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Evaluation of Asteraceae Plants for Control of *Meloidogyne incognita*

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Abstract: Of the 56 species and 43 genera of Asteraceae tested, 9 were highly resistant or immune to *Meloidogyne incognita* and did not form root galls. Twenty-six species and six cultivars had 25% or fewer roots galled and were considered moderately resistant to *M. incognita*. Pre-planting *Cosmos bipinnatus* (F190), *Gaillardia pulchella, Tagetes erecta, Tithonia diversifolia,* or *Zinnia elegans* (F645) reduced root galling and *M. incognita* J2 in and around *Ipomoea reptans*. Amendment of soils with roots, stems, or leaves of *G. pulchella* was effective in controlling *M. incognita* on *I. reptans*. Tissue extracts of *G. pulchella* were lethal to various plant-parasitic nematodes but were innocuous to free-living nematodes. Root exudates of *G. pulchella* were lethal to J2 of *M. incognita* and were inhibitory to the hatch of eggs at the concentration of 250 ppm or higher. *Gaillardia pulchella* could be used to manage *M. incognita* as a rotation crop, a co-planted crop, or a soil amendment for control of root-knot nematode.

Key words: antagonistic plants, Asteraceous plants, *Ipomoea repans,* root exudates, rotation, soil amendment, tissue extracts.

Plant diseases caused by parasitic nematodes are widespread in Taiwan (Tsay, 1997). The most destructive species is *Meloidogyne incognita,* which causes serious problems on a number of economically important agricultural crops including grape (*Vitis vinifera*), papaya (*Carica papaya*), guava (*Psidium guajava*), tomato (*Lycopersicon esculentum*), tobacco (*Nicotiana tabacum*), and watermelon (*Citrullus vulgaris*). *Meloidogyne incognita* also increases the severity of bacterial wilt disease in solanaceous plants caused by *Ralstonia solanacearum* (Yen et al., 1997) and Fusarium wilt of watermelon caused by *Fusarium oxysporum* f. sp. *niveum* (Yen et al., 1998). Moreover, farms infested with *M. incognita* frequently experience a decline of subsequent crops (Tsay, 1996). Current management of *M. incognita* primarily relies on nematicide application (Tsay, 1999). However, because chemicals create a potential hazard to the environment and human health, alternatives to nematicides are desirable.

Various plants have been shown to be effective in controlling nematodes on agricultural crops when grown in rotation, inter-planted with susceptible crops, or used as a soil amendment (Hackney and Dickerson, 1975; Halbrendt, 1996; Wang et al., 2002). Among these plants, marigold (*Tagetes* spp.) is the most commonly studied (Halbrendt, 1996; McSorley and Frederick, 1994). As marigold belongs to the Asteraceae, it is possible that other members of the family also may possess antagonistic properties against plant-parasitic nematodes. This study was initiated to survey species of asteraceous plants for their susceptibility to *M. incognita* and to evaluate their effect on this nematode. From the preliminary data, *Gaillardia pulchella* (blanket flower) showed consistent resistance to *M. incognita*. Because it is a native species widely distributed in Taiwan and has good resistance to drought, it was selected for further study.

Previous studies have shown that pre-planting resistant hosts may effectively reduce nematode populations in the soil (Hu and Huang, 1978; Ploeg, 1999). In Taiwan, fields often need to be continually cash-cropped to maintain profitability. Co-planting is therefore a more practical method compared to pre-planting in this environment, so the efficacy of *G. pulchella* as a biological agent to control *M. incognita* was evaluated via both planting systems and the soil amendment experiments.

In addition, plant extracts and root exudates from *G. pulchella* were tested to determine the mechanism of nematode suppression and the impact of *G. pulchella* on other nematodes including free-living nematodes.

Materials and Methods

The *M. incognita* isolate used in this experiment was started from a single egg mass obtained from bitter melon (*Momordica charantia*) in the field in Nan-Tou County, Taiwan. *Meloidogyne incognita* was maintained on water spinach (*Ipomoea reptans*) in the greenhouse.

Interaction between asteraceous plants and M. incognita: Seeds of asteraceous plants were purchased from Know-You Seed Co. (Kaohsiung, Taiwan) or Taiwan Seed Service Center (Hsinseh, Taichung) or collected from fields (Taipei, Kinmen, and Penghu counties). Seeds were soaked in water at 4 °C for 24 hours and germinated in peat moss in plastic planting tubes. When seedlings developed two fully expanded leaves, they were transplanted to either 9-cm or 12-cm-diam. pots depending on the plant size, and the pots were filled with 250 g or 500 g steam-treated sandy loam soil, respectively. Approximately 500 or 1,000 *M. incognita* J2 were inoculated to each pot, respectively. There were five replicates of each species and cultivar. After inoculation, plants were kept in the greenhouse at an average temperature of 27 °C with 83% relative humidity. Forty days later, plants were taken down and evaluated for host status.

Plant tops were removed and the root systems were washed free of soil, immersed in a 0.5% NaOCl solution for 3 minutes, dried with a paper towel, and stained with a cotton blue/lactophenol solution (0.5 g cotton

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blue per 1 liter 1:4:15 = phenol:lactic acid:water). Within the root system, 10 sections, each 1 cm long, were randomly selected and the galls within the section were observed under a dissecting microscope. Host susceptibility to *M. incognita* was rated using a galling index, where 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% of root galls. Second-stage juveniles (J2) of *M. incognita* were extracted from soil using the modified Baermann-funnel technique (Baermann, 1917); after 36 hours, J2 were collected and counted. The experiment was performed twice.

Effect of inter-planting resistant plants on controlling M. incognita: Ipomoea reptans was used as a model crop to evaluate the efficacy of pre-planting and co-planting resistant plants for control of *M. incognita*. Highly resistant asteraceous plants identified from the previous experiment that had a rapid growth rate and vigorous root system were selected for this experiment. Lettuce (*Lactuca saliva* L.) was chosen as a susceptible control, and *I. reptans* were planted alone as an additional control. In the pre-plant experiment, resistant and susceptible plants were direct-seeded in 12-cm-diam. pots filled with 500 g steam-treated sandy loam soil and maintained in the greenhouse (27 °C, 83% RH). Five hundred *M. incognita* J2 were added to the pots when the plants were 2 weeks old. Twenty-five days after inoculation, plant tops were removed and 2-week-old *I. reptans* were transplanted into the pots and kept in the greenhouse under the same conditions. The control treatments consisted of 2-week-old *I. reptans* planted into pots without any pre-plant rotation. After 40 days, the number of galls on *I. reptans* was counted and 100 g soil from each pot was processed using the modified Baermann funnel technique and the number of *M. incognita* J2 were recorded.

In the co-planting experiment, 1-month-old resistant and susceptible plants were co-planted with 2-week-old model crop *I. reptans* in 12-cm-diam. pots, with an inoculation density of 1 J2/g soil, and maintained in the greenhouse (27 °C, 83% RH). *Ipomoea reptans* were also planted alone as a control. After 40 days, all the plants were evaluated as in the pre-planting experiment. Both experiments had four replicates for each treatment and each was performed twice.

Control of M. incognita by soil amendments with fresh tissues of G. pulchella: Forty-five-day-old *G. pulchella* plants were cut into small pieces and mixed with steam-treated soil at a concentration of 1% and 5% (w/w). Five hundred grams of amended soil was placed in each 12-cm-diam. pot and inoculated with approximately 1,000 J2 of *M. incognita*. Each treatment had four replicates. After 1 week of incubation in the greenhouse (27 °C, 83% RH), a 2-week-old *I. reptans* seedling was transplanted into each pot. The percentage of galled roots and the number of J2 in soil were determined after 40 days using the methods described above. This experiment was performed twice.

Effect of tissue extracts of G. pulchella on nematodes: Various plant part extracts were prepared from tissue that was dried in an oven at 60 °C for 2 days and ground to powder. Ten grams of powder was mixed with 100 ml water in a 250-ml flask and autoclaved for 20 minutes. The mixture was then filtered through Whatman No. 1 filter paper and the solution was used as the testing solution against nematodes.

Effect on different nematodes: Five plant-parasitic nematodes that have different parasitic niches and one free-living nematode were used in this experiment. The origins of the plant-parasitic nematodes tested were as followed: *Aphelenchoides besseyi* was isolated from strawberry (*Fragaria chiloensis* var. *ananassa*) and cultivated on a culture of *Alternaria brassicicola*. *Bursaphelenchus xylophilus* was isolated from *Pinus thunbergii* and cultivated on a culture of *Botrytis cinerea*. *Pratylenchus coffeae* and *Rotylenchus reniformis* were isolated directly from sweet orange (*Citrus sinensis*) and green onion (*Allium fistulosum*), respectively. *Meloidogyne incognita* was the same origin as the previous experiments. The free-living nematode isolate was collected from Taichung and identified to genus only (*Rhabditis* spp.). The toxicity of tissue extracts was determined by adding 1 ml of test solution to each well of a 24 well plate followed by the addition of a 10-µl nematode suspension containing 100 to 200 nematodes. The number of dead nematodes was determined after incubation at 24 °C for 24 hours and the percentage mortality was calculated. Each treatment had four replicates and the experiment was performed three times.

Effect of plant extract on M. incognita hatching rate and the infectivity: One egg mass of *M. incognita* was placed in each well of a 24 well plate containing 1 ml of test solution, and four replicates for each treatment. After 24 hours the solution was removed and the egg mass and hatched nematodes in each well were washed with sterile-distilled water three times at 10-minute intervals; the hatching rate was determined 4 days later. The effect on nematode infectivity was determined by placing approximately 500 J2 of *M. incognita* in 3 ml of test solution in a 6-cm petri plate for 1 hour. The test solution was then removed and the nematodes were washed three times with distilled water at 10-minute intervals and then used to inoculate 2-week-old *I. reptans* seedlings. There were four replicates of each treatment. The percentage of galled roots was recorded after 40 days. The experiment was performed three times.

Effect of root exudates of G. pulchella on M. incognita: A sterile, soil-free growth chamber was developed for collecting root exudates. One end of a glass tube (3.3-cm diam.; 18.4 cm long) was closed with a piece of cheesecloth and placed in the bottom of 500-ml flask containing 15 ml Gamborg's B5 medium (Sigma, St. Louis, MO) supplemented with 1% glucose. The flask was seated with aluminum foil and autoclaved for 20 min-

TABLE 1. Interaction between selected asteraceous plants and *Meloidogyne incognita*.

Species and (cultivar)	Galling index[a]	Number of J2/100 g soil
Ageratum conyzoides	2.7	NT[b]
A. houstonianum (purple)	0.3	227
A. houstonianum (F005)	0.4	150
Bellis confuses (F055)	3.7	5
Bidens bipinnata	0.2	0
B. pilosa	0.8	0
Blumea lacera	2.0	NT[b]
Calendula officinalis (F070)	4.0	5
Callistephus chinensis (F075)	2.0	2
C. chinensis (F080)	1.0	0
Centaurea cyanus (F130)	4.0	4
C. moschata (F135)	2.0	2
Chrysanthemum carillardia (F140)	2.3	2
C. coronarium (V095)	2.0	2
Cichorium endivia (V100)	4.0	4
Coreopsis lanceolsta (F175)	0.8	2
C. tinctoria (F180)	1.3	16
Cosmos bipinnatus (F185)	1.0	1
C. bipinnatus (F190)	0	0
C. bipinnatus (F191)	0.3	0
C. bipinnatus (F195)	0.7	0
C. sulphureus (F200)	0.5	2
C. sulphureus (F210)	0	0
C. sulphureus (F220)	0.5	0
C. sulphureus (f220)	1.7	2
Crassoucephalum crepidioides	0.7	0
Crisium japonica	3.0	6
Crossostephium chinese	3.3	10
Dahlia hybrida (F240)	4.0	11
Dorotheanthus bellidiformis (F460)	1.0	1
Dyssodia tenuiloba	0.7	4
Eclipta prostrata	3.0	192
Emilia sonchifolia (NO.7)	1.0	2
Erigeron bonariensis	0	3
E. canadensis	0.7	0
Gaillardia pulchella (F300)	0	0
Gazania hybrida (F305)	0.5	13
Gerbera jamesonii (F310)	0.5	2
Gynura scolymus	1.0	0
Helianthus annus (F345)	3.0	5
Helichrysum bracteatum (F355)	2.0	3
H. bracteatum (F360)	3.3	4
Hemistepta lyrata	1.0	4
Ixerix chinensis	1.0	1
I. laerigata	1.0	0
Kalimeris indica	4.0	111
Lactuca formosana	2.5	0
L. sativa (V155)	4.0	6
L. sativa (V160)	3.0	8
Sanvitalia procumbens (F575)	2.0	2
Senecio rowleyanus	0	0
Siegesbeckia orientalis	1.0	1
Sonchus arvensis	0.8	4
Tagetes erecta (F590)	0	0
T. patula (F600)	0.7	0
T. patula (F605)	0.3	0
T. patula (NO. 1870)	0.7	1
Tithonia diversifolia (NO. 13)	0	0
Tridax procumbens	0.4	0
Vernonia cinerea	4.0	285
Wedelia prostrata	2.7	57
W. trilobota	0	1
Xanthium strumarium	4.0	8
Yourgia japonica	1.0	0
Zinnia angustifolia (F660)	2.0	2
Z. elegans (F645)	0	0
Z. elegans (F650)	1.0	2
Z. elegans (milor)	1.7	2
Z. elegans (F655)	0.2	0
Z. haageana (F665)	1.0	2

(Table continues)

TABLE 1 (CONTINUED). Interaction between selected asteraceous plants and *Meloidogyne incognita*.

[a] Galling index ranging from 0 to 4 was based on percentage of roots galled. 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%.
[b] Not tested.

utes. Seeds of *G. pulchella* were soaked in water at 4 °C for 24 hours, surface sterilized with 0.5% NaOCl solution for 1 hour, and germinated on 1% water agar. After 3 days in darkness, five seedlings were transferred to the tube in the flask. After incubation at 24 °C at 12 hours light/12 hours darkness for 2 months, the liquid in the flasks was collected and lyophilized into crystals. Root exudate solutions were made by dissolving 3 mg and 1 mg crystals previously mentioned into 1 ml distilled water, respectively, for 3X and 1X root exudates solution. The other two concentrations were made by diluting the 1X solution. Different concentrations of root exudate solution were tested for their effect on *M. incognita* mortality and the egg hatching rate using the methods described previously. Each concentration had four replicates, and the experiment was performed three times.

RESULTS

Interaction between Asteraceous plants and Meloidogyne incognita: Of the 56 species and 12 species cultivars of Asteraceae tested, 9 were highly immune or resistant to *M. incognita* and did not form root galls (Table 1). These were *Cosmos bipinnatus* (F190), *C. sulphureus* (F210), *Erigeron bonariensis* L., *G. pulchella, Senecio rowleyanus* Jacob., *Tagetes erecta* L. (F590), *Tithonia diversifolia* A. Gary, *Wedelia trilobata* (L.) Hitchc., and *Zinnia elegans* Jacq. (F645). Twenty-six species and six cultivars showed 25% or less roots galled (galling index ≦1) and were considered moderately resistant to *M. incognita*. Galls occurred on more than 50% (galling index ≧3) of roots produced by 14 species and 1 cultivar of the species tested. These were considered susceptible to *M. incognita*.

In most pots, the number of J2 was low (less than 10/100 g soil) regardless of whether the host was resistant or highly susceptible (Table 1). However, in a few cases, high numbers of J2 were recovered from both susceptible and resistant hosts such as *Vernonia cinerea* Less. with galling index of 4.0 having 285 J2/100 g soil, and the tolerant *Ageratum houstonianum* Mill. (cv. purple) with galling index of 0.3 having 227 J2/100 g soil.

TABLE 2. Effect of pre-planting with various asteraceous and susceptible plants on root-knot severity of *Ipomoea reptans* and populations of second-stage juveniles of *Meloidogyne incognita.*

Pre-planted species	Galls/plant	J2/100 g soil
Resistant species		
Cosmos bipinnatus (F190)	6 B[a]	0 B
Gaillardia pulchella	1 B	0 B
Tagetes erecta	8 B	0 B
Tithonia diversifolia	8 B	0 B
Zinnia elegans (F645)	1 B	0 B
Susceptible species (controls)		
Lactuca sativa (V155)	142 A	18 A
Check		
Ipomoea reptans	114 A	13 A

[a] Data followed by the same letter in the same column are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

TABLE 4. Control of *Meloidogyne incognita* on *Ipomoea reptans* by soil amendment with tissues of *Gaillardia pulchella.*

Materials for amendment	Gall index of *I. reptans*[a]	Number of J2/100 g soil
1% amendment		
Roots	1.2 B[b]	9 C
Stems and leaves	1.0 C	34 B
Whole plants	0.8 C	6 C
5% amendment		
Roots	1.2 B	3 C
Stems and leaves	0.8 C	8 C
Whole plants	0 C	1 C
Non-amended control	2.0 A	59 A

[a] Gall index ranged from 0 to 4 was based on percentage of roots galled. 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%.
[b] Data followed by the same letter in the same column are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

Effect of inter-planting resistant plants on controlling M. incognita: Of the nine immune and highly resistant plants identified, *C. bipinnatus, G pulchella, Ta. erecta, Ti. diversifolia,* and *Z. elegans* had rapid growth rates and vigorous root systems and were selected for further study. When soils were pre-planted with selected asteraceous plants, the numbers of galls caused by *M. incognita* per plant ranged from 0 to 16, and J2 of *M. incognita* were not detected in the surrounding soil (Table 2). However, when the soils were pre-planted with susceptible *L. sativa,* the subsequent planting of *I. reptans* developed numbers of galls that were significantly higher. The soil also had J2, ranging from 7 to 25 per 100 g of soil.

Co-planting *I. reptans* seedlings with *G. pulchella, Ta. erecta,* or *Ti. diversifolia* reduced the root-knot galling on *I. reptans* significantly, and galls were not found on the roots of the asteraceous plants (Table 3). However, co-planting with *C. bipinnatus* or *Z. elegans* did not reduce root-knot galling on *I. reptans* and the roots of the asteraceous plants were also galled. When *I. reptans* were grown in soil infested with *M. incognita,* the average nematode number was 39/100 g soil; with *C. bipinnatus,* it was increased to 215/100 g soil (Table 3). The numbers of *M. incognita* J2 in soil were significantly greater when *I. reptans* was co-planted with *C. bipinnatus, Ta. erecta, Z. elegans,* and *L. sativa* than with *G. pulchella, Ti. Diversifolia,* or the model crop plant alone (Table 3). *Gaillardia pulchella* and *Ti. diversifolia* were most effective in reducing both the root-knot galling on *I. reptans* and numbers of *M. incognita* J2 in the soil.

Control of root-knot nematodes by soil amendments with fresh tissues of G. pulchella: The amendment of soils with tissues of *G. pulchella* at 1% and 5% was effective in reducing the root-knot severity of *I. reptans* caused by *M. incognita,* and there were no differences between the 1% and 5% amendments (Table 4). Incorporation of the whole plant was the most effective treatment to suppress both galling formation on *I. reptans* and the number of J2 in the soil.

Effects of tissue extracts of G. pulchella on nematodes: After treating with aqueous extracts of roots or stems and leaves of *G. pulchella,* plant-parasitic nematodes but not free-living species (*Rhabditis* spp.) had mortality rates significantly higher than the water control (Table 5).

TABLE 3. Effect of co-planting with various asteraceous and susceptible plants on root-knot severity of *Ipomoea reptans* and populations of second-stage juveniles of *Meloidogyne incognita.*

Co-planting species	Gall index of companion plant[a]	Gall index of *I. reptans*[a]	J2/100 g soil
Resistant species			
Cosmos bipinnatus (F190)	1.0 B	3.0 AB	215 A[b]
Gaillardia pulchella	0 C	1.6 C	20 C
Tagetes erecta	0 C	1.5 C	197 A
Tithonia diversifolia	0 C	1.0 C	11 C
Zinnia elegans (F645)	0.6 BC	2.8 A	112 B
Susceptible species (control)			
Lactuca sativa (V155)	4.0 A	3.4 A	79 B
Check			
Ipomoae reptans	4.0 A	3.5 A	39 C

[a] Gall index ranged from 0 to 4 was based on percentage of roots galled. 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%.
[b] Data followed by the same letter in the same column are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

TABLE 5. Effect of *Gaillardia pulchella* extracts from roots and aboveground parts on the mortality of pathogenic and free-living nematodes.

Nematode	Mortality (%)		
	Root extract	Stem and leaf extract	Water control
Aphelenchoides besseyi	78 A[a]	58 B	8 C
Bursaphelenchus xylophilus	76 A	41 B	12 C
Meloidogyne incognita	82 A	79 A	18 B
Pratylenchus coffeae	56 B	82 A	22 C
Rotylenchus reniformis	92 A	82 A	55 B
Rhabditis spp.	40 A	20 B	41 A

[a] Data followed by the same letter in the same row are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

The root extract was more effective against *A. besseyi* and *B. xylophilus* than stem and leaf extract, but the reverse was true for *P. coffeae*. There were no differences between extracts on mortality of *M. incognita* and *R. reniformis*.

G. pulchella tissue extracts also inhibited egg hatch and reduced the infectivity of *M. incognita* (Table 6). Root extract was more effective in inhibiting egg hatch than the extract from stems and leaves, and exposure to either extract for 1 hour decreased the ability of *M. incognita* to infect *I. reptans*.

Effect of root exudates of G. pulchella on M. incognita: The concentration of root exudate mirrored the mortality of *M. incognita* J2 (Table 7). The 3X root exudate solution killed virtually all the J2 tested and suppressed egg hatching of *M. incognita;* only 7 eggs hatched compared to 94 for the water control.

Discussion

The results of these experiments show that about 60% of the species of Asteraceae tested were either highly resistant or moderately resistant to *M. incognita*. Only 7 of the 35 resistant species were previously reported to be resistant to *M. incognita* or to contain substances toxic to this nematode. These seven were *Ageratum houstinianum* Mill., *C. bipinnatus, Gerbera jamesonii* Bolax ex Hook., *Tagetes patula* L., *Ta. erecta, Ti. diversifolia,* and *Zinnia elegans* (McSorley and Frederick, 1994; Prakash and Rao, 1997).

Our data show that *Calendula officinalis* L., *Helianthus annuus* L., and *Xanthium strumarium* L. were highly susceptible to *M. incognita*. However, soil amended with tissues of *C. officinalis* was reported to reduce the incidence of *M. incognita,* and extracts from *H. annuus* and *X. strumarium* were found to be toxic to this nematode (Prakash and Rao, 1997). This suggests that plants susceptible to a nematode may still contain substances or precursors of substances toxic to that nematode.

In these experiments, soil planted with resistant *A. houstonianum* (purple) had a final density of 227 J2/100 g soil, whereas susceptible *Centaurea cyanus* L. had only 4 J2/100 g soil. It appears that resistant *A. houstonianum* contained substances stimulatory to nematode egg production and this plant may not be suitable for use as an inter-cycle cover crop for management of root-knot nematodes. Susceptible *Ce. Cyanus,* on the other hand, may contain substances inhibitory to egg production and therefore may be an effective nematode-suppressing crop.

In the greenhouse tests, the severity of root knot on *I. reptans* was greatly reduced by pre-planting or co-planting with *C. bipinnatus, G. pulchella, Ta. erects, Ti. diversifolia,* or *Z. elegans*. Only *Ta. erecta* was reported to decrease the number of *M. incognita* when it was grown in infested soil (Prasad et al., 1992).

Although *C. bipinnatus* (F190) and *Z. elegans* (F645) were non-hosts of *M. incognita,* they became poor hosts when co-planted with *I. reptans*. There were two possible explanations: First, root exudates from *I. reptans* may induce susceptibility on *C. bipinnatus* and *Z. elegans*. Second, the host *I. reptans* had supported a large number of *M. incognita;* when *C. bipinnatus* and *Z. elegans* were provided as alternative hosts to select the nematode population, another biotype of *M. incognita* may have been induced. A similar example has been found in the orange and grape inter-planted groves in Taiwan (Tsay et al., 1997), in that the citrus nematode (*Tylenchulus semipenetrans* Cobb) had adapted to parasitize grape (*Vitis vinifera* L.) within 4 years. When introducing a new resistant or non-host into the field for controlling plant-parasitic nematodes, it is important to evaluate the possibilities of resistance breaking down.

Results from this study suggested that *G. pulchella* is an ideal antagonistic plant for use in the management of *M. incognita*. The roots of *G. pulchella* exuded substances that were lethal to the J2 of *M. incognita* and inhibitory to egg hatch. Moreover, tissues of this plant contained substances that were lethal to various plant-parasitic nematodes but not to free-living nematodes. *Gaillardia pulchella* could be used to manage *M. incognita* as a rotation crop, as a co-planted crop, or as a soil amendment for control of root-knot nematode.

Table 6. Effect of *Gaillardia pulchella* extracts from roots and aboveground parts on the egg hatching and infectivity of *Meloidogyne incognita*.[a]

Tissue used for extraction	J2 hatched/ egg mass	Gall index of *I. reptans*[b]
Roots	45 C[c]	1.0 B
Stems and leaves	140 B	1.0 B
Water, control	249 A	3.5 A

[a] For infectivity tests, nematodes were immersed in extracts for 1 hour before being used to inoculate *I. reptans*.
[b] Gall index ranged from 0 to 4 was based on percentage of roots galled. 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%.
[c] Data followed by the same letter in the same column are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

Table 7. Effect of different concentrations of *Gaillardia pulchella* root exudates on *Meloidogyne incognita* mortality and egg hatching.

Concentration	*M. incognita* J2 mortality (%)	J2 hatched/ egg masses
3 X	98 A[a]	7 C
1 X	28 B	20 BC
25×10^{-2}	16 BC	40 B
5×10^{-2}	6 C	94 A
water	4 C	94 A

[a] 1X solution of root exudates was made by dissolving 1 mg dried, lyophilized root exudates in 1 ml distilled water.
[b] Data followed by the same letter in the same column are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

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