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EXPERIMENTS IN SUPPRESSING CITRUS NEMATODE POPULATIONS BY USE OF A MARIGOLD AND A PREDACIOUS NEMATODE [1]

by
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Chemical control of the citrus nematode, *Tylenchulus semipenetrans* Cobb, on established citrus trees in Israel has hitherto been only partially successful. Although treatment with dibromochloropropane, (DBCP) has been effective in killing the nematodes, tree response has not always been consistent and sometimes a phytotoxic effect has been observed (Cohn, 1969). Since the citrus nematode is recognized in Israel as a pest of considerable importance (Cohn *et al.*, 1965), it was decided to assay some less conventional nonchemical control methods. The present paper reports experiments in controlling the citrus nematode by biological means.

The nematicidal effects of marigolds, *Tagetes* spp., have been studied by several workers and much of the work done was reviewed by Winoto Suatmadij (1969). It has been shown that populations of several plant nematode species — both endo and ectoparasitic — have been suppressed when exposed to *Tagetes* roots in the soil. On the other hand, other nematode species — for instance, *Hemicycliophora*, *Paratylenchus* and *Trichodorus* — appear to be unaffected by *Tagetes* cultivars. The effect of *Tagetes* on *T. semipenetrans* does not appear to have been studied previously.

Studies of predacious nematodes have largely been restricted to the mechanism of predation (Esser and Sobers, 1964), while quan-

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titative information appears to be scant. However, Boosalis and Mankau (1965) have demonstrated a seven-fold increase of *Thornia* sp. in the presence of *T. semipenetrans* in pot inoculations, but no significant decline in the citrus nematode numbers. Our attention was attracted by a chance observation of an apparent reduction in population levels of *T. semipenetrans* in our laboratory cultures by a predacious nematode, *Mylonchulus sigmaturus* (Cobb) Altherr and more detailed investigations of this association were therefore carried out.

Procedures and results

Trials with TAGETES

All experiments were carried out with the species *Tagetes patula* L. var. Golden Harmony, which has been reported to be potentially most effective in suppressing nematode populations (Winoto Suatmadji, 1969).

Initially, the effect of *T. patula* on a soil population of the free-living stages of *T. semipenetrans* in the absence of a host was studied. Nematode-infested orchard soil was placed in 200 liter asbestos containers, kept in a screenhouse; in three of them *T. patula* was planted, while three others were left as checks. Soil samples were taken at different time intervals from each container and larvae and males of *T. semipenetrans* were counted. After 20 weeks, the *Tagetes* plant tops were cut, and nematode sampling continued until 14 months after planting. Results of nematode counts, presented in Fig. 1, indicated no rapid suppression of the nematode population in comparison with checks, either during or immediately after the growing period of the marigold.

A second trial was carried out to study the effect of *T. patula* on the rate of increase of *T. semipenetrans* on its host. Twelve-week old sour orange seedlings (*Citrus aurantium* L.) were planted in 10 liter plastic containers in the following combinations: (*a*) nematode-infested soil without *T. patula*; (*b*) nematode-infested soil with three *T. patula* seedlings introduced two months after planting of citrus (Fig. 2, A); (*c*) heat-sterilized soil without *T. patula*; and (*d*) heat-sterilized soil with three *T. patula* seedlings introduced two months

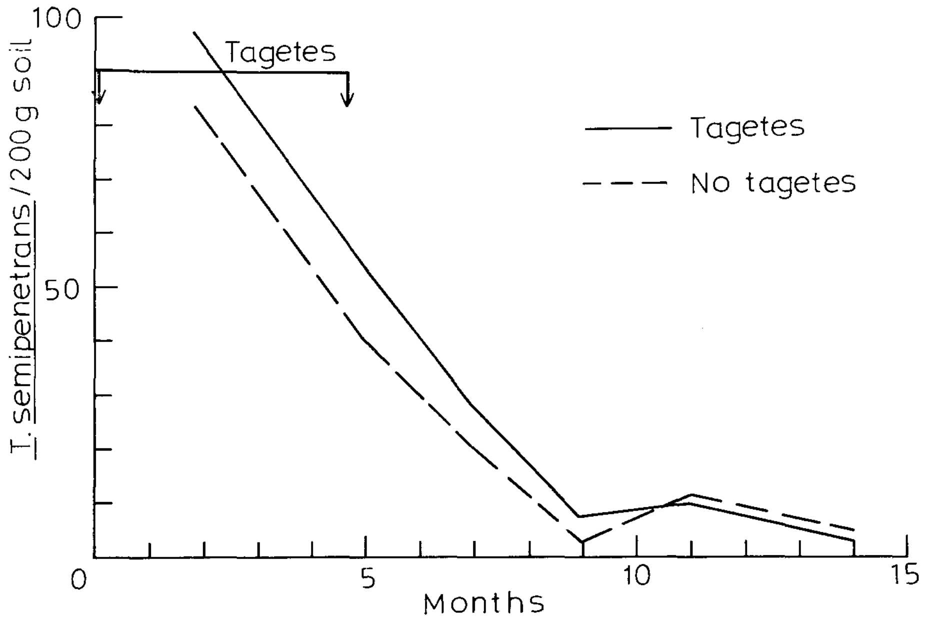

Fig. 1 - Rate of *T. semipenetrans* population decrease in fallow soil in presence and absence of *Tagetes*.

after planting of citrus. There were 18 replications in each of the four categories. The marigold tops were cut after five months' growth, and nematode counts (larvae and males of *T. semipenetrans*) were carried out on soil samples taken at different intervals. The plants were kept in a screenhouse and the experiment was run for 15 months.

Fig. 3 shows that the *Tagetes* again had no suppressive effect on the nematode. The pattern of population change was similar, irrespective of the presence or absence of *Tagetes*, *viz*, an initial drop in the nematode population immediately after planting of the citrus seedlings, followed by a stabilization period and finally an increase in numbers. In fact, population increase was even somewhat faster in the soil with *Tagetes*. It is therefore hardly surprising that the marigold in this trial was harmful rather than beneficial to the citrus seedlings due to its competitive effect on the citrus plant development. This is illustrated in Fig. 4, which presents significant differences in the weight of the citrus plants between the different combinations at the end of the experimentation period.

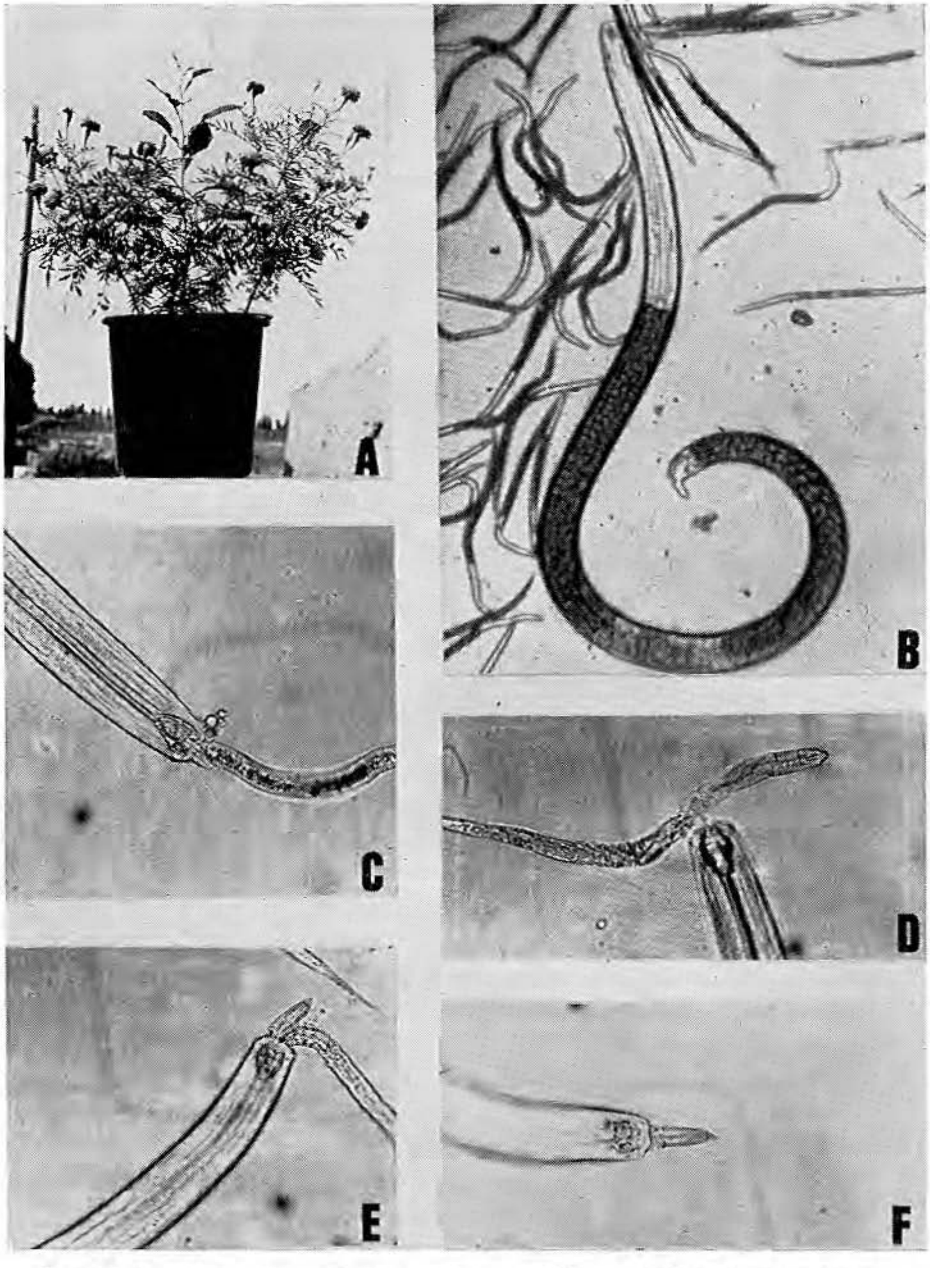

Fig. 2 - A - Container with sour orange seedling and three *T. paluta* plants; B - *M. sigmaturus* female in a population of larvae and males of *T. semipenetrans*; C-F - *M. sigmaturus* feeding on *T. semipenetrans*.

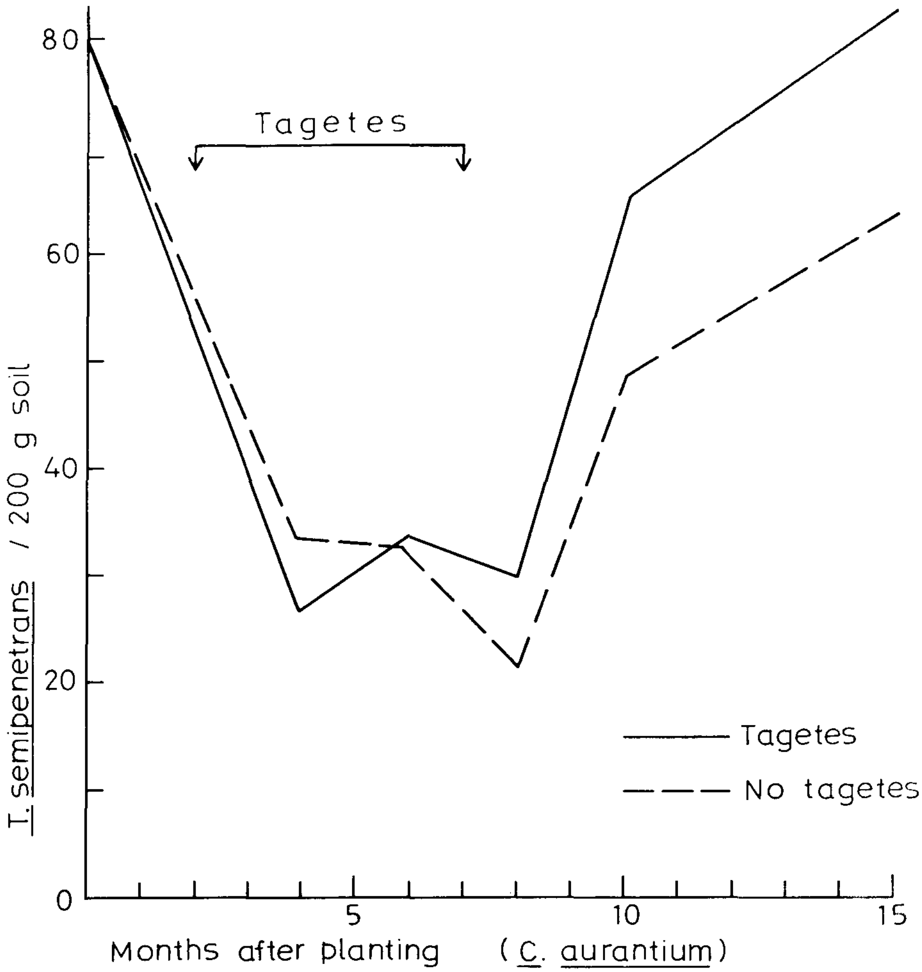

Fig. 3 - Population build-up of *T. semipenetrans* on sour orange seedlings in presence and absence of *Tagetes*.

Trials with the predacious nematode, MYLONCHULUS SIGMATURUS

The efficiency of *M. sigmaturus* as a predator of *T. semipenetrans* was determined in a series of standard trials using a single female of the predator in a population of 50 larvae and males of *T. semipenetrans* (Fig. 2, B-F). The animals were introduced into watch glasses containing tap water and kept in the dark at 26°C. Predation

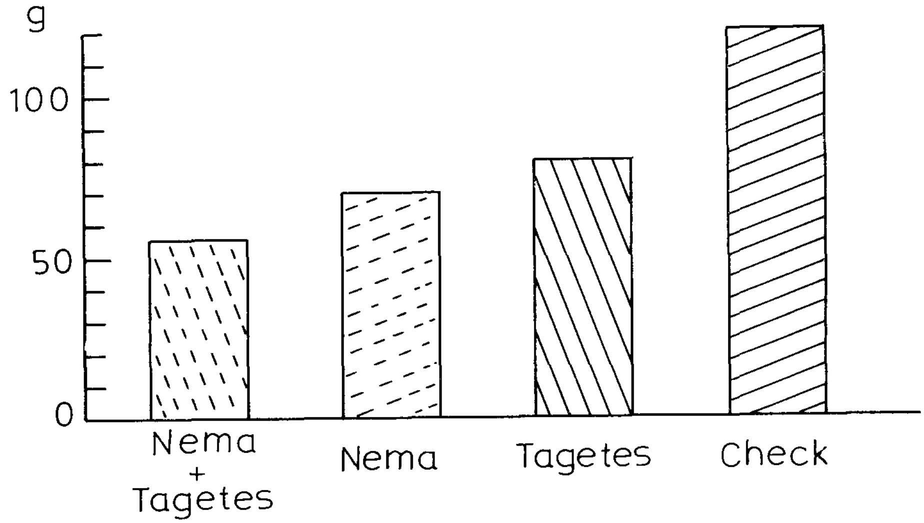

Fig. 4 - Mean weight of sour orange seedlings (n = 18) grown in presence and absence of *T. semipenetrans* and *T. patula*, 15 months after planting.

rate was determined by counting the number of prey animals devoured after varìyng exposure times in five watch glasses, from which means were calculated and plotted. Predation curves followed a more or less constant pattern, and a typical curve is given in Fig. 5. The predator devoured its prey at an average rate of 1.5-2 per hour during the first 25 hours. Subsequently the predation rate dropped. This was presumably due to the low density of the remaining prey population and a consequent difficulty for the predator to find its prey, or possibly also a result of a fouling of the environment. When the predator was transferred to a watch glass with a fresh prey population, the initial predation rate was regained. Up to eight such transfers were carried out — each after 25 hours — without a drop in the predation rate, as compared with predators kept in isolation from prey for different periods of time. This suggests that the predator probably feeds almost incessantly and that satiation does not seem to occur. Similar results have been reported by Yeates (1969), who found that the predation rate of *Mononchoides potohikus* Yeates was not affected by satiation.

The predation rate of *M. sigmaturus* on *T. semipenetrans* was compared with other prey species to determine possible specificity

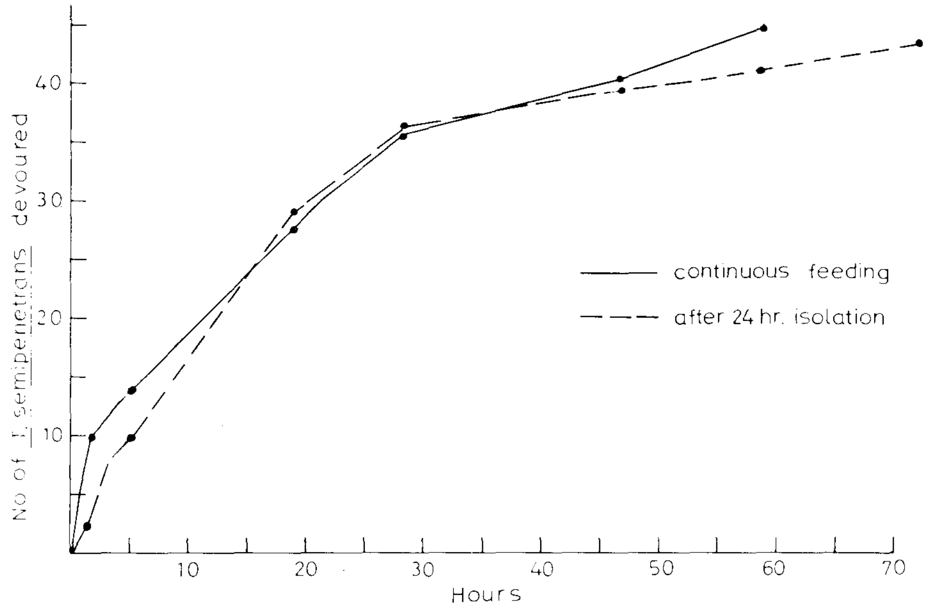

Fig. 5 - Predation rate of a single female of *M. sigmaturus* on larvae and males of *T. semipenetrans* (n = 50). Each point represents the mean of five replications.

in feeding. The species studied were *Meloidogyne javanica* (Kofoid *et* White) Chitw. larvae, and adults of *Helicotylenchus multicinctus* (Cobb) Golden and *Longidorus africanus* Merny. Results, presented in Table I, show that *H. multicinctus* and *L. africanus* were not devoured at all (although attempts at feeding were observed under the microscope) and predation rate on *T. semipenetrans* was almost twice as great as on *M. javanica*. When, however, the two latter species were placed together, no significant difference in the predation rate was observed (Table II). It is interesting to note that there was an additional difference between these two prey species in that some remnants of the cuticle of the *M. javanica* larvae always remained, while the *T. semipenetrans* larvae were devoured entirely and no traces of the animals remained.

The influence of *M. sigmaturus* on *T. semipenetrans* populations was also studied in soil. Natural nematode-infested orchard soil was placed in 500-cc plastic containers, in each of which a 12-week-old sour orange seedling was planted. One week after planting, 50 females of *M. sigmaturus* were introduced into each of 30 containers,

Table I - *Number of nematodes of different species in pure cultures devoured by a single female of* M. sigmaturus *.

Exposure Time (hrs)	T. semipenetrans	M. javanica	H. multicinctus	L. africanus
2	5	2	0	0
4	6	4	0	0
8	12	6	0	0
16	25	11	0	0
24	29	11	0	0
42	31	13	0	0
72	33	14	0	0

* Initial prey population = 50 nematodes; each figure is a mean of five replications brought to nearest whole number.

Table II - *Number of* T. semipenetrans *and* M. javanica *in mixed culture devoured by a single female of* M. sigmaturus *.

Exposure Time (hrs)	T. semipenetrans	M. javanica
2	8	7
4	14	15
22	15	16
46	16	16
70	17	16

* Initial prey population = 20 *T. semipenetrans* + 20 *M. javanica;* each figure is a mean of five replications brought to nearest whole number.

Table III - *Number of* M. sigmaturus *and* T. semipenetrans *in 500 cc soil around sour orange seedlings (each figure is a mean of five replications).*

Exposure Time (Months after planting)	50 M. sigmaturus added to orchard soil		Orchard Soil	
	M. sigmaturus	T. semipenetrans	M. sigmaturus	T. semipenetrans
3	408	67	0	131
7	409	15	0	243
10	465	18	50	344
12	137	3	275	20
14	194	11	805	3
18	820	30	289	28

while the remaining 30 containers were kept as checks. On six dates after planting, five predator-inoculated containers and five checks were removed, and numbers of *M. sigmaturus* and *T. semipenetrans* in the soil were determined. The trial was run for 18 months in a screenhouse. Results, presented in Table III, showed that population of *M. sigmaturus* developed also in the checks after 10 months (probably from very low populations present from the start), but there was a constant relationship in all readings between high population levels of *M. sigmaturus* and low populations of *T. semipenetrans*. On all examination dates, differences in weight of trees growing in soil infested with and without *M. sigmaturus* were not statistically different.

Discussion

From these trials it is apparent that *Tagetes* is ineffective in suppressing populations of *T. semipenetrans*. This is perhaps not too surprising since it has been shown that it is the endoradicular rather than exoradicular influences which are mainly responsible for the *Tagetes* effect (Winoto Suatmadji, 1969). Hence, *Tagetes* has been found effectively to suppress chiefly polyphagous nematodes, and particularly endoparasitic forms such as *Pratylenchus* and *Meloidogyne*. Since *T. semipenetrans* is relatively specific in its host range and does not penetrate *Tagetes* root, it might be expected to elude the nematicidal effect of the marigold.

The predacious nematode, on the other hand, appears to offer some possibilities which merit further study. Although *M. sigmaturus* is a fairly cosmopolitan organism (R. Mankau, *in litt.*), it does seem to show some preference for *T. semipenetrans*, albeit probably largely due to the small size of the free-living stages of the citrus nematode. The mere fact that it interferred with our routine cultures of *T. semipenetrans* — the phenomenon which led us to carry out these studies — is in itself encouraging. In order to investigate its practical potential, it will be necessary to develop techniques for rearing it in large numbers under controlled conditions and for studying its efficacy in different ecological environments. Studies in this direction are now being initiated.

We thank Dr. R. Mankau, University of California, Riverside, for identifying *Mylonchulus sigmaturus*.

SUMMARY

Biological control experiments on the citrus nematode, *Tylenchulus semipenetrans* Cobb, were conducted, using the marigold species, *Tagetes patula* L. and the predacious nematode *Mylonchulus sigmaturus* (Cobb) Altherr. *T. patula* growing in an orchard soil infested with *T. semipenetrans* did not reduce nematode numbers in comparison with checks without the marigold; citrus nematode population build-up on sour orange seedlings during 15 months was not affected by the presence of the marigold. On the other hand, citrus seedling growth was markedly retarded by *T. patula*, because of the competitive action of the marigold. The *in vitro* predation rate of a single female of *M. sigmaturus* feeding on *T. semipenetrans* larvae was 1.5-2.0 per hour. The prey animals were completely devoured and the predation rate was not affected by satiation. *M. sigmaturus* failed to prey on *Helicotylenchus multicinctus* (Cobb) Golden and *Longidorus africanus* Merny despite visible attempts, probably because of the relatively large size of the prey animals. *Meloidogyne javanica* (Kofoid *et* White) Chitw. larvae were preyed on, but generally with less efficiency than on the citrus nematode. Predation studies in soil, over a period of 18 months, revealed a fairly constant relationship between high predator densities and reduced *T. semipenetrans* populations. It is concluded that whereas the marigold is evidently ineffective in controlling citrus nematode populations, the predacious nematode *M. sigmaturus*, offers promising possibilities which merit further investigation.

RIASSUNTO

Esperimenti per sopprimere popolazioni del nematode degli agrumi con *Tagetes* sp. e un nematode predatore.

Prove di lotta biologica contro il nematode degli agrumi, *Tylenchulus semipenetrans* Cobb, sono state intraprese con *Tagetes patula* L. ed il nematode predatore *Mylonchulus sigmaturus* (Cobb) Altherr. *T. patula* seminato in terreno infestato con *T. semipenetrans* non ha ridotto le popolazioni del nematode, anzi ha ritardato con la sua competizione la crescita di piantine di Arancio amaro. In esperimenti *in vitro*, *M. sigmaturus* ha divorato da 1,5 a 2 larve di *T. semipenetrans* per ora. I nematodi sono stati completamente divorati ed i predatori, in apparenza, non hanno mai raggiunto uno stato di sazietà. *M. sigmaturus* non ha predato esemplari di *Helicotylenchus multicinctus* (Cobb) Golden e *Longidorus africanus* Merny, mentre ha divorato con efficienza ridotta larve di *Meloidogyne javanica* (Kofoid *et* White) Chitw. Studi sulla dinamica delle popolazioni dei nematodi condotti per un periodo di 18 mesi hanno dimostrato che esiste una correlazione negativa tra densità di popolazione del nematode predatore e densità di popolazione di *T. semipenetrans*. Si conclude che, mentre il *T. patula* è completamente inefficace nella lotta contro il nematode degli agrumi, *M. sigmaturus* offre promettenti possibilità e sembra opportuno approfondire gli studi in tal senso.

RÉSUMÉ

Expériences pour la suppression de population du nématode des agrumes par Tagetes et un nématode prédateur.

Des expériences de lutte biologique contre le nématode des agrumes, *Tylenchulus semipenetrans* Cobb, sont réalisées avec l'espèce *Tagetes patula* L. et le nematode prédatuer *Mylonchulus sigmaturus* (Cobb) Altherr. *T. patula* élevée

dans une terre infestée de *T. semipenetrans* n'a pas réduit le nombre des nématodes comparativement aux témoins sans la plante; l'accroissement de la population du nématode des agrumes sur les plantules de bigaradier pendant 15 mois n'a pas été influencé par la présence de *Tagetes*. D'autre part, le développement des plantules d'agrumes a été inhibé par la compétition due à *T. patula*. Le taux de prédation *in vitro* d'une femelle de *M. sigmaturus* élevée sur des larves de *T. semipenetrans* a été de 1.5-2.0 par heure. La proie a été dévorée complètement et le taux de prédation n'a pas été influencé par la satiation. *M. sigmaturus* ne s'est pas nourri de *Helicotylenchus multicinctus* (Cobb) Golden et de *Longidorus africanus* Merny malgré des essais visibles, probablement à cause de la taille relativement grande de la proie. Des larves de *Meloidogyne javanica* (Kofoid *et* White) Chitw. ont été dévorée, mais en général, d'une manière moins efficace que le nématode des agrumes. Des études sur la prédation dans le sol, poursuivies pendant 18 mois, ont décelé une relation assez constante de densité de populations élevées du prédateur correspondant à des faibles populations de *T. semipenetrans*. La conclusion à deduire est que, tandis que le *Tagetes* est sans influence dans la lutte contre le nématode des agrumes dans ces conditions, le nématode prédateur *M. sigmaturus* possède des qualités qui méritent des études plus approfondies.

LITERATURE CITED

BOOSALIS M. G. and MANKAU R., 1965 - Parasitism and predation of soil microorganisms. In « Ecology of Soil-borne Plant Pathogens » (K. F. Baker and W. C. Snyder, Eds.), pp. 374-389. University of California Press, Los Angeles.

COHN E., 1969 - The citrus nematode, *Tylenchulus semipenetrans* Cobb as a pest of citrus in Israel. Proc. First International Citrus Symposium. Univ. of California, Riverside, Vol. 2, pp. 1013-1017.

COHN E., MINZ G. and MONSELISE S. P., 1965 - The distribution, ecology and pathogenicity of the citrus nematode in Israel. *Israel J. agric. Res.*, *15*: 187-200.

ESSER R. P. and SOBERS E. K., 1964 - Natural enemies of nematodes. *Proc. Soil Crop Sci. Soc. Fla*, *24*: 326-353.

WINOTO SUATMADJI R., 1969 - Studies on the Effect of *Tagetes* Species on Plant Parasitic Nematodes. Veenman and Zonen N. V., Wageningen, The Netherlands, 132 p.

YEATES G. W., 1969 - Predation by *Mononchoides potohikus* (Nematoda: Diplogasteridae) in laboratory culture. *Nematologica*, *15*: 1-9.

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