Greenhouse Studies on the Effect of Marigolds (*Tagetes* spp.) on Four Root-knot Nematode Species *(Meliodogyne* spp.)

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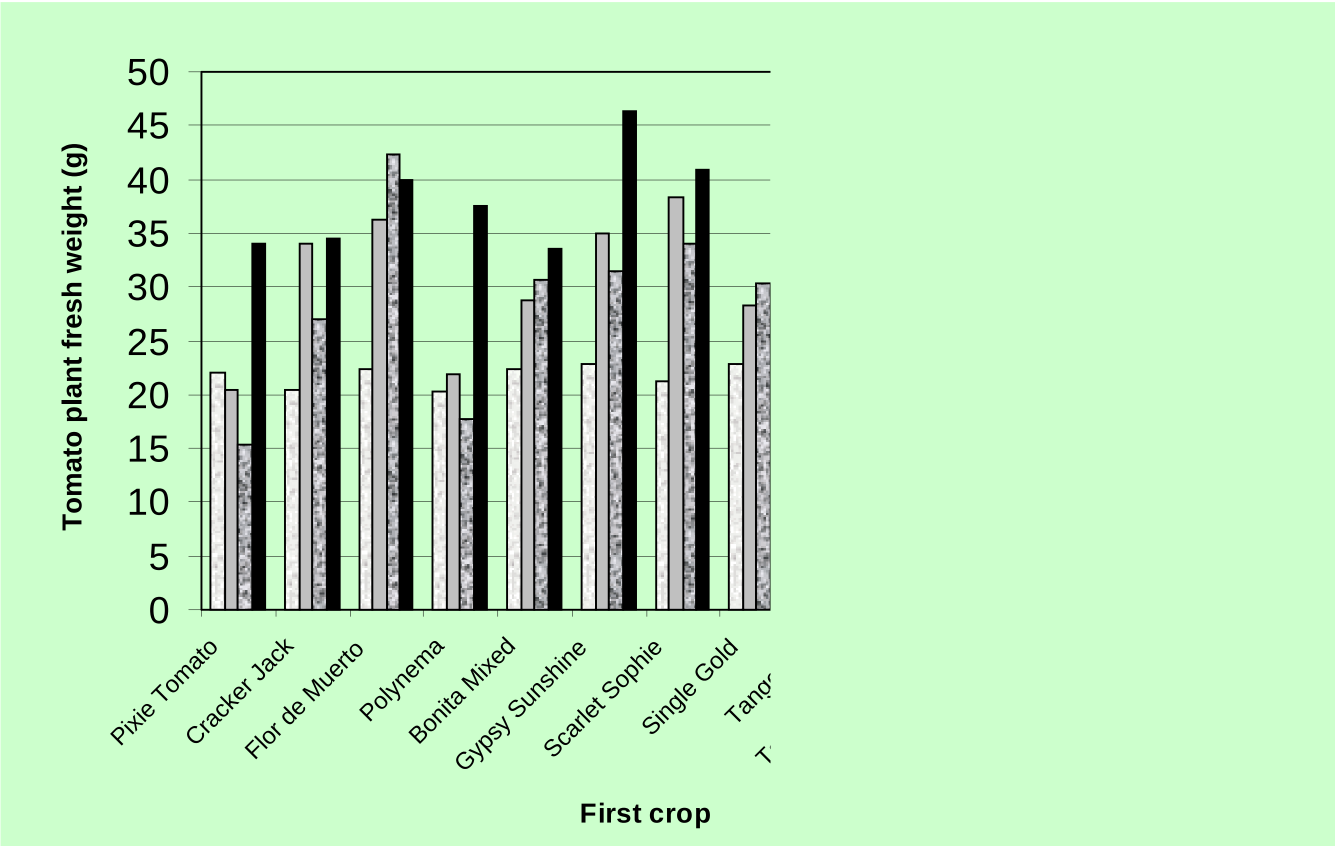
Abstract: The effects of pre-planted marigold on tomato root-galling and multiplication of *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* were studied. Marigold cultivars of *Tagetes patula*, *T. erecta*, *T. signata*, and a *Tagetes* hybrid all reduced galling and numbers of second-stage juveniles (J2) in subsequent tomato compared to the tomato-tomato control. All four *Meloidogyne* spp. reproduced on *T. signata* 'Tangerine Gem.' Several cultivars of *T. patula* and *T. erecta* suppressed galling and reproduction of *Meloidogyne* on tomato to levels lower than or comparable to a fallow control. Phytotoxic effects of marigold on tomato were not observed. It is concluded that several of the tested marigold cultivars are now ready for full scale field evaluation against *Meloidogyne* spp.

Root-knot nematodes (*Meloidogyne* spp.) are the most economically important plant-parasitic nematodes in tropical and subtropical agriculture (Sasser, 1979). In California, control of these nematodes is dependent primarily on nematicides and resistant crop cultivars. However, as the use of nematicides is being severely restricted and resistant cultivars are available for only a limited number of crops (Roberts, 1990), development of alternative control strategies is urgently required. Marigold (*Tagetes* spp.) has long been known to possess nematicidal activity. Initial reports on suppression of root-knot nematodes by marigold (Steiner, 1941; Tyler, 1938) were later confirmed for *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica* (Daulton and Curtis, 1963; Hackney and Dickerson, 1975; McSorley and Frederick, 1994; Motsinger *et al*., 1977; Rickard and Dupree, 1978; Suatmadji, 1969). However, results from these studies were often equivocal. Thus, *T. erecta* did not suppress *M. hapla* in one study (Suatmadji, 1969), but efficiently controlled it in two other studies (Bünte and Müller, 1996; Rickard and Dupree, 1978). In studies with *T. patula*, *M. arenaria* was suppressed (Motsinger *et al*., 1977; Suatmadji, 1969) or not suppressed (McSorley and Frederick, 1994; Rickard and Dupree, 1978). The reasons for these conflicting results are not known, but it is likely that intraspecific differences in the plants and in the nematodes play an important role (Motsinger *et al*., 1977; Suatmadji, 1969). Results with *M. incognita* and *M. javanica* were more consistent. Strong suppression of these nematodes, particularly by *T. patula*, was observed in several studies (Daulton and Curtis, 1963; Hackney and Dickerson, 1975; McSorley and Frederick, 1994; Siddiqi and Alam, 1988; Suatmadji, 1969).

Suppression of lesion nematodes (*Pratylenchus* spp.) by marigolds also has been reported (Hackney and Dickerson, 1975; Hutchinson, 1962; McKenry, 1988; Suatmadji, 1969; Visser and Vythilingam, 1959) and appears less variable than suppression of root-knot nematodes. Despite numerous reports on suppression of lesion- and root-knot nematodes, few studies have included the effects of marigolds on nematode infestation or yields of subsequent crops. Oostenbrink *et al.* (1957) reported yield increases in roses and apple on lesion nematode-infested soils after marigolds comparable to nematicide treatments. Later, Oostenbrink (1960) suggested that marigolds increased yields of lesion nematode-susceptible crops on sandy and peaty soils by 10% to 40%. Significant yield increases in lesion or root-knot nematode-susceptible crops after marigold were also reported by Bünte and Müller (1996), Miller and Ahrens (1969), Siddiqi and Alam (1988), and Suatmadji (1969). Seinhorst and Klinkenberg (1963) obtained significant yield increases after marigold, but suggested that these results might not be attributed solely to nematode control as yields of onions and sugarbeets were 1.4x and 1.2x higher, respectively, after marigolds in fields without plant-parasitic nematodes. In contrast, McKenry (1988, 1991), achieved significant reductions in *P. vulnus* populations, but did not observe expected yield increases in subsequent plum plantings. McKenry (1988) attributed the lack of yield increase to phytotoxicity of marigold that nullified the beneficial effects of nematode control, and concluded that marigold is unlikely to be of use for controlling nematodes in perennial crops.

Given the conflicting results on the suppression of *Meloidogyne* species by marigold and effect of marigold on nematode infestation and yield responses of subsequent crops, this study was initiated to identify marigold cultivars that would effectively suppress *Meloidogyne* populations, decrease infestation levels in a subsequent susceptible crop, and be non-phytotoxic.



Material and Methods

Nematode inocula. Populations of four *Meloidogyne* spp. were used in the experiments: *M. incognita* race 3 from cotton in the San Joaquin Valley, California; *M. javanica* from cowpea, Chino, California; *M. arenaria* race 1, unknown origin; and *M. hapla* from alfalfa in San Bernardino, California. Species and race identifications were done with isoenzyme electrophoresis and on differential host tests (Eisenback and Triantaphyllou, 1991). Populations were increased and maintained on tomato cv. Pixie grown in coarse sand in a greenhouse. Inoculum was prepared by collecting sand and roots from the tomato nematode cultures, cutting the tomato roots into 1-cm-long pieces, and thoroughly mixing infested sand and roots with sterilized sand (ratio infested:sterilized = 1:10).

Suppression of Meloidogyne by marigolds. Three-week-old seedlings of *T. patula* cultivars Single Gold, Scarlet Sophie, Tangerine, and Bonita Mixed, *T. signata* (*T. tenuifolia*) cv. Tangerine Gem, *T. erecta* cultivars CrackerJack and Flor de Muerto, and the *Tagetes* hybrid Polynema were transferred to 200-ml plastic cones (Stuewe and Sons, Corvallis, OR) filled with 250 g of the inoculum sand. Cones planted with tomato cv. Pixie and cones without plants served as controls. Fifteen cones were prepared for each treatment and randomized in plastic holding trays on a greenhouse bench. Plants were fertilized with 3 g of N-P-K (17-6-10) and grown for 60 days. An additional 10 cones were prepared at the start of each experiment for each *Meloidogyne* sp. to determine initial inoculum density. Nematodes from soil in these cones were extracted with sieving and decanting and the resulting suspensions together with root pieces were left for five days on a filter paper supported by a coarse plastic sieve in a petri dish with water at room temperature. Second-stage *Meloidogyne* juveniles (J2) were then counted following extraction at 50x magnification. Sixty days after start of the experiments, five cones from each treatment were randomly collected. The tops and roots of the plants were weighed, the roots were indexed for galling on a scale from 0 (no galls) to 10 (100% of roots galled) (Bridge and Page, 1980), cut into 1-cm-long pieces, and placed in a misting chamber (Seinhorst, 1950) for five days for nematode extraction. Numbers of J2 in the soil from each cone were determined by sieving and decanting as described above.

Infestation of tomato after marigold. The tops of the remaining plants (10 per treatment) were cut and a three-week-old tomato Pixie seedling was planted into each cone. An additional 10 cones were filled with sterilized sand and remained un-infested ("fallow control" treatment). Six weeks later the tomatoes were washed from the cones, tops and roots were weighed, roots were indexed for galling, and nematodes were extracted from the roots as described above.

Statistical analysis. Analysis of variance with SAS software (SAS Institute, Cary, NC) was carried out on plant weight and gall index data and on $\log_{10}(x + 1)$-transformed nematode count data. Treatment means were compared with Duncan's multiple range test at the 5% level of probability.

Results

Meloidogyne hapla: The inoculum density averaged 2,516 J2/cone. Sixty days after starting the experiment the highest numbers of J2 were extracted from tomato, and the fewest from Flor de Muerto. Galls were seen only on roots of tomato and Scarlet Sophie, with average gall indices of 3.0 and 2.6, respectively (Table 1). Effects of marigold cultivars on subsequently grown tomato were minor with regard to plant weight (Figure 1). Tomato after Tangerine Gem had the smallest tops and roots. Tops of tomato after fallow (infested or uninfested) were not significantly different from any of the other treatments.

Tomato after tomato had the highest gall index and number of J2 in the roots. Numbers of J2 in tomato roots following marigold cultivars Tangerine, Cracker Jack, Flor de Muerto, Polynema, and Tangerine Gem were lower than numbers in the fallow treatment (Table 1).

Meloidogyne incognita: The average inoculum density per cone was 2,719 J2. Sixty days after transplanting, roots of tomato were heavily galled, those of Tangerine Gem had intermediate galling, and only very light galling was observed on Flor de Muerto roots, which corresponded with the number of J2 extracted from the roots. In all but the tomato and Tangerine Gem treatments the numbers of J2 were significantly lower than after fallow (Table 1). Correspondingly, galling and numbers of J2 in subsequently grown tomato were highest after Tangerine Gem and tomato. Three of 10 tomato plants after tomato died during the experiment. Roots of these plants were small and severely galled (gall index 10). These plants were not included in the calculation of mean top and root weight or number of J2 in the roots. Top weights of tomato after tomato were lower than after the uninfested control (Figure 1).

Meloidogyne javanica: High numbers of J2 were collected from tomato. Fewer developed on roots of Tangerine Gem and Polynema, whereas no nematodes were recovered from any of the other marigold cultivars (Table 1). These results were reflected in the infestation of subsequent tomato: gall indices and J2 numbers were highest and top weights were lowest after tomato, Tangerine Gem, and Polynema (Figure 1). No J2 were obtained from tomato following any of the other marigold cultivars (Table 1).

Meloidogyne arenaria: Galling and nematode multiplication were highest on tomato, followed by Tangerine Gem and Polynema. The total number of J2 from tomato after the other marigold treatments was significantly lower than after fallow (Table 1). Correspondingly, galling on tomato after tomato, Tangerine Gem, and Polynema was greater than after the other marigolds. The number of J2 extracted from tomato roots six weeks after transplanting was lower than or similar to the number at planting, with the highest numbers on tomato following tomato, Tangerine Gem, Polynema, and fallow (Table 1).

Discussion

Tomato was a better host for all four *Meloidogyne* species than any of the marigolds tested. Correspondingly, gall indices and J2 numbers on tomato after tomato were higher than on tomato after marigold. Each of several marigold cultivars (e.g. Tangerine, Flor de Muerto, CrackerJack) had a consistent effect on all four *Meloidogyne* spp. on subsequent tomato compared to the fallow treatment. However, other marigold cultivars had nematode-specific effects. For example, Tangerine Gem suppressed galling and reproduction of *M. hapla* but not of *M. javanica, M. arenaria,* or *M. incognita* in subsequent tomato. In contrast, planting of Single Gold resulted in a total absence of galls or J2 on subsequent tomato with *M. incognita, M. javanica* or *M. arenaria*, but increased galling and J2 numbers of *M. hapla* on tomato compared to fallow.

All four *Meloidogyne* species were able to reproduce on *T. signata* Tangerine Gem. Similar results were obtained by Rickard and Dupree (1978) for reproduction of four *Meloidogyne* spp. on *T. signata*. In contrast, Siddiqi and Alam (1988) reported control of *M. incognita* by *T. signata*. Efficient suppression of *M. incognita* and *M. javanica* by *T. patula* and *T. erecta* varieties corresponds with results obtained by others (Daulton and Curtis, 1963; Hackney and Dickerson, 1975; McSorley and Frederick, 1994; Rickard and Dupree, 1978;

Table 1. The effect of marigold cultivars on populations of root-knot nematodes

Species	Cultivar	First crop inoculated with nematodes			Tomatoes after first crop	
		Gall Index	J2 in roots	J2 in soil	Gall Index	J2 in roots
Meloidogyne hapla						
Tomato	Pixie	3.0	25,550	5703	5.8	45,025
Tagetes erecta	CrackerJack	0.0	3	76	0.8	796
T. erecta	Flor de Muerto	0.0	5	85	0.8	812
Tagetes hybrid	Polynema	0.0	2	105	1.0	1091
Tagetes patula	Bonita Mixed	0.0	33	96	0.9	2724
T. patula	Gypsy Sunshine	0.0	1011	136	2.2	18,300
T. patula	Scarlet Sophie	2.6	1150	552	2.6	16,920
T. patula	Single Gold	0.0	576	240	2.7	6858
T. patula	Tangerine	0.0	2	155	0.6	843
Tagetes signata	Tangerine Gem	0.0	868	293	0.7	1029
Fallow inoc.					1.2	3610
Fallow control				474		
Meloidogyne incognita						
Tomato	Pixie	7.5	31,400	2124	7.9	2003
Tagetes erecta	CrackerJack	0.0	0	1	0.5	11
T. erecta	Flor de Muerto	0.2	11	0	0.7	10
Tagetes hybrid	Polynema	0.0	0	3	0.7	3
Tagetes patula	Bonita Mixed	0.0	0	7	0.6	8
T. patula	Gypsy Sunshine	0.0	1	7	0.3	12
T. patula	Scarlet Sophie	0.0	0	1	0.1	7
T. patula	Single Gold	0.0	0	4	0.0	0
T. patula	Tangerine	0.0	1	0	0.3	3
Tagetes signata	Tangerine Gem	2.8	2209	253	3.0	283
Fallow inoc.					0.1	4
Fallow control				132		

Suatmadji, 1969). None of the four *Meloidogyne* spp. caused galls or reproduced on *T. patula* cv. Tangerine. This cultivar also was reported to be free of galls and J2 in a previous study with *M. incognita, M. arenaria,* and *M. hapla* as inocula (Motsinger *et al*., 1977). As in the present study, a rapid decline of *Meloidogyne* inoculum to near zero levels under fallow in greenhouse experiments has been observed by others (Hackney and Dickerson, 1975; Rickard and Dupree, 1978; Suatmadji, 1969), resulting in nonsignificant differences between the most suppressive marigold cultivars and fallow. Little is known of the effects of marigold compared to fallow in field experiments with *Meloidogyne* spp. However, Oduor-Owino and Waudo (1994) found significant increases in tomato growth and fruit yield and decreased root galling after *T. minuta* compared to fallow in a field infested with *M. javanica*.

Phytotoxic effects of marigold as reported by McKenry (1988, 1991) were not observed in this study on tomato. Top weights of tomato after six weeks in uninfested soil generally were not different from those after marigold. Whether the results obtained from these greenhouse experiments can be directly translated to field situations remains to be studied. It is possible that differences among marigold cultivars that are not relevant in a greenhouse study, (e.g. in depth of rooting and in growth rate), become important under field conditions. This study provides an initial characterization of the differences among marigold cultivars with regard to the suppression of *Meloidogyne* species. In order to further evaluate the usefulness of marigolds in an integrated pest management system, field studies

Table 1. continued

Species	Cultivar	First crop inoculated with nematodes Gall Index	J2 in roots	J2 in soil	Tomatoes after first crop Gall Index	J2 in roots
Meloidogyne javanica						
Tomato	Pixie	5.2	49,600	11,400	9.7	2506
Tagetes erecta	CrackerJack	0.0	0	1	0.0	0
T. erecta	Flor de Muerto	0.0	0	1	0.0	0
Tagetes hybrid	Polynema	0.0	79	1	3.4	63.3
Tagetes patula	Bonita Mixed	0.0	0	0	0.0	0
T. patula	Gypsy Sunshine	0.0	0	1	0.0	0
T. patula	Scarlet Sophie	0.0	0	1	0.0	0
T. patula	Single Gold	0.0	0	0	0.0	0
T. patula	Tangerine	0.0	0	0	0.0	0
Tagetes signata	Tangerine Gem	0.5	278	4	7.0	528
Fallow inoc.					0.2	5
Fallow control				0		
Meloidogyne arenaria						
Tomato	Pixie	7.5	50,563	4575	6.8	2451
Tagetes erecta	CrackerJack	0.0	0	0	0.0	0
T. erecta	Flor de Muerto	0.0	1	1	0.0	0
Tagetes hybrid	Polynema	0.6	482	36	4.4	535
Tagetes patula	Bonita Mixed	0.0	0	1	0.5	0
T. patula	Gypsy Sunshine	0.0	0	4	0.5	6
T. patula	Scarlet Sophie	0.0	17	27	0.5	0
T. patula	Single Gold	0.0	0	0	0.0	0
T. patula	Tangerine	0.0	0	8	0.3	0
Tagetes signata	Tangerine Gem	1.8	3274	41	4.7	1529
Fallow inoc.					3.5	65
Fallow control				194		

incorporating the effects of marigolds on *Meloidogyne* population development, yield responses, and economic outcome are required.

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