Marigold, Castor Bean, and Chrysanthemum as Controls of Meloidogyne incognita and Pratylenchus alleni[1]

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Abstract: Root and soil populations of *Meloidogyne incognita* were significantly fewer from marigold, castor bean, and chrysanthemum than from tomato roots and soil, but not from fallow soil. Root populations of *Pratylenchus alleni* were significantly fewer from marigold, castor bean, and chrysanthemum than from tomato; marigold had the fewest. Root populations of *M. incognita* and *P. alleni* from tomato simultaneously cultivated with marigold, castor bean, and chrysanthemum were significantly fewer than from tomato cultivated alone. Aborted giant cells and dead *M. incognita* (larvae and females) were observed in roots of marigold and castor bean, but not in chrysanthemum or tomato. Significantly more males than females occurred in castor bean roots. Infection sites of *P. alleni* appeared normal in all hosts. Thin-layer and column chromatography of alcoholic extracts from castor bean revealed no nematicidal thiophene derivatives. *Key Words:* resistance, thiophene derivatives.

As part of a field experiment, we tested nematicides against *Pratylenchus* spp. on pinto bean (*Phaseolus vulgaris* L.) in soil where castor bean (*Ricinus communis* L.) had been grown the previous year. Nematode counts made during the growing season indicated that few *Pratylenchus* spp. were in the control plots in that area. Further, nematode populations in castor bean soil with no nematicides were similiar to those in nematicide-treated plots where corn had been grown the previous season and where populations of *Pratylenchus* spp. were high in nontreated controls. These observations, coupled with reports that marigold reduces populations of *Pratylenchus* spp. and of *Meloidogyne* spp. (6, 14, 19, 20, 21), and that *Chrysanthemum* (8) contains a thiophene derivative similiar to those extracted from marigold, prompted us to conduct greenhouse experiments to measure effects of marigold, castor bean, and chrysanthemum on *Pratylenchus alleni* and *Meloidogyne incognita*. Objectives of the study were: (i) to measure the effects of marigold, castor bean and chrysanthemum as hosts for *M. incognita* and *P. alleni;* (ii) to evaluate the nematicidal potential of marigold, castor bean, and chrysanthemum grown simultaneously with a good host for *M. incognita* and *P. alleni;* and (iii) to determine if castor bean contained thiophene derivatives similar to those reported from *Tagetes* spp.

MATERIALS AND METHODS

Experimental design: The greenhouse experiment was completely randomized. Data were evaluated by a four-way analysis of variance where the main effects were: (i) hosts (marigold, castor bean, chrysanthemum, tomato, or fallow); (ii) nematodes (*P. alleni* or *M. incognita*); (iii) controls (tomato, or no tomato); and (iv) time (30, 60, or 90 days) between transplanting into infested soil and measuring nematode populations. Each treatment group consisted of four replicates of each host plant. Roots from one replicate of each treatment were stained with acid fuchsin in lactophenol (1, 7, 10); nematodes were extracted from roots of the remaining three replicates. All bioassay and soil-count data were based on four replications.

Plant material: Dwarf French marigold (*Tagetes patula* L., 'Lemondrop'), castor bean (*Ricinus communis* L., 'Bronz King'), and tomato (*Lycopersicon esculentum* L., 'Rutgers') seeds were germinated in vermiculite. Two-week-old plants and chrysanthemum (*Chrysanthemum morifolium* Ramat., 'Escapade') rooted cuttings were transplanted into nematode-infested soil in 17.8-cm diameter pots.

Nematodes: Greenhouse cultures of *Meloidogyne incognita* (Kofoid & White) Chitwood on tomato and *Pratylenchus alleni* Ferris on chrysanthemum were diluted in steam-sterilized sandy-loam soil. At transplanting, the soil contained either an average of nine *P. alleni* or twelve *M. incognita* larvae per 25 g soil. Greenhouse temperatures (air and soil) were 24-30 C.

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TABLE 1. Numbers of *Meloidogyne incognita* extracted from fresh roots, from soil around roots, and root-knot indices from bioassay plants grown in soil around roots of 'Lemondrop' marigold, 'Bronz King' castor bean, 'Escapade' chrysanthemum, and 'Rutgers' tomato at three sampling dates.

Treatments	No. females per g root dry wt			No. males per g root dry wt			No. per 25 g soil			Root-knot index - bioassay[a]		
	Days after transplanting			Days after transplanting			Days after transplanting			Days after transplanting		
	30	60	90	30	60	90	30	60	90	30	60	90
Marigold alone	0	3,444	426	0	0	0	8	3	1	1.8	0.4	1.0
Marigold + tomato	3	96,496	39,049	1	42	0	3	2	312	1.6	1.1	4.0
Marigold	0	2,223	13,329	0	0	0						
Tomato	3	94,273	25,720	1	42	0						
Castor bean alone	22	702	254	22	22	7	11	6	10	2.3	2.3	1.9
Castor bean + tomato	8	665,421	95,747	2	34	318	9	3	22	3.0	2.3	3.1
Castor bean	6	618	1,647	0	34	19						
Tomato	2	664,803	94,100	2	0	299						
Chrysanthemum alone	12	729	127	12	78	8	16	3	3	3.3	0.9	0.5
Chrysanthemum + tomato	5	457,344	38,167	5	42	10	16	4	229	2.5	1.4	2.5
Chrysanthemum	3	1,588	3,126	3	42	10						
Tomato	2	455,756	35,041	2	0	0						
Fallow							6	5	1	3.3	0.5	0.4
Tomato alone	5	89,817	11,427	5	0	0	10	6	366	3.5	2.3	3.9

[a]Root-knot indices of tomato where 0 = 0% galled roots; 1 = 0-25%; 2= 26-50%; 3 = 51- 75%; 4 = 76-100% galled roots; 0.5 was added to index value when egg masses were observed.

TABLE 2. Numbers of *Pratylenchus alleni* extracted from roots, soil around roots, and from bioassay plants grown in soil around 'Lemondrop' marigold, 'Bronz King' castor bean, 'Escapade' chrysanthemum, and 'Rutgers' tomato at three sampling times.

Treatments	No. per g root dry wt			No. per 25 g soil			No. per g root dry wt; bioassay[a]		
	Days after transplanting			Days after transplanting			Days after transplanting		
	30	60	90	30	60	90	30	60	90
Marigold alone	0	556	69	17	12	6	31,429	12,422	18,125
Marigold + tomato	0	16,152	3,383	11	9	30	19,453	32,979	47,136
Marigold	0	6,917	238						
Tomato	0	9,235	3,145						
Castor bean alone	6,892	1,122	560	20	8	8	33,125	9,498	9,063
Castor bean + tomato	5,863	10,776	6,054	10	5	8	30,191	60,194	26,111
Castor bean	4,027	684	617						
Tomato	1,836	10,092	5,437						
Chrysanthemum alone	1,408	2,704	2,266	32	9	6	11,032	27,668	26,563
Chrysanthemum + tomato	1,056	14,871	4,690	11	9	12	39,958	80,813	33,125
Chrysanthemum	410	3,291	1,788						
Tomato	646	11,580	2,902						
Fallow				57	11	12	16,436	141,250	12,500
Tomato alone	226	18,105	9,289	23	10	39	20,280	75,052	33,438

[a]Soil bioassay using 'Rutgers' tomato.

TABLE 3. Numbers of galls and egg masses of *Meloidogyne incognita* in roots of 'Lemondrop' marigold, 'Bronz King' castor bean, 'Escapade' chrysanthemum, and 'Rutgers' tomato stained with acid fuchsin in lactophenol at three sampling times.

Treatments	No. galls per 100 cm roots			No. egg masses per 100 cm roots		
	Days after transplanting			Days after transplanting		
	30	60	90	30	60	90
Marigold alone	0	3	0	0	1	0
Marigold + tomato	90	107	200	0	28	54
Marigold	2	1	1	0	0	0
Tomato	88	106	199	0	28	54
Castor bean alone	24	28	11	0	1	1
Castor bean + tomato	19	178	161	0	84	52
Castor bean	10	26	29	0	4	3
Tomato	9	152	132	0	80	49
Chrysanthemum alone	44	92	17	0	1	0
Chrysanthemum + tomato	117	120	263	0	1	34
Chrysanthemum	54	50	55	0	0	1
Tomato	63	70	208	0	1	33
Fallow						
Tomato alone	67	128	282	0	30	31

Nematode counts: P. alleni (all active stages) and *M. incognita* (larvae and males) were extracted from roots over a period of 7 days at 27 ± 1 C using the funnel spray method of Oostenbrink (13). Soil samples were taken every 30, 60, and 90 days with a 2.5-cm diameter soil probe. Nematodes were separated from soil (25 g/sample) with 1.18 sp. gr. sucrose solution and centrifugation (2, 11), and recovered on a 38-μm (400-mesh) sieve and counted. Specimens of stained roots for microscopic examination were obtained by randomly selecting 100 cm of root per plant from each treatment. Bioassays were conducted by removing 500 cc of soil from around the roots of each treatment and transplanting a 'Rutgers' tomato seedling in it at 30, 60, and 90 days after beginning an experiment. Twenty-one days after beginning the bioassay, tomato roots were processed for *P. alleni*, or rated with a root-knot index (16) for *M. incognita*.

Castor bean root extracts: A crude concentrate from castor bean roots was obtained using alcoholic extraction and saponification, as described by Uhlenbroek and Bijloo (19). Crude concentrate was fractionated on a column (57 × 2.2 cm) of, (i) alumina, activated, chromatographic grade, 177-74 μm (80- to 200-mesh) using petroleum ether with increasing quantities of diethyl ether as eluent (19); (ii) alumina, activated, chromatographic grade, 177-74 μm (80- to 200-mesh) with diethyl ether as eluent; and (iii) silica gel, 0.73 mm-74 μm (28- to 200-mesh), grade 12, with absolute ethanol as eluent. Fractions of 10 ml were collected, and the eluate was monitored at 280 nm. An ultraviolet spectrum was made of all fractions (obtained by column chromatography) showing fluorescence at 280 nm.

Thin-layer chromatography, using Eastman Chromagram Sheets (6062 and 6061), was used to screen the crude concentrate for thiophene derivatives according to Curtis and Phillips (3).

TABLE 4. Numbers of *Pratylenchus alleni* in roots of 'Lemondrop' marigold, 'Bronz King' castor bean, 'Escapade' chrysanthemum, and 'Rutgers' tomato stained with acid fuchsin in lactophenol at three sampling times.

Treatments	No. *P. alleni* per 100 cm roots		
	Days after transplanting		
	30	60	90
Marigold alone	2	0	0
Marigold + tomato	63	21	16
Marigold	7	0	9
Tomato	56	21	7
Castor bean alone	52	0	14
Castor bean + tomato	133	9	4
Castor bean	51	0	1
Tomato	82	9	3
Chrysanthemum alone	32	2	3
Chrysanthemum + tomato	75	75	6
Chrysanthemum	35	11	0
Tomato	40	64	6
Fallow Tomato alone	82	35	25

RESULTS

Host-parasite relationships: Typical *M. incognita* infection sites were observed in tomato and chrysanthemum. In castor bean and marigold, giant cells, females, egg masses, and matrix deposition were not typical. In castor bean, many giant cells were small, and some had degenerated. A few females reached maturity and eggs were observed, but most egg masses were entirely within the gall. No ovoviviparity (17) was observed. In marigold, nematodes developed slowly and in many infection sites, giant cells and nematodes were degenerating. Infection sites originated in the stele. Ninety days after inoculation, some females had broken through to the outside of the gall, and small egg masses were produced.

Treatment effects:—1) Individual host.—Tomato was the best host for both *M. incognita* and *P. alleni* (Tables 1, 2). When measured by number of recoverable second-stage larvae from roots (mostly eggs hatched by the mist system) or from soil, marigold, castor bean, and chrysanthemum did not differ ($P \leq 0.05$) as host for *M. incognita*. When measured by tomato bioassay, chrysanthemum and marigold were significantly more effective than castor bean in reducing numbers of *M. incognita* (Table 1). Tomato grown in soil previously planted to marigold or chrysanthemum had root-knot indices of 1.0 and 0.5, respectively–significantly lower than that (1.9) of tomato grown in soil from around castor bean roots. In general, time influenced the effectiveness of marigold or chrysanthemum (Table 1). The only statistically significant difference in numbers of *M. incognita* in stained, infected roots was an increase in males in castor bean roots, but numbers were too few to draw conclusions. Egg masses were not present 30 days after inoculation, but were present after 60 days.

Root populations of *P. alleni* were significantly ($P \leq 0.05$) reduced by marigold, castor bean, and chrysanthemum when compared with tomato; marigold was the most effective (Table 2). Numbers of *P. alleni* recovered from soil in which the four hosts were grown were not significantly different. Tomato bioassays demonstrated that differences in populations of *P. alleni* were significantly related to time: populations were largest after 60 days and smallest after 90 days on all hosts. No significant differences occurred in numbers of adults, larvae or eggs of *P. alleni* in stained roots from different hosts. More were present after 60 days than after 30 or 90 days.

—2) Host combinations.—Marigold, castor bean, and chrysanthemum did not

significantly ($P \leq 0.05$) reduce *M. incognita* or *P. alleni* populations in tomato roots with which they were cultivated simultaneously, when compared with tomato grown alone in infested soil (Table 1, 2). However, after 30 days, no *P. alleni* were recovered from marigold roots or from tomato roots grown with marigold. After 60 days, the population from tomato roots was comparable with that from any other treatment, and the number from marigold grown with tomato was higher than that from marigold grown alone (Table 2). After 90 days, numbers recovered from marigold roots declined. After 90 days, tomato grown with castor bean had 299 *M. incognita* males per gram root dry wt. compared to only 10/g dry wt. recovered from tomato grown with chrysanthemum, and none from tomato grown with marigold or from tomato grown alone (Table 1).

In general, more galls, but not more egg masses of *M. incognita* were produced after 90 than after 60 days, indicating that the life cycle under our conditions was longer than 30 days (Table 3). Relatively few *P. alleni* were in roots of marigold, castor bean, or chrysanthemum when compared with tomato. Marigold, castor bean, chrysanthemum, and fallow reduced the number of *M. incognita* larvae in soil, but marigold and chrysanthemum did not reduce soil populations when grown with tomato for 90 days.

Chemical extractions: Using thin-layer chromatography, crude concentrate obtained from castor bean roots was compared with 2-methyl-thiophene, 2-thiophene-carboxaldehyde, and 3-(2-thienyl)-acrylic acid. With silica gel and methanol, crude concentrate moved with 3-(2-thienyl)-acrylic acid and could not be resolved. No movement was obtained with either alumina and petroleum ether or silica gel and benzene-chloroform (9:1, v/v).

Column chromatography with alumina and either a mixture of petroleum ether with increasing quantities of diethyl ether or diethyl ether as eluent produced fractions that fluoresced at 280 nm and had ultraviolet absorption spectra (solvent ethanol) with maxima at 228 nm, 254 nm, 275 nm, 285 nm, and 335 nm. Residues from cleansed petroleum ether used in natural product extractions and chromatography had maxima at 223 nm, 232 nm, 253 nm, 256 nm, 262 nm, 270 nm, 273 nm, 275 nm, and 288 nm.

Standard 3-(2-thienyl)-acrylic acid in ethanol had maxima at 272 nm, 281 nm, and 312 nm. Using the same standard on an alumina column and eluting with a mixture of petroleum ether with increasing concentrations of diethyl ether produced a fraction with maxima at 271 nm, 281 nm, and 309 nm.

Crude concentrate on a column of silica gel and eluted with absolute ethanol produced a single fraction with an absorption maximum at 264 nm.

No fractions obtained from castor bean extractions showed the ultraviolet absorption maxima reported for marigold extracts containing the nematicidal thiophene derivatives (251 nm, 340 nm, and 251 nm, 350 nm) (19).

DISCUSSION AND CONCLUSION

Meloidogyne incognita reproduced equally well on marigold, castor bean, and chrysanthemum but significantly ($P \leq 0.05$) less than on tomato. The reduced reproduction in marigold and castor bean obviously resulted from aborted giant cells which prevented development of *M. incognita*. In chrysanthemum, giant cell development appeared normal, but there were fewer of them. Fewer egg masses were produced, and thus fewer larvae were recovered. In marigold, few developing larvae and no males were recovered from infected roots. In castor bean, the number of males was relatively greater, and aborted giant cells were common. In chrysanthemum a relatively large number of males was recovered, but the giant cells appeared normal. This suggests that the resistance mechanism in the three plants differed.

Painter (15) thought that resistance involved a triad of basic relationships: antibiosis, tolerance, and preference or nonpreference. Aborted giant cells are common in resistant plants (4, 5) and are a form of antibiosis. Sex reversal during periods of stress, and in unfavorable hosts, also is known for *Meloidogyne* spp. (9, 18). Dropkin (4) pointed out that tolerance should be distinguished from resistance by considering success of the parasite population as well as success of the plant. Whether the antibiosis observed in this study constitutes resistance or tolerance could not be determined because only parasite productivity was measured.

Based on our data, both *M. incognita* and *P. alleni* exhibited early nonpreference (as indicated by infection during the first 30 days) for marigold but did not differentiate among tomato, castor bean, and chrysanthemum. Winoto Suatmadji (21) observed that *Tagetes patula* may resist penetration of *P. penetrans* (Cobb); he based his conclusions on the first 4 weeks after inoculating with 500 larvae/8 ml of sand and sand-perlite mixture in tube culture. After one week, 7 larvae had penetrated the roots, and he recovered 354 from the sand mixture surrounding the roots. We recovered no *P. alleni* from roots of marigold or tomato grown with marigold 30 days after transplanting into infested soil; yet after 60 days, these treatment combinations had many *P. alleni*. We stress recovery rather than penetration because after 30 days, we found about as many *P. alleni* per unit of stained roots grown with marigold as in tomato grown either with another host or alone (Table 4). We have no explanation for nonmigration from tomato roots grown with marigold.

We found no evidence that marigold, castor bean, chrysanthemum, or tomato killed nematodes in soil by nematicidal action. Marigold, castor bean, and chrysanthemum did not significantly reduce nematode populations in tomato plants with which they were cultivated simultaneously, compared with tomato plants grown alone in infested soil (Tables 1, 2).

Oostenbrink et al. (14) found a 90% suppression of *Pratylenchus* by *T. patula* compared with fallow soil, and they suggested that *Tagetes* might alleviate attacks on perennials and prevent damage to new plantings. Good et al. (6) thought South American marigolds were promising as a nematode-reducing cover crop to control *M. incognita acrita, M. incognita incognita,* and others. However, they did not recommend marigolds for reducing populations of *M. hapla* and *M. arenaria*. Their study showed that, of *Crotalaria,* marigold, beggarweed, hairy indigo, sundangrass, millet, and Coastal bermudagrass, *Crotalaria* and marigold were the most effective crops in reducing populations of a wide range of soil nematodes. Winoto Suatmadji (21) found that *Tagetes* spp. were as effective as, or better than, fallow in suppressing *Meloidogyne* spp. He noted that *Meloidogyne* larvae were less persistent that *Pratylenchus* larvae in fallow soil, and that *T. patula* severely suppressed *M. hapla, M. incognita, M. arenaria,* and *M. javanica*. Yields of main crops after *Tagetes* were increased by nematicidal action, growth factors, or both, and *Tagetes* promoted growth of apple seedlings in soil with *P. penetrans* populations up to 167% of fallow-infested soil, although *Tagetes* decreased growth of apple in uninfested soil. Also, direct mulch with a "natural dosage of *Tagetes* roots" suppressed *P. penetrans* much better than did other mulches or fallow. He recommended, therefore, that simultaneously cultivating *Tagetes* with a main crop would be effective around and between trees and woody ornamentals.

Miller and Ahrens (12) reported that marigolds suppressed populations of *P. penetrans* and that their use, in any rotation where cover crops are grown for an entire season and where nematode control is desirable, is economically competitive with ethylene dibromide at 56.2 liters/hectare. They also reported that marigold interplanted with strawberry, tomato, and gladiolus did not increase yields, but acted like a weed in competing for water and nutrients.

In this study, 'Lemondrop' marigold, 'Bronz King' castor bean, and 'Escapade' chrysanthemum reduced populations of *M. incognita* and *P. alleni,* but not significantly more than did fallow. In combination with a good host ('Rutgers' tomato), 'Lemondrop' marigold, 'Bronz King' castor bean, and 'Escapade' chrysanthemum did not reduce numbers of *M. incognita* or *P. alleni*. 'Bronz King' castor bean lacks nematicidal thiophene derivatives present in marigold.

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