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Effect of Temperature on Suppression of *Meloidogyne incognita* by *Tagetes* Cultivars[1]

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Abstract: The suppression of *Meloidogyne incognita* by marigolds differed among six marigold cultivars and five soil temperatures. *Tagetes signata* (syn. *T. tenuifolia*) cv. Tangerine Gem and the *Tagetes* hybrid Polynema allowed reproduction and root galling when grown at 30 °C, and should not be used for control of *M. incognita* at temperatures close to 30 °C. *Tagetes patula* cultivars Single Gold and Tangerine and *T. erecta* Flor de Muerto, when grown within a 20–30 °C soil temperature range, significantly reduced root galling and nematode infestation of subsequent tomato compared to tomato following fallow. When grown at 10 °C or 15 °C, only one of the tested marigold cultivars (*T. erecta* CrackerJack at 15 °C) reduced *M. incognita* infection of subsequent tomato compared to tomato after fallow. Marigolds should be grown at soil temperatures above 15 °C to suppress *M. incognita* infection of a subsequent crop.

Key words: marigold, *Meloidogyne incognita,* nematode, root-knot nematodes, suppression, *Tagetes,* temperature.

The suppression of *Meloidogyne species by Tagetes* spp. (marigolds) was initially reported by Tyler (1938) and Steiner (1941). The potential use of marigolds for the control of *Meloidogyne* species was subsequently studied by others in laboratory and greenhouse experiments (Bünte and Müller, 1996; Daulton and Curtis, 1963; Hackney and Dickerson, 1975; Rickard and Dupree, 1978) and in the field (Motsinger et al., 1977; Oduor-Owino and Waudo, 1994; Oostenbrink, 1960). *Meloidogyne* spp. in these studies included those favoring temperate climates, e.g. *M. hapla* (Good et al., 1965; Oostenbrink, 1960; Suatmadji, 1969) as well as more tropical species, e.g. *M. incognita, M. javanica,* and *M. arenaria* (Daulton and Curtis, 1963; Good et al., 1965; Oduor-Owino and Waudo, 1994; Siddiqi and Alam, 1988; Suatmadji, 1969). Speculation on the mode of action of *Meloidogyne* suppression by marigolds has differed among investigators. Siddiqi and Alam (1988) reported that marigold root exudates strongly inhibited hatching of second-stage juveniles (J2) and were directly nematicidal to hatched juveniles. Christie (1960) suggested that marigold root exudates masked the stimulating effect of other root exudates, and Motsinger et al. (1977) and Suatmadji (1969) concluded that marigolds acted as a trap crop rather than being directly nematicidal. Hackney and Dickerson (1975) and Daulton and Curtis (1963) attributed suppression of *M. incognita* and *M. javanica* to early nonpreference of the nematodes for marigold roots, but Suatmadji (1969) reported that equal numbers of *M. hapla* invaded roots of tomato and marigold.

Soil temperature is one of the most important factors determining *Meloidogyne* egg development and hatching (Goodell and Ferris, 1989; Vrain and Barker, 1978), activity and root penetration (Prot and Van Gundy, 1981; Roberts, 1987; Roberts et al., 1981), and rate of development once juveniles have entered roots (Trudgill, 1995; Vrain et al., 1978). Different species have different temperature thresholds, temperature optima, and numbers of heat units required for their development (Trudgill, 1995; Trudgill and Perry, 1994). Suppression of *Meloidogyne* spp. by marigolds appears to be due to nematodes not completing critical steps during their life cycle (e.g., hatching, root penetration, development inside the roots), and it is likely that suppression is also strongly related to temperature.

The successful use of marigolds as part of an integrated nematode management system will depend on the availability of marigold cultivars that can be easily incorporated

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into existing crop rotation schemes. Therefore, marigold cultivars effective in suppressing *Meloidogyne* spp. over a wide range of soil temperatures would be favored over those effective over a limited temperature range. Our objective was to determine the effects of six marigold cultivars grown at five soil temperatures on *M. incognita* numbers and on infection of subsequently grown tomato plants.

Materials and Methods

Nematode inoculum: An *M. incognita* race 3 population from cotton in the San Joaquin Valley, California, was increased and maintained on tomato (*Lycopersicon esculentum* cv. Pixie) grown in coarse sand in a greenhouse. Inoculum was prepared by extraction of eggs from infected tomato roots with 0.5% NaOCl (Ogallo et al., 1997). The egg concentration was determined by counting subsamples at ×40. The volume of the egg suspension was adjusted with tap water to obtain a concentration of ca. 1,000 eggs/ml.

Experimental design: Marigold cultivars tested were *T. patula* Single Gold and Tangerine, *T. signata* (syn. *T. tenuifolia*) Tangerine Gem, *T. erecta* CrackerJack and Flor de Muerto, and the *Tagetes* hybrid Polynema. For each cultivar, fifty, 3-week-old seedlings were transferred to 200-ml plastic cones (Stuewe and Sons, Corvallis, OR) filled with 250 g of steam-sterilized sandy soil (93% sand, 4% silt, 3% clay). Holes in the bottoms of the cones were covered with tape to confine the root systems to the cones. Cones planted with tomato cv. Pixie and cones without plants served as controls. Ten replicate cones of each cultivar were assigned to each of five temperature treatments (10, 15, 20, 25, and 30 °C) and placed in 1-liter plastic pots (4 cones/pot) filled with sand. Plant treatments within temperatures were assigned to cones at random, and pots with cones were placed in constant-temperature waterbaths at 10, 15, 20, 25, or 30 °C (±1 °C). Three days later, 2 ml of the egg suspension (containing ca. 2,000 eggs) was pipetted into each cone. Plants were fertilized with liquid fertilizer (N-P-K: 15-30-15) and grown for 6 weeks.

After 6 weeks, all cones were removed from the holding pots and the tops of all plants were cut. For each cultivar and temperature, five cones were randomly collected for nematode analysis. Roots were washed and indexed for galling on a scale from 0 to 10 (Bridge and Page, 1980). Root systems were then cut into 1-cm-long pieces and placed on a Baermann funnel for 5 days for nematode extraction. Nematodes were extracted from the soil from each cone by sieving and decanting over a set of four sieves (150, 75, 45, 45-μ aperture). Final extraction was by movement of the nematodes through moist Kleenex tissue into a dish with tap water. Nematodes were collected after 24 hours. Numbers of *Meloidogyne* obtained from the soil and the roots were counted at ×40.

The remaining five cones for each test plant and temperature were randomly distributed over plastic holding trays placed on a greenhouse bench and planted with 3-week-old. Pixie tomato seedlings. Tomatoes were grown for another 8 weeks under greenhouse conditions (25–30 °C, natural daylight). After 8 weeks the tomato plants were removed from the cones, washed, and the root systems were indexed for galling and processed for nematode extraction as described above.

Statistical analysis: Analysis of variance (SAS Institute, Cary, NC) was done on gall-index data and on nematode count data. For normalization, nematode counts were $\log_{10}(x + 1)$-transformed. Treatment means were compared with Duncan's multiple-range test ($P < 0.05$).

Results

Meloidogyne incognita populations after 6 weeks at five temperatures: Root-galling was observed only on Pixie tomato (15, 20, 25, and 30 °C) and Tangerine Gem (25 and 30 °C) and Polynema (30 °C) marigolds. With the exception of tomato at 15 °C, these cultivar × temperature combinations also yielded the highest numbers of J2. The other marigold cultivars reduced the number of J2 to levels similar to or below those of the un-

planted control treatment at all temperatures (Table 1).

Survival of J2 in the unplanted control and on cultivars that remained free of galling was highest at a soil temperature of 10 °C (Table 2). Galling on tomato and on Tangerine Gem and Polynema marigolds was significantly influenced by soil temperature. Whereas galling on tomato roots decreased significantly only at soil temperatures below 20°C, galling on Tangerine Gem and Polynema marigolds at 25 °C was lower than at 30 °C (Table 2).

M. incognita on tomato following marigold: At any temperature, the number of J2 extracted from roots of tomato following the marigold cultivars CrackerJack, Flor de Muerto, Single Gold, and Tangerine was similar to or lower than the J2 number from tomato after the unplanted control (Table 2). Root galling on tomato was low (≤0.6) when these marigold cultivars had been grown at 15 °C or higher, but increased (average 2.25) following these marigolds grown at 10 °C. Tomatoes grown at 20, 25, or 30 °C resulted in severe galling and high numbers

TABLE 1. The effect of different marigold cultivars, tomato, and absence of plants on root galling and numbers of *M. incognita* second-stage juveniles (J2) after 6 weeks at five soil temperatures.

Plant species	Cultivar	Temperature (°C)									
		10		15		20		25		30	
		Gall index[a]									
Lycopersicon esculentum	Pixie	0.0	C a	3.8	B a	7.0	A a	8.0	A a	8.0	A a
Tagetes erecta	CrackerJack	0.0	A a	0.0	A b	0.0	A b	0.0	A c	0.0	A d
	Flor de Muerto	0.0	A a	0.0	A b	0.0	A b	0.0	A c	0.0	A d
Tagetes sp.	Polynema	0.0	B a	0.0	B b	0.0	B b	0.0	B c	0.6	A c
Tagetes patula	Single Gold	0.0	A a	0.0	A b	0.0	A b	0.0	A c	0.0	A d
	Tangerine	0.0	A a	0.0	A b	0.0	A b	0.0	A c	0.0	A d
Tagetes signata	Tangerine Gem	0.0	C a	0.0	C b	0.0	C b	1.4	B b	6.4	A b
		Total J2									
L. esculentum	Pixie	20	B bc	1	C b	26	B a	13,639	A a	6,315	A a
T. erecta	CrackerJack	31	A b	2	B b	0	B b	0	B d	1	B de
	Flor de Muerto	18	A b	1	B b	0	B b	3	B cd	0	B e
Tagetes sp.	Polynema	27	A b	0	B b	1	B b	0	B d	140	A b
T. patula	Single Gold	14	A b	0	C b	0	C b	6	B c	7	B cd
	Tangerine	16	A b	0	B b	0	B b	0	B d	2	B de
T. signata	Tangerine Gem	10	C c	0	D d	0	D d	782	B b	4,463	A a
Unplanted		56	A a	18	B a	1	B b	8	B c	14	B c
		J2 in roots[b]									
L. esculentum	Pixie	0	C a	0	C a	26	B a	9,634	A a	4,242	A a
T. erecta	CrackerJack	1	A a	0	A a	0	A b	0	A c	0	A c
	Flor de Muerto	0	A a	0	A a	0	A b	2	A c	0	A c
Tagests sp.	Polynema	0	B a	0	B a	0	B b	0	B c	47	A b
T. patula	Single Gold	0	A a	0	A a	0	A b	5	A c	0	A c
	Tangerine	0	A a	0	A a	0	A b	0	A c	0	A c
		J2 in soil									
T. signata	Tangerine Gem	0	C a	0	C a	0	C b	623	B b	3,760	A a
L. esculentum	Pixie	20	B bc	1	C b	0	C a	4,005	A a	2,073	A a
T. erecta	CrackerJack	30	A b	2	B b	0	B a	0	B d	1	B d
	Flor de Muerto	18	A b	1	B b	0	B a	1	B d	0	B d
Tagetes spp.	Polynema	27	A b	0	B b	1	B a	0	B d	93	A b
T. patula	Single Gold	14	A b	0	B b	0	B a	1	B d	7	A cd
	Tangerine	16	A b	0	B b	0	B a	0	B d	2	B d
T. signata	Tangerine Gem	10	C c	0	C b	0	C a	159	B b	703	A a
Unplanted		56	A a	18	B a	1	B a	8	B c	14	B bc

Data are from 6 weeks after inoculation. Values are means of five replicates. Significant differences ($P \leq 0.05$) within rows are represented by different capital letters; differences within columns are represented by different small letters. Nematode data were transformed by log (x+ 1) for analysis of variance; non-transformed means are presented.

[a] Gall index: 0 = no galls, 10 = 100% of root system galled.

[b] Second-stage juveniles were extracted from roots placed on Baermann funnels for 5 days.

TABLE 2. Suppression of *M. incognita* root infestation of tomato by six marigold cultivars grown at five soil temperatures.

Plant species	Cultivar	Temperature (°C) 10		15		20		25		30	
		Gall index[a]									
Lycopersicon esculentum	Pixie	3.0	a	1.8	a	7.8	a	8.8	a	9.0	a
Tagetes erecta	CrackerJack	1.8	a	0.0	b	0.0	c	0.0	c	0.2	d
	Flor de Muerto	2.8	a	0.6	ab	0.0	c	0.0	c	0.0	d
Tagetes sp.	Polynema	2.8	a	1.0	ab	0.0	c	0.8	c	6.6	b
Tagetes patula	Single Gold	2.2	a	0.6	ab	0.0	c	0.0	c	0.4	d
	Tangerine	2.2	a	0.4	ab	0.0	c	0.0	c	0.0	d
Tagetes signata	Tangerine Gem	2.2	a	1.4	ab	2.0	b	6.2	b	8.4	a
Unplanted		3.2	a	1.6	a	0.4	c	0.6	c	2.0	c
		J2 in roots[b]									
L. Esculentum	Pixie	970	a	2	b	5,343	a	15,188	a	8,917	a
T. erecta	CrackerJack	548	ab	6	b	5	c	34	bc	9	d
	Flor de Muerto	315	b	23	a	0	c	4	c	15	d
Tagetes sp.	Polynema	408	b	12	ab	0	c	27	bc	2,263	b
T. patula	Single Gold	217	b	13	ab	33	b	1	c	49	d
	Tangerine	253	b	16	ab	4	c	12	bc	11	d
T. signata	Tangerine Gem	687	b	28	ab	317	b	6,322	a	17,425	a
Unplanted		314	b	68	a	10	b	31	b	208	c

Tomato was grown for 8 weeks under greenhouse conditions. Values are means of five replicates. Significant differences ($P \leq 0.05$) within columns are represented by different letters. Nematode data were transformed by log (x+ 1) for analysis of variance; non-transformed means are presented.

[a] Gall index: 0 = no galls, 10 = 100% of root system galled.

[b] Second-stage juveniles were extracted from roots placed on Baermann funnels for 5 days.

of J2 in roots of subsequent tomato. At a soil temperature of 15 °C, however, the effect of tomato on subsequent tomato was no longer evident.

DISCUSSION

Tangerine Gem marigold was a good host for *M. incognita;* consequently, roots of tomatoes grown after Tangerine Gem were severely galled and supported high nematode populations, especially when Tangerine Gem was grown at 20–30 °C. This result agrees with an earlier finding (Ploeg, 1999) that Tangerine Gem was a good host for *M. incognita.* Polynema marigold also allowed *M. incognita* reproduction but only when grown at 30 °C. The temperature-dependent host status of Polynema marigold was recently described by Ploeg and Maris (1999). Galling of tomato roots by *M. incognita* was reduced at temperatures below 20 °C, which agrees with other reports (Prot and Van Gundy, 1981; Roberts, 1987; Roberts et al., 1981). Ploeg and Maris (1999) calculated that at 20 °C *M. incognita* completed its life cycle (J2 to J2) on tomato in approximately 40 days. We harvested tomato grown at 20 °C after 42 days and most of the egg inoculum would have just completed one cycle, occuring on the roots as eggs or females. This would account for the high gall index but low number of J2 on these tomato roots at harvest.

In the absence of a plant, only a small part of the inoculum existed as J2 after 6 weeks. However, more J2 were collected at 10 °C compared to the other soil temperatures. With the exception of the 10 °C soil temperature, the low number of J2 collected after the 6-week period from the unplanted controls cannot have been due to most of the egg-inoculum surviving as eggs, since galling on subsequent tomato was also generally low. The apparent higher survival of *M. incognita* at a low soil temperature was also reported by Bergeson (1959), who found that *M. incognita* J2 infectivity declined much more rapidly at 26.7 °C and 15.6 °C than at 10 °C. The few nematodes

that did survive without a plant were still infective. The significantly higher number of J2 from the unplanted control compared to all other planted treatments after 10 and 15 °C suggests that a plant-associated nematode toxicity occurred. Similar results were reported by Suatmadji (1969), who found that the activity and mobility of *M. hapla* J2 declined more rapidly in the presence of plants, including marigold and tomato, than in unplanted soil.

The higher numbers of J2 from the unplanted controls after 10 and 15 °C compared to the planted treatments were not reflected in the infection of subsequent tomato, (except for CrackerJack marigold at 15 °C, which significantly reduced both galling and numbers of J2 on tomato). Thus, with the possible exception for CrackerJack marigold, cultivation of marigold to suppress *M. incognita* seems of little use if soil temperatures are likely to drop to 15 °C. At higher soil temperatures, when *M. incognita* is more active and infective (Prot and Van Gundy, 1981; Roberts, 1987; Roberts et al., 1981), Flor de Muerto, Single Gold, and Tangerine marigolds significantly reduced root galling and nematode infection of subsequent tomato compared to the unplanted control.

A direct nematicidal effect of marigolds on eggs as suggested by Siddiqi and Alam (1988) does not explain our results. At 10 °C the egg inoculum most likely remained in the egg stage during the 6-week period (Vrain and Barker, 1978). Therefore, infection of tomato after the 10 °C treatments resulted from J2 hatching from eggs after transfer of the cones from 10 °C to greenhouse temperatures. As there were no significant differences among infection rates of tomato after the different marigolds, tomato, or the unplanted control, it can be concluded that marigolds did not affect the viability of the eggs. A more likely explanation for the suppression of *M. incognita* by marigolds is that the marigolds acted as nonhosts preventing nematode root penetration or as trap crops that arrested the development of invaded J2, or a combination or both (Daulton and Curtis, 1963).

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