracts were nematocidal because  $LC_{50}$  values did not change or decrease (Table 1), nor did maximum survival (Fig. 1B), when the extracts were removed and replaced with water. After removal,



**Figure 1.** *Meloidogyne incognita* J2 survival, in extracts from velvetbean plant parts: (A) immediately after 48 h incubation; (B) cumulative survival for the 48 h incubation and the subsequent 24 h rinse in DI water. Data are from two experiments, and each data point is the average of ten replicates. Each error bar represents the standard error of the mean.

**Table 1.** Velvetbean extract concentrations causing 50% reduction in hypocotyl and root length of tomato and lettuce and survival of *Meloidogyne incognita*<sup>a</sup>

| Organism and response                               | Velvetbean plant part    |                          |                           |                        |                          |
|---|--------------------------|--------------------------|---------------------------|------------------------|--------------------------|
|   | Petiole                  | Vine                     | Leaf                      | Main root              | Fine root                |
| Lettuce root <sup>b</sup>                           | 14.4 ( $\pm 1.0$ ) bB    | 15.6 ( $\pm 1.0$ ) bB    | 24.8 ( $\pm 15.5$ ) abABC | 1.2 ( $\pm 1.0$ ) cB   | 25.9 ( $\pm 25.2$ ) aABC |
| Tomato root <sup>c</sup>                            | 64.5 ( $\pm 7.8$ ) aA    | 82.6 ( $\pm 1.0$ ) aA    | 27.6 ( $\pm 1.0$ ) bB     | 1.1 ( $\pm 1.0$ ) cB   | *                        |
| Lettuce hypocotyl <sup>b</sup>                      | 87.2 ( $\pm 3.1$ ) aA    | 75.3 ( $\pm 1.0$ ) aA    | 32.3 ( $\pm 15.1$ ) abABC | 25.6 ( $\pm 3.1$ ) aA  | 82.0 ( $\pm 1.4$ ) aA    |
| Tomato hypocotyl <sup>c</sup>                       | 84.2 ( $\pm 3.1$ ) aA    | 82.1 ( $\pm 3.1$ ) aA    | 39.1 ( $\pm 7.8$ ) aABC   | 45.5 ( $\pm 7.8$ ) aA  | * <sup>d</sup>           |
| <i>Meloidogyne incognita</i> egg <sup>e</sup>       | 65.3 ( $\pm 25.2$ ) aAB  | 42.0 ( $\pm 20.6$ ) aAB  | 30.8 ( $\pm 15.1$ ) aABC  | *                      | 57.7 ( $\pm 1.4$ ) aB    |
| <i>Meloidogyne incognita</i> J2 <sup>f</sup>        | 34.4 ( $\pm 15.1$ ) abAB | 7.8 ( $\pm 1.0$ ) bB     | 19.8 ( $\pm 12.3$ ) abABC | *                      | 39.9 ( $\pm 1.4$ ) aC    |
| <i>Meloidogyne incognita</i> J2 rinsed <sup>g</sup> | 3.3 ( $\pm 2.6$ ) bB     | 12.4 ( $\pm 12.3$ ) abAB | 9.7 ( $\pm 2.6$ ) bC      | 57.6 ( $\pm 20.6$ ) aA | 30.2 ( $\pm 20.6$ ) aABC |

<sup>a</sup> Dry, ground material (8g) was incubated in 120 mL deionized water (1:15 w/v);  $LC_{50}$  is expressed as % v/v concentration of this in deionized water. Values are from two experiments with ten replications for each treatment ( $\pm SE$ ). Within rows, values followed by the same lower-case letter are not different ( $P < 0.05$ ). Within columns, values followed by the same upper-case letter are not different ( $P < 0.05$ ) according to Bon *P*-value adjustments.

<sup>b</sup> After a 3-day exposure to velvetbean extract.

<sup>c</sup> After a 5-day exposure to velvetbean extract.

<sup>d</sup> An asterisk indicates that a 50% reduction in hatch or survival was not obtained.

<sup>e</sup> After a 7-day exposure to velvetbean extract.

<sup>f</sup> After a 48-h exposure to velvetbean extract.

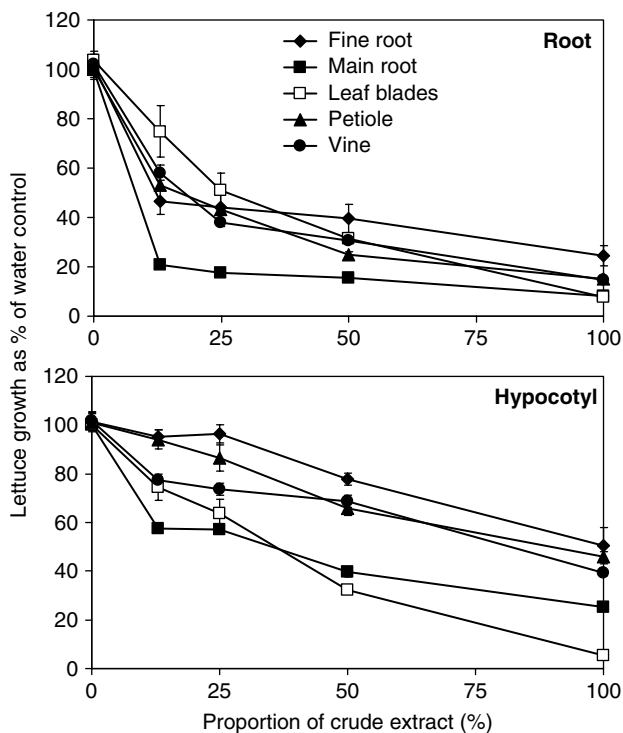
<sup>g</sup> Rinsed with sterile DI water and counted 24 h later.

the extracts from fine and main roots were less toxic to J2 than the extracts from leaf blades and petioles (Table 1). Maximum survival in the undiluted crude extracts ranged from 14 to 35% compared with 81% in the water control (Fig. 1B). When J2 were exposed to the extract from main roots for 48 h, and then held an additional 24 h in sterile DI water, an  $LC_{50}$  value of 57.6% was determined.

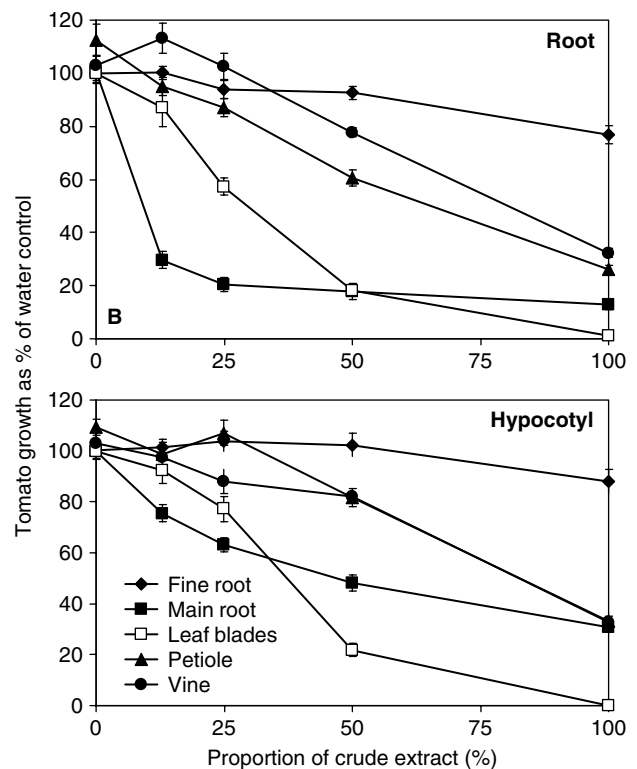
### 3.2 Seed germination and growth

Percentage seed germination was only affected by the full-strength extract from leaf blades, with 41 and 13% germination compared with the water control for lettuce and tomato respectively (data not shown). While there was no difference in maximum reduction in lettuce root growth among the extracts (Fig. 2), the  $LC_{50}$  value was the lowest for the extract from main roots, indicating its greater toxicity (Table 1). The response of tomato root growth to the various extracts was more variable than that of lettuce (Fig. 3). Tomato root response to the extract from fine roots was insufficient to estimate an  $LC_{50}$  value (Table 1). Extracts from main roots, and then from leaf blades, inhibited tomato root growth more at lower concentrations than extracts from vines or petioles, with lower  $LC_{50}$  values for the former plant parts.

In general, the extracts had less effect on hypocotyl growth than on root growth of both tomato and lettuce (Figs 2 and 3). There was no difference in the  $LC_{50}$  values of extracts against tomato or lettuce hypocotyl growth (Table 1). Tomato hypocotyl response to the extract from fine roots was insufficient to estimate the



**Figure 2.** Lettuce root and hypocotyl length, in extracts from velvetbean plant parts. Data are from two experiments, and each data point is the average of ten replicates. Each error bar represents the standard error of the mean.



**Figure 3.** Tomato root and hypocotyl length, in extracts from velvetbean plant parts. Data are from two experiments, and each data point is the average of ten replicates. Each error bar represents the standard error of the mean.

proportion of extract needed to reduce growth by 50%. Maximum inhibition of hypocotyl growth and relative order of toxicity of the extracts were similar between lettuce and tomato, with extracts from leaf blades and fine roots resulting in the most and least inhibition of growth respectively (Table 1; Figs 2 and 3).

The concentration of the extracts necessary to reduce root and hypocotyl growth of tomato and lettuce and survival of *M. incognita* can be used to compare the relative sensitivities of the organisms (Table 1). Root growth of both plant species was much more sensitive to extract from main roots than hypocotyl growth or nematode survival. There was little difference in the sensitivity of the test organisms to extract from leaves. Lettuce root growth and *M. incognita* J2 survival after a water rinse were more sensitive to extracts from petioles and vines than hypocotyl growth or nematode egg hatch.

## 4 DISCUSSION

The present results indicate that velvetbean root degradation products would probably not have a significant suppressive effect on *M. incognita*, contrary to previous findings.<sup>13</sup> Additional nematode suppression would be obtained by incorporating velvetbean material into soil because the above-ground parts of the plant were more toxic to *M. incognita*. The level of suppression of *M. incognita* by the extract from leaf blades in this study was less than reported

in previous studies. An extract from velvetbean leaves (1.0%) reduced *M. incognita* and *Nacobbus aberrans* (Thorne) Thorne & Allen survival completely and reduced galling caused by *M. incognita*.<sup>4</sup> Ethanol stem and root extracts of *Mucuna aterrima* (Piper & Tracey) Holland reduced *M. incognita* egg hatch.<sup>8</sup> The egg is often one of the most resistant stages in the nematode life cycle, possibly owing to its three-layer shell.<sup>14</sup> If the egg stage is the control target, the present data indicate that concentrations of aqueous velvetbean leachates higher than a 6.0% extract would need to be present to accomplish effective control; achieving this concentration in soil may be prohibitive.

The present study demonstrates that plant species vary in their response to extracts from velvetbean, with roots of lettuce and tomato responding differently. Lettuce root growth was the most sensitive indicator of the allelopathic activity of the various extracts. Others have shown that lettuce root growth was sensitive to allelopathic compounds.<sup>11</sup> Root growth of barnyardgrass (*Echinochloa crusgalli* [L.] P. Beauv.), amaranth (*Amaranthus hypochondriacus* L.) and tomato were strongly affected by 1.0% aqueous extract from velvetbean leaves,<sup>4</sup> as was lettuce root growth by 0.7% aqueous extracts from leaves and shoots.<sup>1</sup> In the present study the crude extract from leaf blades reduced lettuce and tomato root growth almost completely. Fujii<sup>1</sup> observed no reduction in lettuce germination with extracts from leaf blades and shoots (0.7%); the present authors did not begin to observe inhibition of germination below a concentration of 1.8% of extract from leaf blades. Both assay plants are relatively small-seeded, and the impact on larger weed seeds should be evaluated, although large seeds are likely to be less sensitive than small seeds.

The chemistry of velvetbean species has been reviewed by Szabo and Tebbet,<sup>15</sup> who concluded that the primary compound of interest in velvetbean was L-dopa (L-3, 4-dihydroxyphenylalanine), which has pharmacological benefits. Other compounds have been described from various plant parts, including tryptamine, serotonin, *N,N*-dimethyltryptamine, 5-methoxydimethyltryptamine and bufotenine.<sup>16,17</sup> Stems, followed by roots, had higher concentrations of L-dopa compared with leaves and pods. In addition to L-dopa, all plant parts (root, stem, leaf, pods and seed) contained 5-methoxydimethyltryptamine, while only leaves contained tryptamine. The chemical composition of velvetbean varied depending on geographic origin, part of the plant examined, time of harvest, soil quality and environment.<sup>15</sup>

The chemistry of *Mucuna* spp. is complicated, and it is not known to which compounds the organisms in this study were exposed. However, it is known that some of the chemical components of *Mucuna* spp. are toxic to pest organisms. The chemical constituents of *M. aterrima*, including L-dopa, at concentrations of 50 µg mL<sup>-1</sup> were toxic to *M. incognita* J2.<sup>8</sup> At lower concentrations, 5 µg mL<sup>-1</sup>, the most active compounds against *M. incognita* were nitrates, β-sitosterol

and stigmasterol. Germinated lettuce and barnyardgrass seeds exposed to concentrations of L-dopa responded differently, with barnyardgrass being more tolerant than lettuce.<sup>18</sup> For both species, root elongation was suppressed more than that of shoots. Whether *M. incognita*, tomato and lettuce were exposed to similar compounds in this study is unknown. Additional research should be directed towards identifying additional *Mucuna*-produced specific compounds that are toxic to weeds and plant-parasitic nematodes.

Determining the toxicity of aqueous extracts involved in seed germination, growth inhibition and plant-parasitic nematode suppression is only part of the information needed to optimize this management tactic. An essential question is whether organisms will be exposed to lethal concentrations of compounds produced by velvetbean in soil. Several studies have determined quantitatively the chemical composition of velvetbean plant material,<sup>15–17</sup> but none has determined whether these concentrations were maintained on the incorporation of plant material into soil.

While the extracts from the velvetbean plant parts varied in their toxicity against lettuce, tomato and *M. incognita*, actual exposure potential in the field will be related to the amount of biomass produced. The proportions of the plant parts from a whole plant of the velvetbean accession PI365315 01 SD, based upon a ten-plant average, were 31% petiole, 26% leaf blades, 24% vines, 15% main roots and 4% fine roots. The extract from main roots had lower LC<sub>50</sub> values against root and hypocotyl growth than against nematode J2 survival or egg hatch. However, the main root only comprises 15% of the plant, and therefore the amount of material needed to achieve concentrations necessary to inhibit root and hypocotyl growth may not be present. With 32 820 plants ha<sup>-1</sup>, this velvetbean accession produced 23.5 t ha<sup>-1</sup> of dry above-ground biomass. In general, for all organism and life stages tested, the leaf blade was the most toxic part of the plant based on LC<sub>50</sub> values. Approximately 7.5 t ha<sup>-1</sup> of dry leaf blades would be incorporated into soil with this velvetbean accession. Whether this would be an adequate amount to suppress weed seed germination and growth or *M. incognita* survival is not known. Further research is needed to understand the fate of compounds produced by velvetbean in soil and their use for control of weeds and plant-parasitic nematodes.

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