# Effect of Temperature on Suppression of *Meloidogyne incognita* by *Tagetes* Cultivars

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*Abstract*: The suppression of *Meloidogyne incognita* by marigolds differed among 6 marigold cultivars and 5 soil temperatures. *Tagetes signata* (syn. *T. tenuifolia*) cv. Tangerine Gem and the *Tagetes* hybrid Polynema allowed reproduction and root galling when grown at 30 $^0$C, and should not be used for control of *M. incognita* at temperatures close to 30 $^0$C. *Tagetes patula* cultivars Single Gold and Tangerine and *T. erecta* Flor de Muerto, when grown within a 20 $^0$C - 30 $^0$C soil temperature range, significantly reduced root-galling and nematode infestation of subsequent tomato compared to tomato following fallow. When grown at 10 $^0$C or 15 $^0$C only one of the tested marigold cultivars (*T. erecta* CrackerJack at 15 $^0$C) reduced *M. incognita* infection of subsequent tomato compared to tomato after fallow. Marigolds should be grown at soil temperatures above 15 $^0$C to suppress *M. incognita* infestation of a subsequent crop.

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, both in lab or 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* species in these studies included those favoring temperate climates, e.g. *M. hapla* (Good et al., 1965; Oostenbrink, 1960; Suatmadji, 1969) as well as subtropical 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 Mashkoor-Alam, 1988; Suatmadji, 1969). Speculations on the mode of action of *Meloidogyne* suppression by marigolds differed among these researchers. Siddiqi and Mashkoor-Alam (1988) reported that marigold root-exudates strongly inhibited the hatching of second stage juveniles (J2s) from the eggs and were directly nematicidal to hatched juveniles, Christie (1960) suggested that marigold root-exudates masked the stimulating effect of any 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 non-preference of the nematodes for marigold roots but Suatmadji (1969) reported that equal numbers of *M. hapla* invaded roots of tomato and marigold.

It is well established that for *Meloidogyne* species soil temperature is one of the most important factors determining 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 the rate of development once inside the 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). As suppression of *Meloidogyne* species by marigolds appears to be due to the nematodes not being able to complete critical steps during their life-cycle (e.g. hatching, root penetration, development inside the roots) 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 varieties which can be easily incorporated into existing crop rotation schemes. Marigold varieties, therefore, which are effective in suppressing *Meloidogyne* over a wide range of soil temperatures would be favored over those effective over a limited temperature range. Here we report on the effects of 6 marigold varieties grown at 5 soil temperatures on *M. incognita* population levels and on infestation of subsequently grown tomato plants.

**Materials and Methods**

*Nematode inoculum*: A *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 using 0.5% NaOCl (Ogallo *et al*., 1997). The egg concentration was determined by counting of subsamples at 40x magnification. The volume of the egg suspension was then adjusted with tap water to obtain a concentration of ca. 1000 eggs/ml.

*Experimental design*: Marigold varieties tested were *T. patula* Single Gold and Tangerine, *T. signata* (syn. *tenuifolia*) Tangerine Gem, *T. erecta* CrackerJack

**Table 1. The effect of 5 soil temperatures on root-galling and numbers of second stage *M. incognita* juveniles on tomato, marigolds and "no plant" 6 weeks after inoculation.**

| | Soil Temperature | | | | |
|---|---|---|---|---|---|
| Plant Tested | 30° C | 25° C | 20° C | 15° C | 10° C |
| ***gall index (0= no galls to 10= 100% of roots galled)*** | | | | | |
| tomato | 8.0 | 8.0 | 7.0 | 3.8 | 0.0 |
| CrackerJack | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Flor de Muerto | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Polynema | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 |
| Single Gold | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Tangerine | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Tangerine Gem | 6.4 | 1.4 | 0.0 | 0.0 | 0.0 |
| no plant | N/A | N/A | N/A | N/A | N/A |
| ***total J2 (second stage juvenile nematodes)*** | | | | | |
| tomato | 6315 | 13639 | 26 | 1 | 20 |
| CrackerJack | 1 | 0 | 0 | 2 | 31 |
| Flor de Muerto | 0 | 3 | 0 | 1 | 18 |
| Polynema | 140 | 0 | 1 | 0 | 27 |
| Single Gold | 7 | 6 | 0 | 0 | 14 |
| Tangerine | 2 | 0 | 0 | 0 | 16 |
| Tangerine Gem | 4463 | 782 | 0 | 0 | 10 |
| no plant | 14 | 8 | 1 | 18 | 56 |

Nematode data were transformed by log(x+1) for analysis of variance.

and Flor de Muerto and the *Tagetes* hybrid Polynema. For each test plant variety 50 3-week-old seedlings were transferred to 200 ml plastic cones (Stuewe and Sons Inc., 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. For each test plant variety 10 cones were assigned to one of 5 temperature treatments (10, 15, 20, 25 and 30 $^0$C) and placed in 1 L plastic pots (4 cones per pot). The space between the cones and the pot was then filled with sand. The placement of the cones over the pots was randomized for each temperature. The pots with cones were then transferred to waterbaths running at 10, 15, 20, 25 and 30 $^0$C (± 1 $^0$C). Each temperature/ plant combination occurred 10 times. Three days later 2 ml of the egg suspension (containing ca. 2000 eggs) were 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 test plant variety and temperature 5 cones were randomly collected for nematode analysis. The roots were washed and indexed for galling on a scale from 0 to 10 (Bridge and Page, 1980). The root systems were then cut into ca. 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 4 sieves (150, 75, 2 x 45 µm aperture) and further extraction of the suspension for 24 hours over moist Kleenex tissue paper. Numbers of *Meloidogyne* J2s obtained from the soil and the roots were counted at 40x magnification.

The remaining 5 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 tomato Pixie seedlings. These tomatoes were grown for another 8 weeks under greenhouse conditions (25 $^0$C - 30 $^0$C, natural day-light). 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 with SAS software (SAS Institute, Cary, NC) was done on gall index data and on $\log_{10}(x + 1)$-transformed nematode count data. Treatment means were compared with

**Table 2. Suppression of *M. incognita* root-infestation of tomato by 6 marigold varieties grown at 5 soil temperatures.**

| | Soil Temperature | | | | |
|---|---|---|---|---|---|
| Previous Plant | 30° C | 25° C | 20° C | 15° C | 10° C |
| ***gall index (0= no galls to 10= 100% of roots galled)*** | | | | | |
| tomato | 9.0 | 8.8 | 7.8 | 1.8 | 3.0 |
| CrackerJack | 0.2 | 0.0 | 0.0 | 0.0 | 1.8 |
| Flor de Muerto | 0.0 | 0.0 | 0.0 | 0.6 | 2.8 |
| Polynema | 6.6 | 0.8 | 0.0 | 1.0 | 2.8 |
| Single Gold | 0.4 | 0.0 | 0.0 | 0.6 | 2.2 |
| Tangerine | 0.0 | 0.0 | 0.0 | 0.4 | 2.2 |
| Tangerine Gem | 8.4 | 6.2 | 2.0 | 1.4 | 2.2 |
| no plant | 2.0 | 0.6 | 0.4 | 1.6 | 3.2 |
| ***J2 from roots (second stage juvenile nematodes)*** | | | | | |
| tomato | 8917 | 15188 | 5343 | 2 | 970 |
| CrackerJack | 9 | 34 | 5 | 6 | 548 |
| Flor de Muerto | 15 | 4 | 0 | 23 | 315 |
| Polynema | 2263 | 27 | 0 | 12 | 408 |
| Single Gold | 49 | 1 | 33 | 13 | 217 |
| Tangerine | 11 | 12 | 4 | 16 | 253 |
| Tangerine Gem | 17425 | 6322 | 317 | 28 | 687 |
| no plant | 208 | 31 | 10 | 68 | 314 |

Nematode data were transformed by log(x+1) for analysis of variance.

Duncan's multiple range test at the 5% level of probability.

**Results**

*M. incognita on test plants and in soil after 6 weeks at 5 temperatures:* Root-galling was observed only on tomato Pixie (30 $^0$C, 25 $^0$C, 20 $^0$C, and 15 $^0$C) and marigolds Tangerine Gem (30 $^0$C and 25 $^0$C) and Polynema (30 $^0$C) (Table 1). Correspondingly, compared to the other plant varieties tested the recovery of J2s was higher after tomato Pixie grown at 30 $^0$C, 25 $^0$C or 20 $^0$C, after marigolds Tangerine Gem grown at 30 $^0$C or 25 $^0$C and Polynema grown at 30 $^0$C. The other marigold varieties reduced the number of J2s to levels similar to or below those of the 'no plant' control treatment at all temperatures.

Comparing the effect of soil temperature on *M. incognita* infestation levels for the plant varieties tested, showed that from the marigold varieties which remained free of root-galling and from the 'no plant' control significantly more ($P < 0.05$) J2s were recovered after soil temperatures of 10 $^0$C than after the other soil temperatures (Table 1.). Galling on tomato and on marigolds Tangerine Gem and Polynema was significantly influenced by soil temperature. Whereas galling on tomato roots decreased significantly ($P < 0.05$) only when soil temperatures were below 20 $^0$C, galling on marigolds Tangerine Gem and Polynema decreased significantly ($P < 0.05$) when soil temperatures decreased from 30 $^0$C to 25 $^0$C.

*M. incognita on tomato after the different test plants and soil temperatures:* The number of J2s extracted from roots of tomatoes following the marigold varieties CrackerJack, Flor de Muerto, Single Gold and Tangerine grown at any of the tested temperatures was similar to or lower than the number of J2s from tomato after the 'no plant' treatment (Table 2). The highest average gall-index on tomatoes following any of these marigold varieties was 0.6 when marigolds had been grown at soil temperatures of 15 $^0$C or higher. However, when these marigolds had been grown at 10 $^0$C the gall indexes on subsequent tomatoes increased to ca. 2.4 and were not different from the other treatments. Tomatoes

grown at temperatures of 30 $^0$C, 25 $^0$C, or 20 $^0$C resulted in severe galling and high numbers of J2s in roots of subsequent tomato. At a soil temperature of 15 $^0$C, however, the negative effect of tomato on subsequent tomato was no longer evident (Table 2).

**Discussion**

Marigold Tangerine Gem did not suppress *M. incognita* but rather acted as a host plant allowing nematode reproduction and root-galling. Correspondingly, roots of tomatoes grown after Tangerine Gem were severely galled and supported high nematode populations, especially when Tangerine Gem had been grown at 30 $^0$C, 25 $^0$C or 20 $^0$C. This agrees with earlier findings (Ploeg, 1998) showing Tangerine Gem to be a good host for *M. incognita*. Hybrid marigold Polynema also allowed *M. incognita* reproduction but only when grown at 30 $^0$C. This temperature-dependent host status of Polynema was recently described by Ploeg and Maris (1998). The reaction of tomato to *M. incognita* was also affected by soil temperature. Below 20 $^0$C root galling significantly decreased. This agrees with earlier research (Prot and Van Gundy, 1981; Roberts, 1987; Roberts *et al*., 1981) which showed that below 18 $^0$C the activity and penetration rate of *M. incognita* J2s significantly decreased. From results by Ploeg and Maris (1998) it can be calculated that, at 20 $^0$C, completion of the *M. incognita* life cycle would require ca. 40 days. This explains why at 20 $^0$C a high gall-index was associated with low numbers of J2 as the nematodes at the time of extraction were just past completion of their life cycle, and therefore were mainly in the egg stage.

At soil temperatures above 15 $^0$C marigolds CrackerJack, Flor de Muerto, Single Gold and Tangerine strongly reduced galling and nematode numbers on subsequent tomato, compared to the tomato after tomato treatment.

In the absence of a plant only a small part of the inoculum was recovered as J2s after 6 weeks. However, more J2s were recovered after 10 $^0$C compared to the other soil temperatures. 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 more rapidly at 26.7 $^0$C and 15.6 $^0$C than at 10 $^0$C. The few nematodes which did survive without a plant were still infective as they were able to cause galling and reproduce on subsequent tomato. The significantly higher recovery of J2s from the 'no plant' treatment compared to all other planted treatments after 10 $^0$C and 15 $^0$C suggests that a general plant-associated nematode toxicity occurred. Similar results were reported by Suatmadji (1969) who found that the activity and motility of *M. hapla* J2s declined much more rapidly in the presence of plants, marigolds as well as tomato, than in unplanted soil.

The higher recovery of J2s from the unplanted controls after 10 $^0$C and 15 $^0$C compared to the planted treatments was not, however, reflected in the infection of subsequent tomato (except for marigold CrackerJack at 15 $^0$C which significantly reduced both galling and numbers of J2s in subsequent tomato). Thus, with the possible exception of marigold CrackerJack, the cultivation of marigolds to suppress *M. incognita* seems of little use when soil temperatures drop to ca. 15 $^0$C. At higher soil temperatures, which correspond to temperatures at which *M. incognita* is most active and infective (Prot and Van Gundy, 1981; Roberts, 1987; Roberts *et al*., 1981), the marigold varieties Flor de Muerto, Single Gold and Tangerine significantly reduced root-galling and/or nematode infection of subsequent tomatoes compared to the 'no plant' control. Whether the temperature range at which suppression is effective against *Meloidogyne* species changes according to the temperature thresholds and optima for the respective *Meloidogyne* species remains unknown, but has important practical implications.

The suppression of *M. incognita* by marigolds was most likely due to marigolds acting as non-hosts preventing nematode root penetration, trap crops, arresting the development of invaded J2s, or a combination of these as suggested by Daulton and Curtis (1963). A direct nematicidal effect of marigolds on eggs as suggested by Siddiqi and Mashkoor-Alam (1988) does not explain our results. At 10 $^0$C the egg-inoculum most likely remained in the egg stage during the 6-week period (Vrain and Barker, 1978). Therefore, the infection of tomato after the 10 $^0$C treatments resulted from J2s hatching from eggs after transfer of the cones from 10 $^0$C to greenhouse temperatures. As there were no significant differences between infection of tomato after the different marigolds, tomato or the 'no plant' control, it can be concluded that marigolds did not directly affect the infectivity of the eggs.

Suppression of lesion nematodes (*Pratylenchus* spp.) by marigolds occurs inside the roots and therefore is dependent on invasion of the roots by the nematodes (Gommers and Bakker, 1988). Whether the mechanism responsible for the suppression of *M. incognita* is the same remains to be studied.

**Literature Cited**


Bergeson, G. B. 1959. The influence of temperature on the survival of some species of the genus *Meloidogyne*, in the absence of a host. Nematologica 4:344-354.

Bridge, J., and S. L. J. Page. 1980. Estimation of root-knot nematode infestation levels on roots using a rating chart. Tropical Pest Management 26:296-298.

Bünte, R., and J. Müller. 1996. Influence of resistant oil radish genotypes on the population dynamics of *Meloidogyne hapla* and *M. incognita*. In German. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 103:527-534.

Christie, J.R. (1960). Some interrelationships between nematodes and other soil-borne pathogens. pp. 432-436. In: J.N. Sasser and W.R. Jenkins (eds.). Nematology, Fundamentals and Recent Advances. Chapel Hill, North Carolina Press.

Daulton, R. A. C., and R. F. Curtis. 1963. The effects of *Tagetes* spp. on *Meloidogyne javanica* in Southern Rhodesia. Nematologica 9:357-362.

Gommers, F. J., and J. Bakker. 1988. Physiological changes induced by plant responses or products. pp. 3-22 In G. O. Poinar, and H. B. Jansson (eds.) Diseases of Nematodes. Volume 1. Boca Raton, FL: CRC Press Inc.

Good, J. M., N. A. Minotn, and C. A. Jaworski. 1965. Relative susceptibility of selected cover crops and coastal bermudagrass to plant nematodes. Phytopathology 55:1026-1030.

Goodell, P. B., and H. Ferris. 1989. Influence of environmental factors on the hatch and survival of *Meloidogyne incognita*. Journal of Nematology 21:328-334.

Hackney, R. W., and O. J. Dickerson. 1975. Marigold, castor bean and chrysanthemum as controls of *Meloidogyne incognita* and *Pratylenchus alleni*. Journal of Nematology 7:84-90.

Motsinger, R. E., E. H. Moody, and C. M. Gay. 1977. Reaction of certain French marigold (*Tagetes patula*) cultivars to three *Meloidogyne* spp. Journal of Nematology 9:278.

Oduor-Owino, P., and S. W. Waudo. 1994. Comparative efficacy of nematicides and nematicidal plants on root-knot nematodes. Tropical Agriculture 71:272-274.

Ogallo, J.L., P.B. Goodell, J. Eckert, and P.A. Roberts. 1997. Evaluation of NemX, a new cultivar of cotton with high resistance to *Meloidogyne incognita*. Journal of Nematology 29:531-537.

Oostenbrink, M. 1960. *Tagetes patula* L als voorvrucht van enkele land- en tuinbouwgewassen op zand- en dalgrond. Mededelingen van de Landbouwhoogeschool en Opzoekingsstations Gent 25:1065-1075.

Ploeg, A. T. 1998. Greenhouse studies on the effect of marigolds (*Tagetes* spp.) on four *Meloidogyne* species. Journal of Nematology - submitted.

Ploeg, A. T., and P. C. Maris. 1998. Effects of temperature on the duration of the life cycle of a *Meloidogyne incognita* population. Fundamental and Applied Nematology - submitted.

Prot, J. C., and S. D. Van Gundy. 1981. Influence of photoperiod and temperature on migrations of *Meloidogyne* juveniles. Journal of Nematology 13:217-220.

Rickard, D. A., and A. W. Dupree. 1978. The effectiveness of ten kinds of marigolds and five other treatments for control of four *Meloidogyne* spp. Journal of Nematology 10:296-297.

Roberts, P. A. 1987. The influence of planting date of carrot on *Meloidogyne incognita* reproduction and injury to roots. Nematologica 33:335-342.

Roberts, P. A., S. D. Van Gundy, and H. E. McKinney. 1981. Effects of soil temperature and planting date of wheat on *Meloidogyne incognita* reproduction, soil populations and grain yield. Journal of Nematology 13:338-345.

Siddiqi, M. A., and M. Mashkoor-Alam. 1988. Control of plant parasitic nematodes by *Tagetes tenuifolia*. Revue de Nématologie 11:369-370.

Steiner, G. 1941. Nematodes parasitic on and associated with roots of marigolds (*Tagetes* hybrids). Proceedings of the Biological Society Washington 54:31-34.

Suatmadji, R. W. 1969. Studies on the effect of *Tagetes* species on plant parasitic nematodes. H. Veenman & Zonen N. V. - Wageningen, The Netherlands. 132 pp.

Trudgill, D. L. 1995. An assessment of the relevance of thermal time relationships to nematology. Fundamental and Applied Nematology 18:407-417.

Trudgill, D. L., and J. N. Perry. 1994. Thermal time and ecological strategies - a unifying hypothesis. Annals of applied Biology 125:521-532.

Tyler, J. 1993. Development of the root-knot nematode. Hilgardia 7:391-414.

Tyler, J. 1938. Proceedings of the root-knot nematode conference held at Atlanta, Georgia, February 4, 1938. Plant Disease Reporter Supplement 109:133-151.

Vrain, T. C., and K. R. Barker. 1978. Influence of low temperature on development of *Meloidogyne incognita* and *M. hapla* eggs in egg masses. Journal of Nematology10:311-313.

Vrain, T. C., K. R. Barker, and G. I. Holtzman. 1978. Influence of low temperature on rate of development of *Meloidogyne incognita* and *M. hapla* larvae. Journal of Nematology 10:166-171.



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