STUDIES ON THE EFFECT OF *TAGETES* SPECIES ON PLANT PARASITIC NEMATODES

(Met een samenvatting in het Nederlands)

PROEFSCHRIFT

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AAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN,
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STELLINGEN

I

Rohde (1965) en Wallace (1963) concluderen ten onrechte uit het onderzoek van Oostenbrink *et al* (1957) en Uhlenbroek & Bijloo (1958, 1959) dat door *Tagetes* soorten specifieke nematicide stoffen in de grond worden afgescheiden.

ROHDE, R. A., 1965, Phytopathology **55**, 1159–1167
WALLACE, H. R., 1963, The biology of plant parasitic nematodes, Arnold, London, p. 193
OOSTENBRINK M,. *et al*, 1957, Nematologica Suppl. **2**, 424–433
UHLENBROEK, J. H. & BIJLOO, J. D., 1958, Rec. Trav. Chim. Pays-Bas Belg. **77**, 1004–1009
– & –, 1959, Rec. Trav. Chim. Pays-Bas Belg. **78**, 382–390

II

De opzet van de proeven van Koen (1966) en Daulton & Curtis (1963) over penetratie van *Tagetes* wortels door nematoden is onjuist; het resultaat wordt door andere processen vertroebeld.

KOEN, H., 1966, S. Afr. J. agric. Sci. **9**, 981–992
DAULTON, R. A. C. & CURTIS, R. F., 1963, Nematologica **9**, 357–362

III

Groeiende planten hebben in het algemeen rondom hun wortels een dodend effect op parasitaire nematoden, dat van grote invloed kan zijn op de populatiedichtheid van deze laatsten.

IV

De wilde flora biedt nog goede mogelijkheden voor het bestrijden van parasitaire nematoden, door middel van wisselbouw of tussenteelt.

V

In de discussies over het ontstaan van plant-insect relaties wordt oecologische specialisatie dikwijls te weinig in de beschouwing betrokken.

KENNEDY, J. S., 1965, Ann. appl. Biol. **56**, 317–322
THORSTEINSON, A. J., 1960, A. Rev. Ent. **5**, 193–218.

VI

Bij de toenemende handel in en gebruik van bestrijdingsmiddelen in Indonesia dient de instelling en de uitvoering van een 'bestrijdingsmiddelenwet' sterk te worden bespoedigd.

VII

In ontwikkelingslanden verdient verhoging van de produktie van conventionele consumptie-eiwitten de voorkeur boven introductie van onconventionele industriële eiwitten.

VIII

Een traditioneel ingestelde opvoedingsstijl in gezins- en gemeenschapsverband kan een der belangrijkste remmende factoren zijn voor de ontwikkeling in de tropen.

IX

In ontwikkelingslanden is veredeling om sociaal psychologische redenen een bij uitstek geschikt middel ter verhoging van de produktie in de landbouw. Gezien het grote belang van het kwekerswerk in deze landen dienen maatregelen ter erkenning, beloning en bescherming van deze arbeid te worden geëntameerd of gestimuleerd.

R. WINOTO SUATMADJI
Wageningen, 28 maart 1969

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CHAPTER 1

GENERAL

1.1. Literature survey

Several plant parasitic nematodes combine endoparasitic mode of life with pronounced polyphagy. The best known genera are *Pratylenchus* Filipjev with 29 described species up to 1968 and *Meloidogyne* Goeldi with 23; several species are widely distributed and some of them are extremely noxious for valuable crops.

The finding of a non-host, especially when it is a trap crop or enemy plant of these nematodes, is intriguing for the study of host-parasite relationships and for its possible application to control damage by these nematodes. Marigolds, *Tagetes* species, are recorded as exceptional plants which demonstrate strong nematicidal effects or extreme resistance.

The earliest reports on the resistance of *Tagetes* to nematode infestation, by Tyler (1938) and Steiner (1941), pertain to rootknot nematodes, *Meloidogyne* species. The latter reported that larvae of *Meloidogyne* entered roots of *Tagetes* in large numbers, but generally failed to develop and to reach sexual maturity. It was not until about 15 years later that *Tagetes* reappeared in nematological literature, then for its suppressive effect on populations of free-living root parasites of the genus *Pratylenchus*. Van den Berg-Smit (1953), a Dutch commercial grower, reported favourable results when *Tagetes* preceded *Narcissus* in root-rot-infested soils. Slootweg (1956) confirmed this observation and suggested a relation to numbers of *Pratylenchus penetrans* (Cobb) Filipjev & Schuurman Stekhoven in these soils since this nematode was the primary cause of rootrot in *Narcissus*. Oostenbrink & Slootweg (in Slootweg, 1956) stated that *Tagetes* reduced *Pratylenchus* populations to about a tenth of the level with other crops.

Extensive experimental evidence of marked suppression of *Pratylenchus* populations in the soil by *Tagetes* was given by Oostenbrink, Kuiper & s'Jacob (1957) The experiments were mainly on *P. penetrans* and *P. crenatus* Loof, syn. *P. pratensis* Thorne nec (De Man). These nematodes were suppressed more than by fallow. *Tagetes* roots were not damaged and very few nematodes were found among the roots of full-grown plants. Subsequently, other *Pratylenchus* species were reported to be killed by growing *Tagetes: P. convallariae* Seinhorst (Cayrol & Ritter, 1962) and *P. loosi* Loof (Visser & Vythilingam, 1959; Hutchinson, 1962). However, in population dynamical studies Seinhorst (1966; 1967) found some multiplication of *P. penetrans* and *P. crenatus* on *T. erecta*. Tarjan (1960) found some effect of *T. erecta* L. on the endoparasitic *Radopholus similis* in greenhouse tests, but no significant reduction in the nematode density of infested field plots, if *Citrus*, a good host plant, was present. Different species of *Meloidogyne* (considered one species until classified by Chitwood in 1949) were also reported not to breed on *Tagetes*. These were *M. hapla* Chitwood (Oostenbrink, 1960), *M. javanica* (Treub) Chitwood (Visser & Vythilingam, 1959; Daulton & Curtis, 1963) and *M. incognita* (Nirula & Bassi, 1965).

Most of the studies were with *Tagetes patula* L., *T. erecta* or *T. minuta* L. In Rhodesia Martin (1958) noticed no or few swellings and no egg masses on roots of *T. patula* in soils infested by *M. hapla* and *M. arenaria* (Neal) Chitwood, but on *T. minuta* egg masses of both *Meloidogyne* spp. could easily be found. Later, in the United States, Good *et al.* (1965) found *T. minuta* susceptible also for *M. hapla* and *M. arenaria* but resistant to *M. incognita.* Differences in performance of the same species of *Tagetes* with different *Meloidogyne* species may evidently occur. In comparing results obtained by different workers, intraspecific differences in the plants as well as in the nematodes may be determinant.

So far the effect of *Tagetes* on polyphagous endoparasitic root nematodes has been mentioned. Most data point to suppression of populations of these nematodes by *Tagetes* species. The more host-specific *Heterodera* species, at any rate larvae of *H. rostochiensis* Wollenweber occurring in cysts in the soil, are not found to be affected by *Tagetes* (Kuiper & Paetzold in Oostenbrink, 1960b; Omidvar, 1962). Ectoparasitic root nematodes varied in their reaction to the presence of *Tagetes* species. Prolific breeding on these plants was reported for *Criconemoides mutabile* Taylor (Steiner 1941), *Trichodorus teres* Hooper (Kuiper, 1963) and *Hemicycliophora similis* Thorne (Seinhorst & Klinkenberg, 1963). *Tagetes* is also a good host of *Longidorus maximus* (Bütschli) Thorne & Swanger and attack by these nematodes causes damage to the plant (Sturhan, 1963). According to Oostenbrink (1960b), *Rotylenchus robustus* (de Man) Filipjev and a *Paratylenchus* species maintained moderate populations, whereas *Tylenchorhynchus dubius* (Bütschli) Filipjev was suppressed to low levels. Seinhorst (1966, 1967b), however, showed that multiplication of *Tyl. dubius* on *T. erecta* is possible.

The *Tagetes* effect therefore varies, perhaps between *Tagetes* species and varieties, but certainly between nematode genera and perhaps species and strains. Literature records are available on the influence of *Tagetes* species on 14 genera of plant nematodes listed below.

Pratylenchus Filipjev: Oostenbrink, s'Jacob & Kuiper, 1957; Oostenbrink, Kuiper & s'Jacob, 1957; 1960; Visser & Vythilingam, 1959; Hutchinson, 1962; Cayrol & Ritter, 1962; Decker, 1963; Seinhorst, 1966; 1967;

Meloidogyne Goeldi: Tyler, 1938; Steiner, 1941; Gaskin & Crittenden, 1956; Martin, 1958; Visser & Vythilingam, 1959; Oostenbrink, 1960b; Daulton & Curtiss, 1963; Good, Minton & Jaworski, 1965; Nirula & Bassi, 1965; Koen, 1966;

Rotylenchulus Linford & Oliveira: Linford & Yap, 1940;

Radopholus Thorne: Tarjan, 1960;

Heterodera A. Schmidt: Oostenbrink, 1960b; Omidvar, 1962;

Tylenchorhynchus Cobb: Oostenbrink, Kuiper & s'Jacob, 1957; Oostenbrink, 1960b; Seinhorst, 1966, 1967;

Rotylenchus Filipjev: Oostenbrink, Kuiper & s'Jacob, 1957; Meyneke & Oostenbrink, 1958; Oostenbrink, 1960b;

Paratylenchus Micoletzky: Oostenbrink, 1960b;

Criconemoides Taylor: Steiner, 1941;

Hemicycliophora de Man: Seinhorst & Klinkenberg, 1963; Kuiper & Oostenbrink, 1962;
Trichodorus Cobb: Kuiper, 1963;
Longidorus (Micoletzky) Filipjev: Sturhan, 1963;
Ditylenchus Filipjev (Emend. Thorne): Oostenbrink, 1961;
Aphelenchoides Fischer: Steiner, 1941.

Despite the hitherto incomplete and complex picture, the *Tagetes* effects on *Pratylenchus* and *Meloidogyne* spp. were striking enough to evoke interest in the causal mechanism and in its practical application.

A possible explanation of the effect was given by Uhlenbroek & Bijloo (1958, 1959) who found that roots of *T. erecta* were exceptional because they contained highly nematicidal compounds: α-terthienyl and a certain bithienyl compound. The former chemical had earlier been shown to occur in aerial parts of *Tagetes* (Zechmeister & Sease, 1947), and was recorded as a rare chemical in nature, having no antibiotic action against bacteria. Christie (1960) suggested that *Tagetes* roots produce diffusates which mask or neutralize the stimulating effect of other root diffusates on nematodes. He further suggested that *Tagetes* acts as a "nematistat" rather than as a nematicide and that nematodes which come incidentally into contact with roots are able to enter and develop, whereas others fail to reach the roots because they are not stimulated to move either in any direction or in the direction of the roots. Seinhorst & Klinkenberg (1963) decry the importance of polythienyl action on the nematodes and explain it by low survival of *P. penetrans* because of the tough necrosis it causes over *Tagetes* roots Nematodes which penetrate do feed and deposit eggs, but movement of young larvae may be severely hampered by the necroses. Wallace (1963) referring to the findings of Oostenbrink *et al.* (1957) and Uhlenbroek & Bijloo (1958, 1959) stated that there is evidence that *Tagetes* inhibits nematode activity by the secretion of toxic substances into the soil, though the authors he refers to refrained from accepting this mechanism. Interesting as the views on possible killing effect of *Tagetes* outside the roots may be, support by experimental evidence was not given. Two mechanisms of resistance of *Tagetes* which are seated in the host tissue, the presence of nematicidal substances and necrosis of plant tissue penetrated by P. penetrans, seem to be well established.

Koen (1966) in a study of the reaction of *M. javanica* in the rhizosphere and roots of susceptible and resistant plants, including *T. patula*, *T. erecta* and *T. minuta*, found that roots of *Tagetes* spp. attracted larvae and although fewer penetrated resistant than susceptible plant species, this was still fairly high with *Tagetes* spp. He further stated that root exudates of *Tagetes* spp. neither inhibited hatching nor masked the attractive effect of root exudates of susceptible plants; root extracts of *T. minuta* had a nematostatic effect on *M. javanica* larvae. He suggested that control of root-knot nematodes is to be attributed to *Tagetes* acting as a trap crop.

Whatever mechanism may be responsible for the marked reduction of nematode numbers by *Tagetes*, its value for nematode control is evident. Practical results were reported by Oostenbrink, Kuiper & s'Jacob (1957), Visser &

Vythilingam (1959), Oostenbrink (1960), Daulton & Curtiss (1963) and Nirula & Bassi (1965). Several questions needing to be answered before *Tagetes* could be used for soil sanitation were reviewed by Meijneke & Oostenbrink (1958). Oostenbrink (1959a) considered *Tagetes* a good crop to precede crops susceptible to *P. penetrans* and found it also a good green manure. In a later publication (1960b) he suggested that *Tagetes* increased yield of most crops on sandy and peaty soils usually by 10–40%. He concluded that this was probably caused by a direct suppression of root-infesting nematodes, rather than by green manure or nitrogen effects of the crop. Wallace (1963) stated that little was known about the relationship between *Tagetes*, nematodes and growth of the main crop, and that it was not clear how much of the increased yield after *Tagetes* must be attributed to kill of nematodes. The fact that *Tagetes* reduced the number of certain plant nematodes did not necessarily mean that plants yielded more. It was first necessary to know whether the nematodes present were causing damage or whether they were among those having no deleterious effect on the crop. Seinhorst & Klinkenberg (1963) illustrated effects of *Tagetes* apart from nematode suppression by field and pot trials. In a field trial onion yields after *Tagetes* were 1.4 times as much as after other crops, without nematode injury being considered to be the cause of this difference. In a pot trial rye yields were 1.2 times as much after *Tagetes* as after beets. Nematicidal and non-nematicidal effects may occur together at the same time; both have to be evaluated for their practical significance.

1.2 Scope of the investigations

The purpose of this study was:

1. To determine the occurrence and significance of *Tagetes* effects on plant nematodes, especially *Pratylenchus* and *Meloidogyne* species
2. To elucidate the causal mechanism or mechanisms of the effects
3. To obtain data which may contribute to the application of *Tagetes* in practical cropping systems.

CHAPTER 2

MATERIALS AND METHODS

Regularly employed materials and methods are mentioned in this chapter. Techniques used occasionally are indicated elsewhere.

2.1 Plant material and its culture

Three *Tagetes* spp. were frequently used in experiments. These were *T. patula* Golden Harmony, *T. erecta* Aurantiaca and *T. minuta* (syn. *T. glandulifera* Schrank). Incidentally *T. signata* Bartl. (syn. *T. tenuifolia* Cav.) cv. Golden Gem and *T. lucida* Cav. were used. Seed of *T. minuta* and *T. lucida* was supplied by the Botany Department of the University of Nijmegen and those of the other species by the firm of Tubergen in Haarlem, the Netherlands.

Seed from one single batch was used in all experiments. Unless mentioned otherwise, the *Tagetes* spp. in this text pertains to the cultivars indicated above.

In field trials 3–5 seeds were sown per plant site at a depth of about 1 cm or about 5 kg seed per ha was sown in rows about 25 cm apart. The number of seedlings per plant site was generally reduced to one when they were sufficiently established about 10 days after germination. Plants were normally grown in a square pattern 25 cm apart.

For greenhouse trials seeds were germinated on a glass-covered bench in steam-sterilized standard potting soil. Germination took 3–4 days for *T. patula*, *T. erecta* and *T. signata* and about 7 days for *T. minuta*. Seedlings were then transplanted into wooden boxes with the same soil in the greenhouse and used for trials about one week later. For comparisons good hosts of the nematode species used were also included in the trials: usually tomato *(Lycopersicon esculentum* L.*)* Moneymaker, red clover *(Trifolium pratense* L.*)* Inlands, and apple *(Malus pumila* L.*)* Bittenfelder. They were raised from seed in a similar way to *Tagetes*, except for apple, whose seeds were subjected to 4 °C temperature for at least 40 days and then mixed with moist sand before germination.

The temperature was maintained at 22 °–25°C. From the beginning of October until the beginning of May the next year extra light was supplied with 80 W (4300K) daylight incandescent lamps so that plants received a total of 16 hours light.

Greenhouse trials with plants grown in soil heavily infested with plant nematodes usually lasted up to 3 or 4 months. Plants were usually grown in cylindrical glass tubes 2.5 cm wide and 16.5 cm high, filled with about 80 ml of soil, or in glass tubes 3.5 cm wide and 20 cm high with about 160 ml of soil. The column of soil in the tubes was always placed on top of 2 cm of porous gravel. A plastic straw connected the layer of porous gravel with the free air above the tube, thus allowing aeration of the tube. Adequate amounts of water were added to the top of the tube or through the plastic straw to the bottom part of the tube by a laboratory water bottle; fertilizers were not added unless stated otherwise. Such tubes appeared to have advantages over clay pots 12 cm or more in dia-

meter with larger amounts of soil. The soil in the tubes was rooted more uniformly and contact of nematodes with roots of test plants was secured better in tube cultures than in pot cultures. Also less inoculum was needed for the experiments, and populations could be more easily assessed for the entire system.

For inoculation of plants a suspension of 2–4 ml with known number of nematodes was carefully applied drop by drop with a pipette into holes of different depths made in the soil around the plant.

2.2 Nematodes and soils for experiments

For greenhouse and laboratory experiments stock soils with monospecific cultures of *Pratylenchus penetrans*, *Meloidogyne incognita*, *M. hapla*, *M. arenaria* and *M. javanica* were prepared and maintained in a separate greenhouse. For this purpose soil suitable for the nematode species was partially sterilized by heating at 60°C for 3 hours and subsequently exposed to the free air in the greenhouse for at least a month to regain physical, chemical and biological stability. Pots filled with this soil were planted with good hosts and inoculated with well identified nematodes.

P. penetrans was cultured on *Zea mais* L., red clover or apple seedling in a peaty sand soil from Nieuwe Pekela, which originally harboured a dense population of this nematode and was therefore a suitable biotope. Stocks of severely infested plants and soils were easily maintained in this way. When *Tagetes* had to be compared with one of the good hosts, e.g. red clover, soil and nematode material was used which was prepared by the cultivation of maize or apple.

The *Meloidogyne* species were reared from single masses of eggs on tomato in a steam-sterilized mixture of peat, sand, clay and leaf mould 75:10:3:12 by volume. Cultures of *Meloidogyne* spp. had to be renewed now and then so that severely infested tomato plants and soils were available whenever needed.

In most trials with other nematode species and in some trials with *Pratylenchus* and *Meloidogyne* spp., selected field populations and soils in which they occur in high densities were used. They are mentioned elsewhere in the text.

For trials with *P. penetrans*, soils with well established stock cultures were usually used. A month before the experiment began, entire host plants were removed from the pots. Any severed roots were removed from the soil as much as possible by sieving. At the start of the trial remaining bits of roots were decayed to a large extent. Most nematodes were then free in the soil as in a plantless habitat. Especially in work with *P. penetrans* this method was superior to the one in which nematodes were directly inoculated into partially sterilized soil. High population densities are maintained in the first technique, whereas direct inoculation often kills most of the nematodes.

Sometimes *Pratylenchus* or *Meloidogyne* species had to be directly inoculated into soils or other media. The nematodes were then obtained from host roots of the stock cultures. *P. penetrans*, for example, was extracted by a standard procedure (Section 2.4), after which the suspension liquid was repeatedly replaced by clean water so that the final suspension was practically free from dissolved

plant cell saps For this purpose the original suspension was shaken in a beaker and kept at 4°C for several hours to allow the nematodes to settle The supernatant was then carefully removed as far as possible by siphoning or by suction applied by hand pipette. The beaker was then refilled with tap water and the process repeated at least thrice. The final suspension consisted of nematodes in clear water practically free from plant juices, at all stages of development. In some trials the *P. penetrans* inoculum consisted of undifferentiated larvae only. The preparation of such inocula is described later. In preparing inocula of *Meloidogyne* larvae the same procedure was followed except that *Meloidogyne* spp. other than *M. hapla* were allowed to settle at normal room temperature and that the supernatant liquid of the original suspension was replaced by fresh water only once. *Meloidogyne* larvae must be inoculated as soon as they emerge from the macerated tomato roots.

2.3 Estimation of nematode densities in soil

In field trials soil samples were often taken from plots of about 3 m^2 or 7.5 m^2 within the area bordered by the outermost row of plants. From each plot 30–50 cores were taken with an auger of one cm width to a depth of 30 cm. Soil samples were deposited in bags of waxed paper or polythene and stored in a cold room at 4°C until extraction.

After discarding large debris and other material by sieving, the samples were thoroughly mixed and subsamples of 100 ml were taken. Active nematodes were extracted with Oostenbrink elutriators and the resulting nematode suspension was analysed as by Oostenbrink's (1960d) method.

In greenhouse trials with tube cultures, the contents of tubes were processed as a whole at the end. Soil and roots were usually found tightly fitted to the tube wall. To free them, water was forced through the aeration straw into the bottom of the tube to lift the column of soil with roots which is loosened by the water. The entire root system with the soil can then normally be taken from the tube for further processing. The roots are separated from the soil by rinsing them several times in a pan with water; adhering debris were removed with forceps. Pieces of roots remaining in the soil were collected with forceps or with sieves and added to the root system. After drying between filter papers, roots were weighed and processed for nematode extraction or for direct counting (Section 2.4). All water used in this procedure was collected in the pan with soil. Nematodes in the soaked soil were extracted as indicated before.

2.4 Estimation of nematode densities in plant tissues

Active nematodes were normally extracted by rapidly macerating the roots and filtering through cotton wool as described by Stemerding (1960).

In greenhouse trials with plants grown in infested soils, *Pratylenchus* species were used at high initial population densities. Measurable numbers of them can be found in roots of *Tagetes* at the end. In laboratory trials employing smal-

ler numbers of nematodes and younger plants, whole counts of nematodes in entire rootsystems were made under the dissecting microscope after roots have been fixed in formaline-acetic-alcohol (FAA) mixture of a composition suited for fixing plant material and after differential staining of the eelworms in the fixed roots by the cottonblue-lactophenol method (Goodey, 1963).

Meloidogyne species were similarly counted in whole root systems because few of these nematodes were usually found within or attached to *Tagetes* roots and because developing stages were immobile. *Meloidogyne* specimens were then classified according to developmental stage and to sex; since under the binocular microscope the various larval stages could not be differentiated only three categories were classified: slender and motile second stage larvae of morphologically undetermined sex, juvenile swollen individuals of different shapes, and mature females usually bearing egg masses. Juvenile specimens within the larval skin could often be clearly determined as males; they were recorded separately within the category of obese juveniles.

2.5 Evaluation of plant growth

Plant growth sometimes had to be evaluated. This was by the common techniques of measuring, weighing and counting. The quantities used for the evaluation of plant growth in relation to nematode attack were usually units of root weight, plant length or increase of shoot length, or for some crops yield. We did not usually evaluate differences in quality of the crop.

2.6 Statistical treatment of data

The significance of differences between nematode densities or between yields were usually calculated from original or logarithmically transformed figures by analysis of variance, covariance, or by Student's t test. Logarithmic transformations were of original figures or of these figures plus one. In several cases numerical data were expressed in percentages and subjected to arcsine transformation for further analysis. Least significant differences (L.S.D.) were calculated for significant comparisons. Comparisons were made at the 0.05 and 0.01 level of probability ($p = 0.05$ and $p = 0.01$). Cf. Snedecor, 1962; Cochran & Cox, 1964. The trials and type of the data sometimes required other statistical tests.

CHAPTER 3

OCCURRENCE AND SIGNIFICANCE OF *TAGETES* EFFECTS ON PLANT NEMATODE POPULATIONS

3.1 Introduction

The effect of *Tagetes* spp. on nematode populations concerns a special plant-nematode relationship, in which the suppressive effect, or resistance, of the plants is extreme. It is probable from the literature survey that effectiveness of *Tagetes* spp. as well as susceptibility of nematode species to effects of *Tagetes* differ, and that these differences must be determined in more detail than as yet. Each plant-nematode relationship is in fact a relation between a certain plant variety and a certain nematode strain or trophotype. In defined conditions it can be illustrated by the reproduction curve, which indicates the different rates of nematode reproduction at different initial densities within a certain time. The corresponding mathematical formulae (Oostenbrink, 1966; Seinhorst, 1966) are derived from the logistic equation for population increase. In these equations a certain constant describes the plant's properties for population increase, therefore its effect on the population. The *Tagetes* effect could thus be expressed in each experiment where *Tagetes* is grown on soil with several different densities of a nematode. This theoretically exact approach was not followed in our trials for two reasons. Firstly, experiments with several nematode densities are time-consuming. Secondly, the effect of *Tagetes* is so outstanding that it suppresses susceptible nematode populations often to practically zero, whatever the initial population density. Under these circumstances comparisons between *Tagetes*, a suitable host plant and fallow (= no plant) on soil with a known heavy nematode infestation were considered practical and efficient ways of acquiring information.

Experiments were with a wide range of nematodes: species of *Pratylenchus*, *Meloidogyne*, *Tylenchorhynchus*, *Rotylenchus* and *Helicotylenchus* Steiner (emend Golden), other ectoparasitic root nematodes, *Ditylenchus* and *Aphelenchoides*. They are described in Sections 3.2 to 3.8.

3.2 Pratylenchus spp.

The effect of *Tagetes* spp. on *Pratylenchus* populations was studied in tube cultures (Section 3.2.1) and in field trials (Section 3.2.2), in comparison with one or more suitable host plants and with fallow.

There were five tube-culture trials: one lasting four months with *P. penetrans* inoculated directly onto five species of *Tagetes* in comparison to red clover and fallow, and a series of four comparable tests with established populations of *P. penetrans*, *P. crenatus*, *P. neglectus* (Rensch) Schuurmans Stekhoven, *P. thornei* Sher & Allen in their original soils. In each of these four tests the same three *Tagetes* spp. were compared with wheat *(Triticum aestivum* L.*)* and fallow; they

were started at weekly intervals but were otherwise similar in set-up, treatment and evaluation, and all lasted nine weeks.

The field trials comprised an introductory trial for one season with different *Tagetes* spp. and cultivars and 12 long-term rotation trials on different soils with different *Pratylenchus* spp. with *T. patula* cv. Harmony grown continuously or in two-year rotations with other crops. The rotation trials were all cross trials, according to a scheme described by Oostenbrink (1959b), in which a fixed series of crop strips was grown in one direction every other year and perpendicular to this direction in the years between, so that monoculture of each crop and all possible two-year rotations occurred. Some field trials were expressly designed for this study and others were made available to the author to study the *Tagetes* effects; all these field trials were supervised by field stations of the Plant Protection Service except one by the Agricultural University.

3.2.1. *Tube cultures*

a. *P. penetrans* inoculated directly onto plants in potting soil

Seventy tubes were filled with the same weight of partially sterilized standard potting soil equivalent to about 80 ml. Seedlings of *T. patula, T. erecta, T. minuta, T. lucida, T. signata* and red clover were planted in 6 series of 10 tubes respectively on 10 July 1964, and one series was left unplanted as control. A week after planting, 1500 *P. penetrans* of different developmental stages from a monospecific culture on maize were inoculated as a suspension in water into each of the tubes including controls. All inoculated plants thrived under the experimental conditions chosen, except *T. lucida* and *T. signata* which remained rather small and rooted only poorly.

Four months after inoculation the fresh weight of the root systems were determined and the nematodes in roots and soil were extracted, and counted for all tubes separately. The results are summarized in Table 1. The average numbers of nematodes of all replicates are given and also the averages of the logarithms of these numbers.

Less than 10% of the inoculum survived in the control. Such a high mortality is not uncommon in inoculation experiments with this nematode. It is nevertheless a drawback for the interpretation of the results obtained in this experiment.

In judging the effect of the *Tagetes* spp. on nematode density two criteria may be used. One is the number of nematodes in the soil (Col. 3 in Table 1), for nematodes found in the roots may not relate with the final infestation in the soil. This is unlikely for nematodes in roots of a good host as red clover, but it is possible for *Tagetes* which eliminate penetrated nematodes. According to this criterion, all *Tagetes* spp., except *T. signata*, show a distinct suppressive effect in this test.

Another criterion is the total number of nematodes in roots and soil (Col. 5), accepting that the nematodes penetrating the roots will survive and contribute to the final population. *P. penetrans* may even be able to multiply as much or more in the roots when plants continue to grow after the four months' period of the experiment. The values in Column 5 show that populations of *P. penetrans*

TABLE 1. Effect of five *Tagetes* species and red clover on *P. penetrans* in a tube culture. Planting date: 10–7–1964. Inoculated density 1500 *P. penetrans* per tube with 80 ml of soil. Final densities and root weights determined 4 months after inoculation; average figures of 10 replicates; between brackets averages of logarithmically transformed figures and least significant differences (= L.S.D.) at 5% and at 1% levels.

1	2	3	4	5	6
		Final number of *P. penetrans*			
Plant	Fresh weight of roots, in mg	In soil	In roots	Total 3 + 4	Per 10 g of roots
T. patula	830	0.3 (0.08)	0.3 (0.08)	0.6 (0.14)	6.0 (0.28)
T. erecta	2130	7 (0.78)	95 (1.61)	102 (1.66)	397 (2.26)
T. minuta	1790	12 (0.90)	99 (1.73)	111 (1.78)	573 (2.47)
T. lucida	415	19 (1.06)	9 (0.68)	28 (1.20)	160 (1.72)
T. signata	319	498 (2.49)	206 (2.13)	703 (2.70)	7845 (3.66)
Red clover	770	4757 (3.44)	8741 (3.62)	13497 (3.93)	99361 (4.78)
No plant	–	114 (2.01)	–	114 (2.01)	–
L.S.D. 5%		(0.395)	(0.522)	(0.352)	(0.658)
1%		(0.526)	(0.698)	(0.469)	(0.879)

are much more suppressed by *T. patula*, and by *T. lucida*, than by fallow in the control tubes. Populations under *T. erecta* and *T. minuta* were slightly lower than in the control tubes, populations under *T. signata* were significantly denser.

Differences in effect of the *Tagetes* spp. can also be judged from the number of nematodes per 10 g of roots (Col. 6). *T. patula* harboured the fewest of *P. penetrans* followed by *T. lucida*. *T. erecta* and *T. minuta* were also unsuitable hosts, but it is doubtful whether they suppressed the nematode population more than fallow.

The figure for *T. signata*, more than 7800 nematodes per 10 g of roots, does not suggest a suppressive effect at all under these circumstances. The high nematode density in the red clover roots gives an impression of the development of *P. penetrans* populations in a good host. The nematode-suppressing effect of the different species of *Tagetes* (Col. 3 or 5) seems to be inversely proportional to the number of nematodes present in the roots of the four-months-old plants (Col. 4 or 6), as would perhaps be expected.

b. *P. penetrans* as established culture in peaty sand

Thirty tubes were filled with well mixed sandy peat soil harbouring an established monospecific population of *P. penetrans* bred on maize and prepared as described in Section 2.2. Each tube comprised the same weight of soil equivalent to about 180 ml. The *P. penetrans* density, counted as an average of five samples of 180 ml, was found to be 3096. Four series of six tubes were planted on 20 December 1966 with a seedling of *T. patula*, *T. erecta*, *T. minuta* and wheat, respectively, and one series was left unplanted as control. All plants grew well and the tubes were well rooted a few weeks later. Nine weeks after planting fresh

TABLE 2. Effect of three *Tagetes* spp. and wheat on an established population of *P. penetrans* in a tube culture. Planted on 20.12.1966. Initial density 3096 *P. penetrans* per tube with 180 ml of soil. Final densities and root weights determined 9 weeks after planting; average figures of 6 replicates; between brackets averages of logarithmically transformed figures and least significant differences (L.S.D.) at 5% and at 1% levels.

1	2	3	4	5	6
		Final number of *P. penetrans*			
Plant	Fresh weight of roots, in mg	In soil	In roots	Total 3 + 4	Per 10 g of roots
T. patula	2419	0.8 (0.23)	22 (1.32)	23 (1.33)	91 (1.90)
T. erecta	3668	138 (2.09)	1259 (3.02)	1397 (3.07)	3631 (3.47)
T. minuta	2289	101 (1.99)	1044 (2.98)	1145 (3.03)	5004 (3.65)
Wheat	1949	1198 (3.03)	6895 (3.80)	8093 (3.85)	36955 (4.52)
No plant	–	1790 (3.25)	–	1790 (3.25)	–
L.S.D. 5%		(0.239)	(0.330)	(0.273)	(0.376)
1%		(0.325)	(0.456)	(0.373)	(0.520)

roots were weighed and nematodes in roots and soil of all tubes were counted. Values are summarized in Table 2.

The number of *P. penetrans* in the fallow soil dropped to about 60% of the initial density, which was much less than in the inoculation experiment of Section 3.2.1a. This can be interpreted as a technical improvement.

Nematode counts in soil (Col. 3) were again lowest under *T. patula*, followed by *T. erecta* and *T. minuta*. The effect of all three species was highly significant compared to wheat or fallow. Unlike *Tagetes* spp. wheat increased total populations, as is evident from the numbers in Col. 4 and 5.

Total counts of *P. penetrans* were strongly suppressed by *T. patula*. The other two *Tagetes* spp. suppressed the total population slightly more than fallow, but the difference was not or hardly significant. In the tubes with *T. patula* only 1,3% of *P. penetrans* survived in comparison to the controls. The roots of *T. erecta* and *T. minuta* harboured large numbers of nematodes, 3631 and 5004 per 10 g, respectively. More than 90% of the nematodes surviving in all *Tagetes* treatments were inside the roots. In tubes with wheat the total count of *P. penetrans* was 4.5 times that of controls; 85% of the nematodes were in the roots.

In the *T. patula* tubes, counts of *P. penetrans* were extremely low in soil as well as in roots. This is not so or less obvious for *T. erecta* and *T. minuta*. These differences in effectivity between the three *Tagetes* spp. are also reflected by the numbers in Col. 6.

c. *P. crenatus* as natural population in loamy sand

A similar trial to that in Section 3.2.1b. was made with *P. crenatus*. It began a week later, on 27 December 1966, and the infested soil was taken from a trial plot on sandy loam in Wageningen on which maize had been the last crop. The soil harboured a mixed population in which *P. crenatus* was the most numerous

species followed by the ectoparasitic *Tylenchorhynchus dubius* and *Rotylenchus robustus*. Determination of 40 *Pratylenchus* females revealed no species other than *P. crenatus*. The *Pratylenchus* population in the soil was therefore regarded as pure *crenatus*, also because analyses for other purposes in preceding years had always indicated the presence of this species only. The mean count of *P. crenatus* and of *Tyl. dubius* and of *R. robustus* per tube as determined from five samples taken from the whole batch of soil was 1327, 740 and 287, respectively.

As in preceding experiments all plants grew well and nine weeks after planting roots were weighed and nematodes counted. Values are shown in Table 3.

Pratylenchus populations in the control tubes were fallen to 82% only at the end of the experiment. In all planted tubes the soil contained considerably fewer *P. crenatus* than in the control tubes (Col. 3), but it appears again from Col. 4 and 5 that the number of nematodes was reduced only with *Tagetes*. Total number of nematodes at the end of the test (Col. 5) was 4%, 10%, 46% and 144%, respectively, for *T patula, T. erecta. T. minuta* and wheat. Of these numbers 37%, 53%, 73% and 86% were found in the roots of the plant species in the same order. Hence, there was a tendency to increase in both series of percentages. *T. patula* suppressed populations of *P. crenatus* more than *T. erecta*, and *T. erecta* was more effective than *T. minuta*. This is markedly reflected in the values in Col. 6. *T. patula* proved again to be the most effective species for *Pratylenchus* and *T. minuta* the least.

The effects on *Tyl. dubius* and *R. robustus* were less marked; they will be discussed in Section 3.4.1 and 3.5.1.

d. *P. neglectus* as natural population in clay

A similar trial was started, again a week later on 3 January 1967 with a clay soil from a trial field in Uithuizen heavily infested with *P. neglectus* and lightly with other species among which was *Tylenchorhynchus nothus* Allen. The last crop had been oil rape, *Brassica napus* L. *oleifera*.

The average number of *P. neglectus* and *Tyl. nothus* per tube as determined by counting nematodes in 5 samples of the same batch of soil was 974 and 594, respectively. All plants grew well and the experiment was evaluated again 9 weeks after planting. Table 4 shows the results.

Numbers of *P. neglectus* hardly decreased in the control tubes without plant: to 96% of the initial population density.

After 9 weeks total counts (Col. 5) were with *Tagetes* only slightly lower than in the control tubes without plant: 90%, 92% and 98% of control for *T. patula, T. erecta* and *T. minuta*, respectively. The difference between these species were not significant, but again *T. minuta* was the least effective according to numbers per 10 g of roots.

Wheat allowed *P. neglectus* to breed and multiply to a density which was 180% of the control and which was also higher than the initial density (Col. 5). Of this number 83% was present in the roots.

Only 1–2% of the total number of nematodes was found inside the roots of *Tagetes* spp. *P. neglectus*, therefore, was decreased little by *Tagetes* spp. in this

TABLE 3. Effect of three *Tagetes* spp. and wheat on a natural population of *P. crenatus*, *Tyl. dubius* and *R. robustus* in a tube culture. Planted on 27.12.1966. Initial densities: 1327 *P. crenatus*, 740 *Tyl. dubius* and 287 *R. robustus* per tube with 180 ml soil. Final densities and root weights determined 9 weeks after planting; average figures of 6 replicates; between brackets averages of logarithmically transformed figures and least significant differences (L.S.D.) at 5% and 1% levels.

1	2	3	4	5	6	7	8
Plant	Fresh weight of roots, in mg	Final number of *P. crenatus*: In soil	In roots	Total 3+4	Per 10 g of roots	Final number of *Tyl. dubius* In soil	Final number of *R. robustus* In soil
T. patula	1244	27 (1.37)	16 (1.13)	43 (1.63)	139 (2.00)	215 (2.31)	59 (1.72)
T. erecta	1771	50 (1.70)	55 (1.70)	105 (2.00)	318 (2.43)	310 (2.48)	379 (2.54)
T. minuta	1369	136 (2.12)	366 (2.57)	502 (2.68)	2669 (3.42)	208 (2.28)	225 (2.34)
Wheat	1155	219 (2.28)	1344 (3.12)	1563 (3.17)	11823 (4.05)	2751 (3.41)	475 (2.63)
No plant	–	1083 (3.03)	–	1083 (3.03)	–	594 (2.77)	169 (2.18)
L.S.D. 5%		(0.247)	(0.301)	(0.145)	(0.345)	(0.191)	(0.283)
1%		(0.337)	(0.417)	(0.198)	(0.477)	(0.260)	(0.386)

TABLE 4. Effect of three *Tagetes* spp. and wheat on a natural population of *P. neglectus* and *Tyl. nothus* in tube cultures.
Planted on 3.1.1967. Initial densities 974 *P. neglectus* and 594 *Tyl. nothus* per tube with 180 ml of soil. Final densities and root weights determined 9 weeks after planting; average figures of 6 replicates; between brackets averages of logarithmically transformed figures and least significant differences (L.S.D.) at 5% and at 1% levels; n.s. = not significant.

1	2	3	4	5	6	7
Plant	Fresh weight of roots, in mg	Final number of *P. neglectus:* In soil	In roots	Total 3+4	Per 10 g of roots	Final number of *Tyl. nothus:* in soil
T. patula	1191	832 (2.90)	10 (1.02)	842 (2.92)	85 (1.87)	338
T. erecta	1762	859 (2.93)	8 (0.90)	867 (2.93)	44 (1.62)	423
T. minuta	965	897 (2.95)	13 (1.07)	910 (2.95)	134 (2.05)	436
Wheat	819	280 (2.42)	1395 (3.10)	1675 (3.20)	16515 (4.22)	302
No plant	–	933 (2.97)	–	933 (2.97)	–	365
L.S.D. 5%		(0.132)	(0.336)	(0.150)	(0.351)	n.s.
1%		(0.180)	(0.465)	(0.204)	(0.485)	n.s.

experiment and the bulk of the nematodes remained alive in the soil. The rate of penetration into *Tagetes* was apparently very low. *P. neglectus* extracted from tubes with *Tagetes* showed no difference in appearance or activity from those extracted from tubes with wheat or from the control tubes.

Tyl. nothus was also not significantly influenced in this trial (Section 3.4.1).

e. *P. thornei* as natural population in heavy clay

This trial similar to the previous one, was started again a week later on 10 January 1967, with a heavy clay of a good structure from a field in Wageningen where *P. thornei* had reached a high density under wheat. No *Pratylenchus* spp. other than *thornei* were found on determination of 100 *Pratylenchus* specimens from this soil. There were on an average 2121 *P. thornei* per tube as determined from numbers found in five samples, and also 369 *Tylenchorhynchus brevidens* Allen.

All plants grew fairly well during the trials though some remarks are necessary. *Tagetes* spp. formed extensive root systems in the heavy clay. The soil which formed a column of loosely packed crumbles in the tube was uniformly rooted and its favourable structure was well retained throughout the trial. This was not true with wheat. Wheat formed considerably fewer roots than *Tagetes*. The soil was sparsely rooted and watering tended to affect its structure, crumbles disintegrating to form compact parts of clay in the tube. This happened to some layers of soil bordering the glass wall near the bottom of tubes as water was added through the plastic tube. In such compacted parts of the soil, roots were soon killed and were bordered by a bluish-black anaerobic zone of clay.

Table 5 give values from the different treatments at the end of the trial, 9 weeks after planting.

Decline of *Pratylenchus* population in the control tubes was almost negligible: 3% on average. Total counts of *P. thornei* (Col. 5) suggest a slightly greater decline in tubes with *T. patula*, *T. erecta* and wheat, and a slight increase in tubes with *T. minuta*. These differences, however, are not significant.

Although wheat is known as a good host of *P. thornei* (Sher & Allen, 1953), there was no increase in the total population. This was probably due to the poor development and decline of the roots. The count of *P. thornei* in individual tubes with wheat varies greatly and fewest occurred in tubes with many dead roots in anaerobic soil sections. Healthy wheat roots harboured many *P. thornei*, as appears from the figures in Col. 4 and especially Col. 6. This was not so in the roots of the *Tagetes* spp.

The vast majority of *P. thornei* stayed in the soil medium in *Tagetes*-planted tubes, whereas in wheat-planted tubes most of the nematodes were in the roots.

Differences in effect between the three *Tagetes* spp., with *T. patula* as most effective, appear from the numbers in Col. 6, although the population as a whole was hardly suppressed by any of the species in the experiment.

There was only a slight effect of the *Tagetes* spp. on *Tyl. brevidens* (Section 3.4.1).

TABLE 5. Effect of three *Tagetes* spp. and wheat on a natural population of *P. thornei* and *Tylenchorhynchus brevidens* in tube cultures. Initial density 2121 *P. thornei* and 369 *Tyl.* species[1]) per tube with 180 ml of soil. Final densities and root weights determined 9 weeks after planting; average figures of 6 replicates; between brackets averages of logarithmically transformed figures and least significant differences (L.S.D.) at 5% and at 1% levels; n.s. = not significant.

1	2	3	4	5	6	7
Plant	Fresh weight of roots, in mg	Final number of *P. thornei*: In soil	In roots	Total 3+4	Per 10 g of roots	Final number of *Tyl.* species: in soil
T. patula	1478	1934 (3.28)	2 (0.47)	1936 (3.28)	16 (1.02)	248 (2.37)
T. erecta	2537	1964 (3.30)	26 (1.37)	1990 (3.30)	101 (1.93)	248 (2.36)
T. minuta	1419	2148 (3.33)	32 (1.45)	2180 (3.33)	230 (2.28)	176 (2.23)
Wheat	540	822 (2.90)	1094 (3.00)	1916 (3.28)	16243 (4.18)	1197 (3.02)
No plant	–	2056 (3.30)	–	2056 (3.30)	–	296 (2.46)
L.S.D. 5%		(0.101)	(0.378)	(n.s.)	(0.500)	(0.248)
1%		(0.137)	(0.523)	(n.s.)	(0.692)	(0.339)

[1]) This initial density may have included a few *Trophurus imperialis* Loof.

3.2.2. *Field trials*

a. Effect of different *Tagetes* spp. and cultivars on a field population with *P. crenatus* and *P. penetrans.*

A sandy soil with many *P. crenatus* and some *P. penetrans* (ratio 37:3) at Zeijerveld was planted in 1964 with 8 cultivars of *T. patula,* 6 of *T. erecta* one of *T. lucida* and *T. signata* and with *T. minuta,* barley *(Hordeum vulgare* L.), potato or fallowed. It was a randomized block experiment with four plots of 1.5 m × 1.8 m per treatment. The plants were sown on the plots in rows 25 cm apart in mid May, except for some *Tagetes* plots (4, 5, 7, 8, 15 and 16 in Table 6) which were sown in the greenhouse and transplanted three weeks later to the plots. The whole field was evenly fertilized with an adequate amount of NPK granules and kept free of weeds, and pests and diseases of aerial parts as much as possible. All plants grew well throughout the season. Soil samples were taken from each plot before and after the experiment and *Pratylenchus* densities were counted per 100 ml of each. The final populations were expressed as percentages of the initial population in each plot. Results are summarized in Table 6 as far as they are relevant to this chapter of the study. The results, also for plant characters, will be discussed in more detail in Chapter 5 on possible application of *Tagetes* spp. in practice.

The *Pratylenchus* population dropped to 79 % of its initial density in the course of 6 months in the fallow plots. It increased under barley, which is known as a good host of both *Pratylenchus* spp. to 282 % and under potato, which is known as a suitable host of *P. penetrans* but not of *P. crenatus* to 160 %.

All eight *T. patula* varieties tested appeared to suppress the nematode population very effectively in this field test. The variety Harmony was similar in effect to the variety Golden Harmony.

T. erecta Aurantiaca showed hardly more effect than control. This result may have been influenced by the percentage transformation of the figures, due to the fact that the initial density noted for the Aurantiaca plots was least of all objects. The final density was 169 *Pratylenchus* per 100 ml against 358 in the Control.

Percentage transformation of the figures may not correctly reflect suppression of populations which are so low that the equilibrium density under the test plant is reached in the course of the experiment, so count of nematodes in the post-plant population, as used before would be more significant. The conclusions from the trial, however, would also then be that the effect of *T. erecta* Aurantiaca has been much less than with any of the *T. patula* treatments. This holds for most *T. erecta* cultivars. There is, however, much variation in this group: the most effective cultivar could be compared to the least effective *T. patula* cultivar.

T. lucida and *T. signata* were moderately effective. *T. minuta* was here, contrary to earlier experiments, not effective at all: it increased the *Pratylenchus* population to 150 % and left a final density of 420 *Pratylenchus* per 100 ml of soil, therefore higher than in the fallow plots.

Comparisons within sets of means of densities were also analysed with Scheffé's multiple F test. For this purpose final densities were corrected relative

to the corresponding initial levels; the differences in the corrected data for pairs of species or varieties were then expressed as units of their estimated standard deviation and compared with the critical value at a level of significance of 0.05. According to this procedure significant differences were found between all *Tagetes* spp. individually and barley and potato, except for *T. erecta* Hawaii, *T. erecta* Cupido Orange, and *T. minuta*, which did not differ significantly from potato. Comparisons within *Tagetes* spp. and fallow, however, yielded differences which were sometimes notable but often not significant.

When data only for *T. patula* Golden Harmony, *T. erecta* Aurantiaca and *T. minuta* were analysed, which were the most frequently used test plants in this study, the differences were significant. *T. patula* cv. Golden Harmony and Harmony were not significantly different.

Thus the cultivars of *T. patula* were generally most effective in this field trial and the cultivar Harmony used in most rotation trials discussed later, is about as effective as Golden Harmony which was used in the test-tube experiments described in Section 3.2.1.

b. Effect of monoculture and two-year rotation with *T. patula* on soils with different *Pratylenchus* spp.

Cross trials, as described before, with *T. patula* Harmony as one of the crops, were maintained on 12 widely different soils with different *Pratylenchus* spp. or mixtures. In all these trials the effect of *Tagetes* was compared with a good host plant and a poor host (or fallow if present) as monocultures over several years. Also two series of 2-year rotations, with *Tagetes* and other crops alternated, became available from each trial field, namely a series beginning with *Tagetes* and a series beginning with alternate crops. Soil samples were examined between growing seasons in the autumn or spring of each year. Autumn samplings are generally considered equivalent to spring samplings, because no difference was noticed in comparison of the population densities, but they are nevertheless marked in summarizing the results. In this arrangement initial density of the second year is also the final density of the first year, and so on. The results of all twelve trials, I to XII, are graphically represented in Fig. 1 for the monocultures; the relevant data of each experiment are indicated in the graphs. Fig. 2 represents the results of both series of two-year rotations of Trials I to IV. The graphs of Figs 1 and 2 are restricted to results for the first years after the onset of each trial, and are confined to *Pratylenchus* densities.

Table 7 lists initial and final densities of all nematode genera or groups of the populations in the *Tagetes* monoculture plots on the same field trials I to XII. The final populations given here are based on the most recent analysis made and the trial periods are longer than those illustrated in the graphs of corresponding trials in Fig. 1. It will be necessary to refer to Table 7 also in the sections on other nematode genera than *Pratylenchus*.

Table 7 shows that longterm monoculture of *T. patula* suppressed *Pratylenchus* populations to a low density in all fields, comprising different nematodes species and different soil types. The final densities per 100 ml of soil varied from

TABLE 6. Effect of 17 *Tagetes* spp. and varieties and barley, potato and fallow in a field trial at Zeijerveld with a mixed population of plant nematodes.

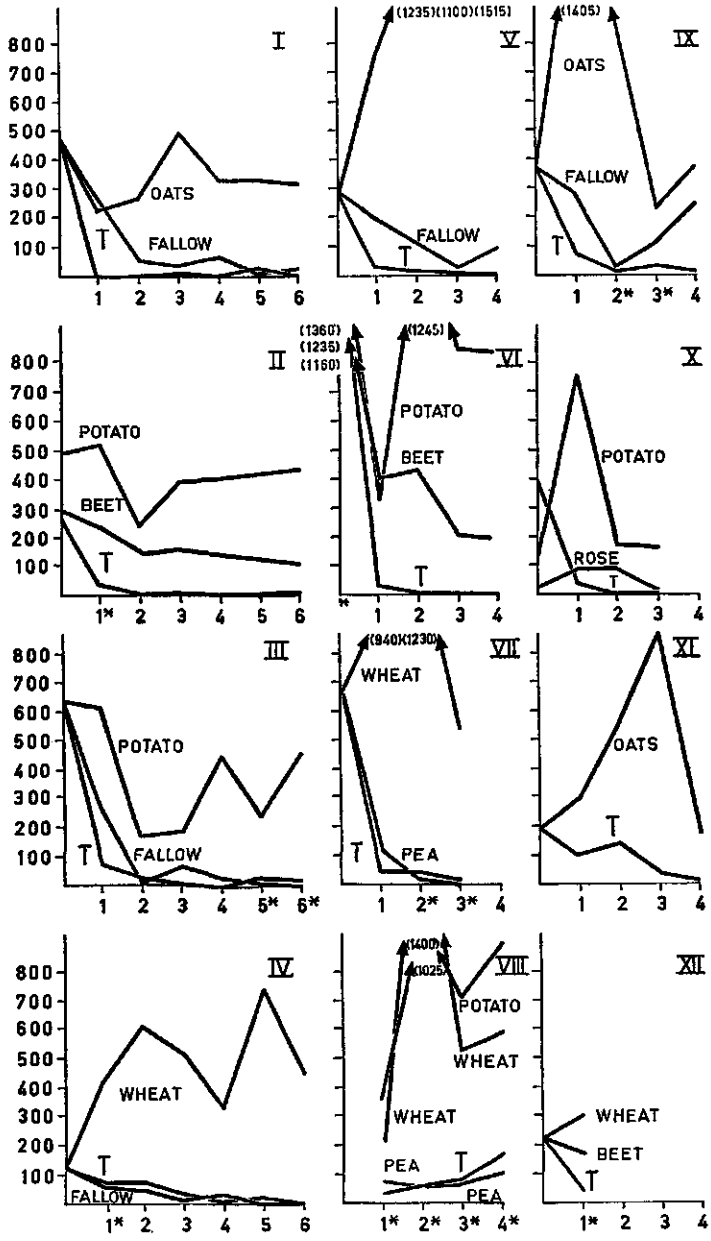
The average height of *Tagetes* varieties is added between brackets under the plant names; first figure is measured in this experiment, second figure or range according to catalogue. Four replicate plots per treatment; planting date 15.5.1964 and evaluation date 15.–30.11.1964. Average nematode figures per 100 ml soil; between brackets averages of logarithmically transformed figures and least significant differences (= L.S.D.) at 5% and 1% levels, using analysis of covariance; n.s. = not significant.

Under P. and T. average final density as a percentage of initial density is added.

P. = *Pratylenchus crenatus* and *penetrans*, T. = *Tylenchorhynchus dubius*, R. = *Rotylenchus robustus*, Pa. = *Paratylenchus* sp., Het.l. = *Heterodera* larvae mainly *H. avenae*, O. = other stylet-bearing nematodes, S. = saprozoic nematodes.

	Number of active nematodes per 100 ml soil						
	P.	T.	R.	Pa.	Het. l.	O.	S.
Initial density: (overall average)	374	455	4	2	88	424	1870
Final densities:							
1. *T. patula* Golden Harmony (32; 30)	29 (1.45) = 11%	71 (1.82) = 18%	0 (0)	0 (0)	19 (0.75)	610 (2.72)	3598 (3.53)
2. *T. patula* Harmony (33; 60)	83 (1.85) = 13%	141 (2.10) = 29%	0 (0)	0 (0)	109 (1.43)	451 (2.64)	3179 (3.45)
3. *T. patula* Flaming Fire (92; 100)	33 (1.45) = 9%	90 (1.95) = 15%	1 (0.20)	16 (0.82)	39 (0.86)	641 (2.76)	3856 (3.51)
4. *T. patula* Tangerine (47; 30–35)	74 (1.85) = 17%	155 (2.17) = 29%	0 (0)	9 (0.40)	73 (1.51)	675 (2.84)	2624 (3.53)
5. *T. patula* Spry (Lilliput) (34; 20–25)	30 (1.32) = 9%	131 (2.10) = 38%	1 (0.20)	5 (0.50)	81 (1.73)	580 (2.74)	4291 (3.59)
6. *T. patula* Petite Orange (26; 20–25)	35 (1.50) = 10%	148 (2.07) = 35%	0 (0)	1 (0.20)	38 (1.27)	471 (2.68)	2239 (3.32)

7. *T. patula* Petite Yellow (20; 20–25)	84 (1.85) = 21%	140 (2.10) = 33%	0 (0)	1 (0.20)	34 (0.91)	645 (2.77)	4165 (3.59)
8. *T. patula* Brownie (31; 30–35)	31 (1.40) = 8%	201 (2.30) = 25%	1 (0.20)	1 (0.20)	39 (1.26)	706 (2.83)	2439 (3.36)
9. *T. erecta* Aurantiaca (74; 70–80)	169 (2.20) = 76%	155 (2.20) = 33%	0 (0)	16 (0.97)	38 (1.47)	465 (2.63)	3720 (3.54)
10. *T. erecta* Burpee Gold (76; 70–80)	156 (2.20) = 43%	103 (1.97) = 26%	1 (0.20)	35 (1.22)	113 (1.72)	431 (2.59)	2900 (3.45)
11. *T. erecta* Julizon (48; 50–55)	83 (1.92) = 20%	129 (2.10) = 34%	0 (0)	23 (1.07)	46 (1.16)	576 (2.73)	3785 (3.56)
12. *T. erecta* Hawaii (55; 50–55)	366 (2.57) = 85%	131 (2.10) = 26%	1 (0.20)	35 (1.12)	10 (0.82)	421 (2.64)	2733 (3.41)
13. *T. erecta* Cupido Orange (27; 20)	321 (2.50) = 97%	109 (2.00) = 24%	4 (0.45)	4 (0.30)	19 (0.78)	524 (2.73)	3566 (3.52)
14. *T. erecta* Spungold (37; 20)	99 (1.90) = 38%	94 (1.95) = 28%	3 (0.40)	166 (1.55)	34 (1.08)	543 (2.71)	3015 (3.46)
15. *T. minuta* (140; –)	420 (2.47) = 150%	158 (2.17) = 31%	1 (0.20)	60 (1.00)	53 (0.97)	436 (2.55)	2886 (3.40)
16. *T. lucida* (45; –)	196 (2.30) = 44%	151 (2.15) = 46%	4 (0.45)	700 (2.67)	161 (1.12)	536 (2.71)	2874 (3.46)
17. *T. signata* Golden Gem (40; –)	135 (2.07) = 39%	103 (1.95) = 24%	5 (0.65)	180 (2.15)	55 (1.12)	338 (2.54)	3215 (3.47)
18. Barley	978 (2.97) = 282%	1336 (3.12) = 241%	3 (0.25)	3 (0.25)	240 (2.17)	491 (2.68)	1958 (3.27)
19. Potato	666 (2.80) = 160%	260 (2.32) = 54%	3 (0.25)	3 (0.40)	116 (1.70)	495 (2.68)	1785 (3.25)
20. Fallow	358 (2.52) = 79%	386 (2.57) = 92%	5 (0.65)	5 (0.50)	81 (1.25)	304 (2.47)	1120 (3.03)
L.S.D. 5%	(0.360)	(0.337)	n.s.	(0.852)	n.s.	(0.202)	n.s.
1%	(0.480)	(0.448)	n.s.	(1.134)	n.s.	n.s.	n.s.


I
OATS
FALLOW
T
V
(1235)(1100)(1515)
FALLOW
IX
(1405)
OATS
FALLOW
II
POTATO
BEET
VI
(1360)
(1235)
(1160)
(1245)
POTATO
BEET
X
POTATO
ROSE
III
POTATO
FALLOW
VII
(940)(1230)
WHEAT
PEA
XI
OATS
IV
WHEAT
FALLOW
VIII
(1400)
(1025)
POTATO
WHEAT
WHEAT
PEA
PEA
XII
WHEAT
BEET

0 to 20 on the 8 light soils, but were slightly higher on 3 of the 4 heavy soils with final densities of 35, 0, 70 and 48 in Fields IV, VII, VIII and XII, respectively. The fields which had carried *Tagetes* crops for 7 years or longer all harboured low final densities. Zero density was found in only 4 of the 12 fields and may be due to the sample size. It is probable from the fluctuation of figures in successive years that absolute zero was not reached under *Tagetes*.

Fig. 1 shows that monoculture of *T. patula* was nearly always more suppressive than other poor hosts or than fallow and that the differences were most strikingly in the first year. These graphs also show that the decrease in the heavy soils IV, VIII and XII, all with *P. thornei* and *P. neglectus*, was less marked or occurred slower than on other fields. The graphs of Fig. 1 indicate that fallow may reach about the same low *Pratylenchus* density as *Tagetes* but that it takes more years. Under fallow absolute zero is not normally obtained. Under good hosts *Pratylenchus* density fluctuates sharply at a high level, as would be expected.

Fig. 2 on biennial rotations with *Tagetes* on Fields I – IV gives additional insight and is very illustrative. Series a with *Tagetes* first as well as Series b with

FIG. 1. The effect of monoculture of *T. patula* (T) compared to fallow or a poor host and to a good host plant (as indicated in the graphs) on different *Pratylenchus* spp. in field trials I–XII on different soils.
Ordinate of individual graphs: population density of *Pratylenchus* per 100 ml soil determined in spring, or in the autumn before if the corresponding year on the abscissa is marked with an asterisk.
Some individual densities are recorded in the graphs.
Abscissa of individual graphs: number of years for which the crops have been cultivated.

I. *P. crenatus* in a loamy sand at Wageningen; first crop in 1958.
II. *P. penetrans* and *P. crenatus* in a peaty sand at Nieuw Buinen; first crop in 1961.
III. *P. neglectus* and *P. penetrans* (9:1) in a sandy soil at Hijken; first crop in 1959.
IV. *P. thornei* and *P. neglectus* (9:1) on a heavy clay (80% of soil particles $< 16\mu$) at Hagestein; first crop in 1959. Initial density in first year was determined too late and is therefore disputable.
V. *P. crenatus* in a sandy loam at Ellecom; first crop in 1959.
VI. *P. penetrans* and *P. crenatus* in a sandy soil at Zeijerveld; first crop in 1961. Initial density of first year determined in autumn 1960.
VII. *P. neglectus* in a clay soil (19% $< 16\mu$) at Uithuizen; first crop 1962.
VIII. *P. thornei* and *P. neglectus* (3:1) in a clay soil (22% $< 16\mu$) at Ried; first crop in 1961. The initial population density in the spring of 1961 was unrecorded, but all plots were cultivated before with wheat in 1960.
IX. *P. crenatus* with some *P. neglectus* in a sandy soil at Spier; first crop in 1960.
X. *P. penetrans* and *P. crenatus* (2:1) in a sandy peat at Nieuwe Pekela; first crop in 1961. *Tagetes* monocultural plots had been grown with oats in 1958, 1959 and 1960. The potato and rose monocultures were started on the same plots in 1958.
XI. *P. neglectus* and *P. crenatus* (1:1) in a sandy soil at Doorn; first crop in 1958. On the *Tagetes* plots *T. patula* was erroneously replaced by a *T. erecta* variety in the first two years.
XII. *P. thornei* with some *P. neglectus* in a heavy clay soil at Elst. Initial and final densities determined in 1960.

TABLE 7. Effect of longterm monoculture of *T. patula* on the final composition of nematode communities in different soils. Final densities per 100 ml of soil after the last crop of *Tagetes* as compared with initial densities in the same trial fields mentioned in the legenda of Fig. 1.
P = *Pratylenchus;* Pa. = *Paratylenchus;* T = *Tylenchorhynchus;* R. = *Rotylenchus;* Het. l. = *Heterodera* larvae; Mel. l. = *Meloidogyne* larvae; Tr. = *Trichodorus;* Di. = *Ditylenchus dipsaci;* O. = other tylenchids; S. = saprozoic nematodes.

Experimental field	Initial number of nematodes								Numberops	Final number of nematodes								
	P.	Pa.	T.	R/H	Het. l.	Mel. l.	O.	S.	*Tagetes* crops	P.	Pa.	T.	R/H	Het. l.	Mel. l.	Tr.	O.	S.
I. Wageningen 1958–1968	472	4	550	274	1		380	2434	10	0	0	0	385	10		15	180	4405
II. Nieuw Buinen 1961–1967	265		115				655	3510	6	10	5	110	5	5		0	280	970
III. Hijken 1959–1967	638	458	36	1	126	1	165	1327	8	20	165	0	0	0	0	0	250	2095
IV. Hagestein 1955–1966	138	43	51	33			231	604	7	35	0	10	0	0	0		170	175
V. Ellecom 1959–1967	385		856	224		10	143	738	8	5	0	25	510	5	30	0	1430	2780
VI. Zijerveld 1961–1967	1235	0	1455	25	5	0	745	3375	6	0	25	20	0	20	20	25	355	1120
VII. Uithuizen 1962–1967	681	1079	131	5	34		614	1180	5	0	20	20	0	0		60	760	1970
VIII. Ried[1] 1961–1965	220	210	135	55			440	870	5	70	50	215	15	0		0	535	2645
IX. Spier 1960–1967	428	3	352	6			62	908	7	0	10	105	0	0		35	180	1950
X. Nw. Pekela 1961–1964	390		15				85	1275	3	5		10				0	320	2580
XI. Doorn 1958–1963	186	5	283	13	5		15	1424	5	5	0	50	5	0	0	25	355	1120
XII. Elst 1960–1961	225	20	14	67			83	1845	1	48	11	7	23				88	1320

[1]) Preplant density in 1961 unrecorded, but all plots were grown with wheat in 1960. Instead of the initial density in 1961, the density after the first crop of wheat is given in this table.

good hosts first show that biennial rotations do suppress *Pratylenchus* populations about as effectively as permanent *Tagetes*. The populations increase incidentally on a good host to sizeable densities, but they are repressed immediately by the following *Tagetes* crop. The heavy soil of Field IV, with mainly *P. thornei* is exceptional again in sofar that *Tagetes* suppresses this population markedly nearly every year, but that the effect is not strong enough to prevent a good host maintaining high densities, which therefore fluctuate heavily from year to year.

3.2.3 *Discussion*

The effect of *Tagetes* spp. on *Pratylenchus* spp. is clearly established in the field as well as in laboratory trials. But there are differences in effect between *Tagetes* spp. and varieties and soil type or perhaps nematode species influence the result as well.

T. erecta was used by van den Berg-Smit (1953) when he demonstrated the marked effect against Narcissus root rot, thus against *P. penetrans*, for the first time and by Uhlenbroek & Bijloo (1958, 1959) when they found the presence of nematicidal polythienyls. The varieties of this species, however, are less effective than the varieties of *T. patula* (Table 6). *T. erecta* Aurantiaca (Tables 1 and 2) suppresses *P. penetrans* strongly compared with a good host and slightly compared with fallow, but it still harbours many nematodes in its roots even after several months.

The most effective species tested is *T. patula*, and this seems to hold for all its varieties (Table 6). *T. patula* Golden Harmony left a significantly lower density in soil and in roots than did other species in the tube trials with *P. penetrans* (Tables1, 2) and with *P. crenatus* (Table 3). There was hardly any effect in the tube trials with *P. neglectus* (Table 4) and with *P. thornei* (Table 5) in heavy soils. The reason may be circumstantial, for these nematodes were suppressed by *T. patula* Harmony in the field trials depicted in Table 7 and Figs. 1 and 2 although it takes longer. The effect against *Pratylenchus* spp. seems to decrease in the order *T. patula*, *T. erecta*, *T. minuta*, which are the species used in most trials.

The possibility that nematode species and not environment was responsible for the failing influence of *Tagetes* in the tube trials with *P. neglectus* (Table 4) and *P. thornei* (Table 5) may be excluded for *P. neglectus*, which was suppressed strongly in all field trials. For *P. thornei* it is not completely excluded. *P. thornei* was suppressed by *T. patula* in the field trials (Table 7; Trials IV, VIII, XII in Fig. 1; and especially Trials IV a and b in Fig. 2), but the effect is definitely less than with the other *Pratylenchus* spp. One reason is probably that the *P. thornei* populations studied all occurred in heavy clay soils. The influence of plant growth on *Pratylenchus* population in clay soils is often little; this was at any rate so in the tube trials depicted in Tables 4 and 5, in which the influence of the good host wheat was also small or not significant. Possible interpretations will be discussed in Chapter 4.

Tagetes plots and fallow plots both end up with a very low final density of *Pratylenchus* spp. The difference is that *Tagetes* suppresses the populations in a few months and fallow in a few years, which illustrates the drastic suppression

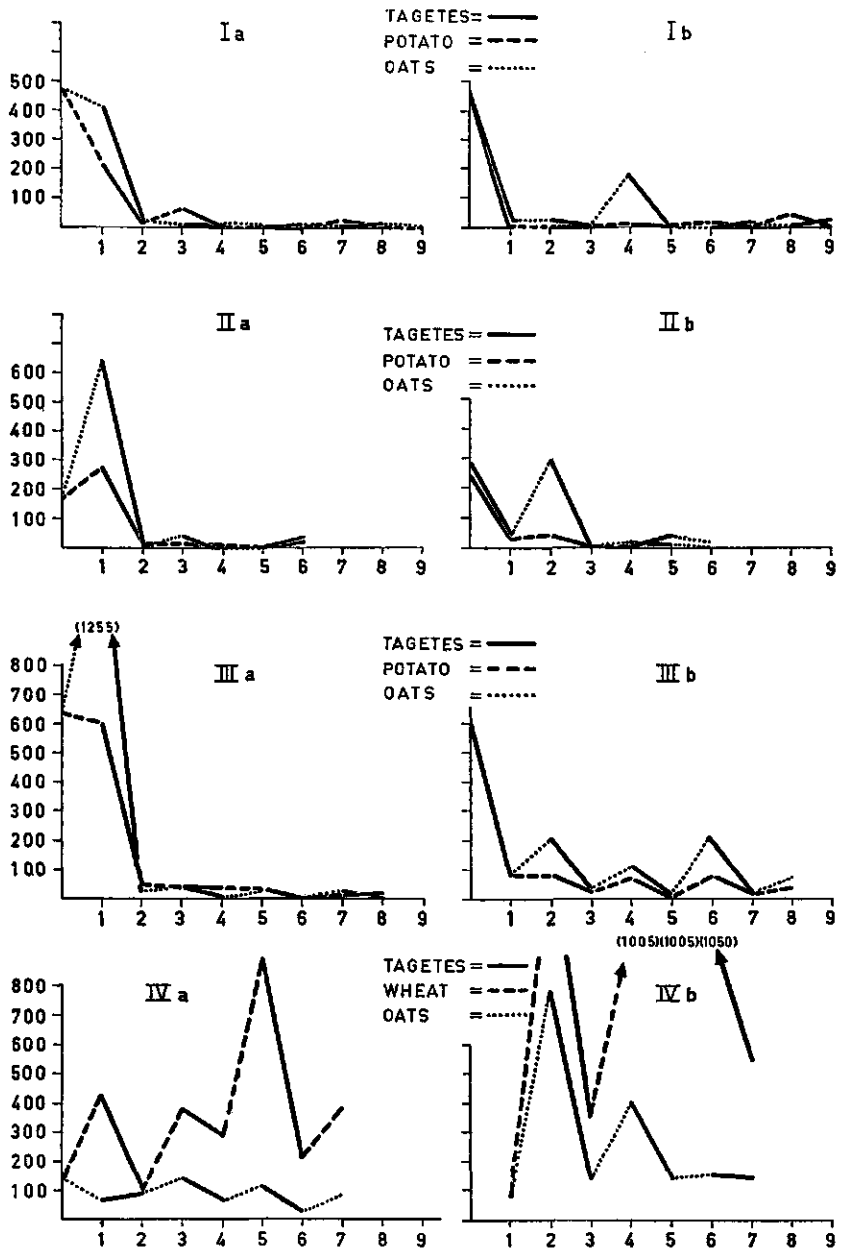


FIG. 2. The effect of two-year rotations in which *T. patula* was grown alternately with a good host plant (as specified in the graphs) on the *Pratylenchus* spp. present in Field Trials I, II, III and IV mentioned in Fig. 1. Two sets of two-year rotations, namely one starting with the good hosts (a) and one starting with *T. patula* (b) in each trial. See Fig. 1 for explanation of ordinate, abscissa and trial number.

of persistent populations. The final densities after some years are in both cases low but not zero. This may be an artefact, namely a small population maintaining itself on stray weeds which are always present in field trials (Oostenbrink, 1966). The tube trials and later field trials (Fig. 7 and Table 20), however, indicate that a very limited reproduction of *P. penetrans* occurs even in *T. patula* after 15–20 weeks, and therefore that the low equilibrium density of this plant-nematode relationship is part of the final population after a full-season *Tagetes* culture. This may hold for other *Tagetes-Pratylenchus* combinations as well.

Reproduction on weeds may explain anomalies in two plots recorded in Fig. 1: the significant increase in the fallow plot of Trial IX and in the *Tagetes* plot of Trial VIII. Field VIII was exceptionally wet and weedy, including the *Tagetes* plots. The population increased in the 3rd and 4th year, but dropped again in later years (Table 7) and does therefore not suggest a break in the resistance of *Tagetes*. The low final densities in all plots with *Tagetes* culture for 7 years or longer indicate that broken resistance of *Tagetes* or appearance of nematode strains resistant to *Tagetes* effects did not occur in any of these cases.

The sharp changes in population from year to year under good hosts (Fig. 1) are normal. The incidental peaks in the biennial rotations of good hosts and *Tagetes* (Fig. 2) may be due to yearly differences in reproduction rate on the good hosts, but they may also be due to differences in the final densities of *Tagetes* as are also the initial densities of the good hosts. These densities are so low that differences can hardly be assessed, whereas these differences may appear very striking on an untransformed scale after multiplication of the populations. It is certainly clear that a full-season *Tagetes* crop once in 2 years kept population of *P. penetrans*, *P. crenatus* and *P. neglectus* at a low level, whereas *P. thornei* in heavy soil fluctuated strongly at a higher level.

3.3. Meloidogyne species

Three species of *Tagetes* were tested for their effect on 4 different *Meloidogyne* spp., in comparison to tomato, a good host plant for all 4 species, and fallow soil as a control. All trials were tube cultures as for *Pratylenchus* (Section 3.2). There was one introductory test lasting 3 months with *M. hapla* inoculated directly to the test plants, and a series of 4 tests with established populations of *M. hapla*, *M. incognita*. *M. arenaria* and *M. javanica* in their original soils. The series of 4 tests were similar in arrangement and all lasted 10 weeks.

3.3.1. *Tube cultures*

a. *M. hapla* inoculated directly onto plants in potting soil

Forty glass tubes were filled with the same weight of partially sterilized standard potting soil equivalent to about 80 ml. Five series of 8 tubes were planted on 5 October 1964 with a seedling of *T. patula*, *T. erecta*, *T. minuta* and tomato, and one series was left unplanted. A week later 2000 second-stage larvae of *M. hapla* were inoculated as a suspension in water into each of the tubes including Controls. The inoculum was prepared by macerating and extracting tomato

TABLE 8. The effect of three *Tagetes* spp. and tomato on *Meloidogyne hapla* in tube cultures. Planted on 5.10.1964. Inoculation density 2000 second-stage larvae (l_2) per tube with 80 ml of soil. Root weights and final densities of undeveloped l_2, developed larvae and mature females determined 3 months after planting; each figure is the mean of 8 replicates; between brackets logarithmically transformed figures and least significant differences (L.S.D.) at 5% and at 1% levels, calculated from individual replicates; n.s. = not significant.

Plant	Fresh weight of roots in mg	Final number of *M. hapla*			
		In soil: Undeveloped l_2	In roots: Undeveloped l_2	Developed larvae	Mature females
T. patula	1575	49 (1.45)	11 (0.95)	0.6 (0.16)	0 (0)
T. erecta	3544	25 (1.14)	35 (1.44)	3[1]) (0.57)	1 (0)
T. minuta	3356	15 (1.12)	46 (1.62)	0 (0)	0 (0)
Tomato		33167 (4.29)			
No plant		139 (2.10)			
L.S.D. 5%		(0.522)	(0.432)	(0.228)	(n.s.)
1%		(0.705)	(n.s.)	(n.s.)	(n.s.)

[1]) of which 0.4 males

roots heavily infested by *M. hapla* which originated from a single egg mass. The inoculation caused well developed galls with many egg-laying females on and numerous larvae around the tomato roots.

Three months after inoculation the roots and the soils were assessed for *Meloidogyne* infestation. The results are in Table 8, and the qualitative assessment is described below.

Only 7% of the inoculated larvae were found alive and active in the unplanted soil after 3 months. These were significantly less in all *Tagetes* soils and the larvae were here in an inactive or slowly moving state and contained no reserve material in the body. New larvae were not found and could not be expected here because practically no egg-laying females were formed during the 3 months. Final soil populations had therefore little value as a criterion for suppression in this experiment.

A better criterion was the number of nematodes in the roots and the stage of development and reproduction of the nematodes. The nematodes in tomato made syncytia, performed their life cycle and reproduced profusely; several egg-laying females were often found in one root knot. In the *Tagetes* roots only 1–2% of the originally inoculated number of nematodes were recovered; together with the inactive nematodes in the soil about 3% were recovered.

Only some root tips were infested in *Tagetes*. Healthy root tips were present in much larger numbers than infested ones. There was, thus, no shortage of sites for the eelworms. As a rule only one nematode, developed or undeveloped, was found in an infested root tip. There were some differences between the *Tagetes* spp. tested. In *T. patula* syncytia were absent or very rare and the number of

rootborne larvae was least. Some larvae were found of a slightly obese shape, but no adults were noticed.

In *T. minuta* the head end of penetrated larvae was normally associated with a small, compact mass of plant tissue which stained strongly with cotton blue and was apparently a rudimentary syncytium. There was at most a very weak thickening on infested root tips. Although none of the larvae was found developing there, they seemed to maintain themselves alive for some time.

Nematodes could survive slightly better on *T. erecta* than on the other *Tagetes* spp. More larvae caused swelling of root tips and syncytia were larger than in *T. minuta*. The data in Table 8 indicate that some nematodes on *T. erecta* can partially develop in 3 months, because an occasional female with protruding egg-mass was observed.

Thus all 3 *Tagetes* spp. cleared the soil of *M. hapla* in comparison with tomato and fallow. *T. patula* and *T. minuta* seemed to be more effective than *T. erecta*, which allowed a number of larvae to develop beyond the infective second stage and one specimen even to maturity.

b. *M. hapla* as natural population in loamy sand

The soil used was a loamy sand from a trial field in Wageningen heavily infested with *M. hapla*. Carrots had been grown for several years in succession. The soil was cleared of carrot roots and other coarse particles by sieving and thoroughly mixed. Twenty glass tubes were filled with the same weight of soil equivalent to about 200 ml. On the same day 4 random samples of the amounts of soil used to fill tubes indicated an average initial density of 1914 second-stage larvae of *M. hapla* per tube. A day later, 4 series of 4 tubes were planted, on 21 October 1966 with a seedling of the test plants and one series was left unplanted. The plants grew well and the test plant, tomato, was readily infested. Table 9 shows results of root weights and final nematode infestations 10 weeks after transplanting the seedlings.

Of the initial number of 1914 larvae only 168 on average were recovered alive from the unplanted tubes at the end of the trial.

The tomato roots held an estimated 800 adult females and an uncounted large number of indivuals of all possible earlier stages of development and more than 6000 larvae per tube were found in the soil. Most adult females occurred singly at random heights on the roots. Few root knots contained more than one female, unlike the situation in the previous trial. Probably knots with many females appear at a later stage, and the difference is attributed to the fact that the first trial with *M. hapla* lasted 3 months against 10 weeks here.

Development of larvae in the *Tagetes* spp. seemed to be related again to how much the root tissue reacted by forming nourishing tissue. *T. erecta* was again the only *Tagetes* sp. which allowed development and even a moderate reproduction of *M. hapla*, as shown by the number of adult females and developed larvae in the table, as also by the number of larvae in the soil and by their condition. Several larvae found in the soil were vigorous and dark because of much reserve material in the body and were evidently newly formed. The soil population in

TABLE 9. The effect of three *Tagetes* spp. and tomato on a natural population of *Meloidogyne hapla* in tube cultures. Planted on 21.10.1966. Initial density 1914 second-stage larvae (l_2) per tube with 180 ml of soil. Root weights and final densities of undeveloped l_2, developed larvae and mature females determined 10 weeks after planting; each figure is the mean of 4 replicates; between brackets logarithmically transformed figures and least significant differences (L.S.D.) at 5% and 1% levels calculated from individual replicates; n.s. = not significant.

Plant	Fresh weight of roots, in mg	Final number of *M. hapla*			
		In soil: Undeveloped l_2	In roots: Undeveloped l_2	Developed larvae	Mature females
T. patula	1.887	125 (2.00)	18 (1.17)	0.3[1]) (0.07)	0 (0)
T. erecta	3.080	615 (2.72)	52 (1.32)	26.0 (1.17)	20 (1.22)
T. minuta	2.152	245 (2.35)	87 (1.90)	0 (0)	0 (0)
Tomato	3.207	6658 (3.82)			
No plant		168 (2.20)			
L.S.D. 5%		(0.449)	(n.s.)	(0.699)	(0.410)
1%		(0.630)	(n.s.)	(n.s.)	(0.667)

[1]) = immature female

the *T. erecta* tubes must therefore be considered infective. In *T. minuta* the number of nematodes in the root system was as high as in *T. erecta*, but no nematodes had developed beyond the initial stage. Few larvae were found in *T. patula* and they were not associated with the presence of small syncytia. One exceptional nematode in one of the replicates had developed on this species into a preadult female. The results of this trial, therefore, broadly agree with the first on *M. hapla.*

c. *M. incognita* as established culture in potting soil

A trial like the previous one was started a week later with *M. incognita* in standard potting compost. The soil was heavily infested with a culture of this nematode on tomato started from a single mass of eggs. Sieved and mixed soil contained 1448 larvae per tube with 180 ml of soil on average of 4 samples. The effect of the different treatments as found 10 weeks later is depicted in Table 10.

Here the initial 1448 decreased to 353 on average in the unplanted tubes. The tomato roots were heavily galled at the end; galls often covered the entire length of the roots and were densily occupied by nematodes at all stages of development. Often 60 or more adult females were counted per cm root.

The initial population of larvae was, therefore, certainly infective at the start. Nevertheless all *Tagetes* spp. tested were practically free from infestation. Frequently *Tagetes* roots with typical symptoms of attack were detected, but they no longer contained a nematode or only contained remains of one. These roots had a swollen tip; it had stopped growing and frequently formed a lateral. They

TABLE 10. The effect of three *Tagetes* spp. and tomato on *Meloidogyne incognita* in tube cultures.
Planted on 28.10.1966. Initial density 1448 second-stage larvae (1_2) per tube with 180 ml of soil. Root weights and final densities of undeveloped 1_2, developed larvae and mature females determined 10 weeks after planting; each figure is the mean of 4 replicates; between brackets logarithmically transformed figures and least significant differences (L.S.D.) at 5 % and 1 % level calculated from individual replicates.

Plant	Fresh weight of roots, in mg	Final number of *M. incognita*			
		In soil: Undeveloped 1_2	In roots: Undeveloped 1_2	Developed larvae	Mature females
T. patula	1329	334 (2.52)	0	0	0
T. erecta	1094	242 (2.25)	2.2	0	0
T. minuta	1693	264 (2.40)	0	1	0
Tomato	3278	17725 (4.22)			
No plant		353 (2.52)			
L.S.D. 5%		(0.638)			
1%		(0.895)			

also usually had a characteristic dark-stained cluster of cells where the nematode apparently had tried to continue feeding. This could be seen most conspicuously in *T. erecta*.

Larvae in the soil at the end, except those in the tomato tubes, were not considered infective because they were extremely weak.

All 3 *Tagetes* spp., therefore, can be rated as equally effective suppressors of this *M. incognita* population.

d. *M. arenaria* as established culture in potting soil

This trial was started on the same day and in the same manner as the previous but with *M. arenaria*. The soil in which the nematode was multiplied as a monospecific culture on tomato, harboured 210 larvae per tube of 180 ml at the time when seedlings of the test plants were planted. The infestation was enhanced by inoculating 3000 larvae of the same population to the seedlings one week after planting, and again 3000 another week later. Nematode populations and root weights were assessed 10 weeks after the last inoculation. Although 6000 larvae were added to the 210 already present per tube, it is assumed that the number which acted as effective inoculum was much lower. Most of inoculated larvae may have succumbed during manipulation and transfer, as in the inoculation trials with *M. hapla* (Section 3.3.1a) and *P. penetrans* (Section 3.2.1a). On an average only 2 of the 6210 larvae originally present were recovered from the unplanted controls at the end. Nevertheless the inoculum seems to have been abundant enough to cause heavy infection, for most tomato roots were heavily galled and dead at

TABLE 11. The effect of three *Tagetes* spp. and tomato on *Meloidogyne arenaria* in tube cultures.
Planted on 28–10–1966. Initial density 210+3000+3000 (cf. text) second-stage larvae per tube with 180 ml of soil. Root weights and final densities of undeveloped 1_2, developed larvae and adult females determined 12 weeks after planting (10 weeks after the last inoculation); each figure is the mean of 4 replicates; between brackets logarithmically transformed figures and least significant differences (L.S.D.) at 5% and 1% levels calculated from individual replicates.

Plant	Fresh weight of roots, in mg	Final number of *M. arenaria*			
		In soil: Undeveloped 1_2	In roots: Undeveloped 1_2	Developed larvae	Mature females
T. patula	2350	4 (0.30)	0 (0)	0 (0)	0 (0)
T. erecta	4754	10 (0.82)	0.2 (0.07)	0 (0)	0 (0)
T. minuta	2681	1330 (3.07)	332 (2.42)	279 (2.37)	356 (2.55)
Tomato	2538	3910 (3.55)			
No plant		2 (0.32)			
L.S.D. 5%		(0.732)	(0.447)	(0.381)	(0.158)
1%		(1.026)	(0.677)	(0.576)	(0.239)

the end. Living roots contained an estimated 20 adult females per cm, and galls on them were found to hold from one to 15 adult females. Table 11 records root weights and final numbers of nematodes.

T. minuta differed strikingly from the other Tagetes spp. Its root system bore numerous galls with many egg-laying females and larvae of different stages of development, whereas practically no *M. arenaria* was sustained on the other two species. The *T. minuta*, however, was not as good a host plant as tomato. This is illustrated by the difference in number of larvae in the soil. The number of motile second-stage larvae in the roots of *T. minuta* recorded in the table is an underestimate of the actual number. They could hardly be counted individually, because many short lateral roots developing near galls were massively invaded by newly hatched larvae. Such larvae were also found in widespread areas of older roots at random distances from the tips.

These results show that *T. patula* and *T. erecta* suppressed *M. arenaria* effectively, whereas *T. minuta* acted as a rather suitable host plant.

e. *M. javanica* as established culture in potting soil

In this trial, started on the same day as the one in Section 3.3.1b, the larvae had an initial density of 2097 *M. javanica* per tube. It was a monospecific culture on tomato. Ten weeks later the trial was evaluated; results are summarized in Table 12.

The initial number of 2097 larvae dropped to 53 on average in the unplanted tubes. Tomato roots were well infested and developed 3 or more galls per cm

TABLE 12. Effect of three *Tagetes* spp. and tomato on an established culture of *Meloidogyne javanica* in tube cultures.
Planted on 21.10.1966. Initial density 2097 second-stage larvae per tube with 180 ml of soil. Root weights and final densities of undeveloped 1_2, developed larvae and adult females determined 10 weeks after planting; each figure is the mean of 4 replicates; between brackets logarithmically transformed figures and least significant differences (L.S.D.) at 5% and 1% levels calculated from individual replicates.

Plant	Fresh weight of roots, in mg	Final number of *M. javanica*			
		In soil: Undeveloped 1_2	In roots: Undeveloped 1_2	Developed larvae	Mature females
T. patula	1675	52 (1.60)	0.3	0	0
T. erecta	3283	45 (1.65)	2.0	0	0
T. minuta	3944	87 (1.92)	0.3	0.3	0.5
Tomato	5337	1505 (3.15)			
No plant		53 (1.65)			
L.S.D. 5%		(0.454)			
1%		(0.636)			

root; each of these galls sustained one to 24 adult females. This is reflected also in the high larval density in the soil. These larvae had a normal vigorous appearance.

In contrast, all *Tagetes* soils harbour only weak larvae, evidently of the initial population. With the exception of 3 individuals on *T. minuta*, 2 of which were egg-mass bearing females, none of the larvae in the *Tagetes* roots had undergone any development.

T. patula and *T. erecta* were therefore totally effective and *T. minuta* nearly so, against *M. javanica* in this trial.

3.3.2. *Discussion*

Tomato and fallow both acted as controls in all 5 tube trials. Tomato was a good host for all 4 *Meloidogyne* spp.: inoculation caused galls with many egg-laying females and immature stages in the roots as well as a high density of active second-stage larvae around the roots. Fallow reduced the inoculated densities of *M. hapla* to 7% in 3 months and of *M. arenaria* to less than 1%. This may partly be due to the inoculation process. Initial densities of established populations, however, were also severely reduced in 10 weeks in fallow: *M. hapla* to 8%, *M. incognita* to 24%, *M. javanica* to 3%. Second-stage larvae of *Meloidogyne* spp., therefore, are not very persistent. Wallace (1966) found that *Meloidogyne* larvae, although active, lose their infectivity in soil within 4 days. Fallow may therefore be an effective procedure to suppress *Meloidogyne* populations. *Tagetes* spp. were generally as effective or better than fallow in suppressing *Meloido-*

gyne spp., with some notable exceptions. There were also some marked differences when the effects on *Meloidogyne* spp. are compared with *Pratylenchus* spp.

T. patula severely suppressed 4 *Meloidogyne* spp. One larva only, of *M. hapla* in the trial in loamy sand (Table 9), developed to an immature female. Few larvae survived in the roots, there were hardly any specimens beginning to develop after infection, and formation of feeding syncytia was absent or rare.

T. erecta certainly demonstrated a suppressive effect against *M. incognita*, *M. arenaria* and *M. javanica*. Development and, hence, reproduction were fully checked in these species. The effect against *M. hapla*, however, was incomplete and significantly less than those caused by the other 2 *Tagetes* spp. in both trials (Tables 8 and 9). *M. hapla* could reproduce and maintain a small population under*T. erecta*.This level was lower than under tomato but may be high enough to enable rapid increase to a high density when a suitable host is cultivated afterwards. The greater suitability of *T. erecta* as a host for *M. hapla* than *T. patula* was also evident from the more general occurrence of syncytia of a greater size. In fact *T. erecta* showed slightly stronger syncytia formation and a slightly higher survival with all 4 *Meloidogyne* spp. This suggests that *T. erecta* is less effective than *T. patula* in general, as was already found for *Pratylenchus* spp.

T. minuta, however, differs qualitatively from the above species. It suppressed *M. hapla* and *M. incognita* completely; suppression of *M. javanica* was incomplete, since some reproduction could take place. But *M. arenaria* could breed on *T. minuta* and reach considerable population densities on both roots and soil, whereas both *T. patula* and *T. erecta* did not allow reproduction of the nematode (Table 11).

This result indicates the occurrence of two types of differences between *Tagetes* spp. in their effect on *Meloidogyne* spp. There is a difference in effectiveness: *T. patula* was more effective than *T. erecta* against all *Meloidogyne* spp. tested, as in the trials with *Pratylenchus* spp. *T. patula* was also more effective than *T. minuta* in suppressing *Pratylenchus* spp. *T. minuta*, however, equalled *T. patula* in suppressing *Meloidogyne* spp., except one, namely *M. arenaria*. *T. minuta* could probably be a useful plant in a test series for identification of *Meloidogyne* spp. (cf. Sasser, 1954).

From the above results all 3 *Tagetes* spp. can be considered ideal suppressors of the *Meloidogyne* spp. tested, except for *T. minuta* with *M. arenaria*, and perhaps *T. erecta* with *M. hapla* and *M. javanica*. Also in these cases populations following the *Tagetes* spp. are much lower than those following tomato and the *Tagetes* spp. may be less suitable hosts than many other plants. They are, however, more dense than in fallow soil; there is no doubt that reproduction has taken place, and the corresponding plant-nematode associations may not completely eradicate nematodes and may even not be advisable for practical control of the corresponding *Meloidogyne* population.

These experimental results and conclusions closely agree with most data in the literature when such data are carefully examined. None of the marked suppression effects of *Tagetes* spp. against *Meloidogyne* spp., recorded in general terms or for certain species (Tyler, 1938; Steiner, 1941; Martin, 1958; Visser &

Vythilingam, 1959; Daulton & Curtis, 1963; Good *et al.*, 1965; Nirula & Bassi, 1965; Koen, 1966) should be questioned on the basis of our observations. Some records on qualitative and quantitative differences, however, have to be scrutinized.

Data given by Daulton & Curtiss (1963) reveal that *M. javanica's* infection index of tomato following *T. patula* was always lower than following *T. erecta* in Rhodesia. Earlier Visser & Vythilingam (1959) recorded at most a negligible infestation of *Tagetes* roots by *M. javanica* in tea soils in Ceylon, but closer examination of their data shows that in one instance *T. erecta* harboured 170 root-knot nematodes per 10 g roots against none in *T. patula.* This indicates that *T. patula* was a more effective suppressor of *M. javanica* than *T. erecta,* also in those countries.

Koen (1966), in pot trials with *M. javanica* in South Africa, found egg sacs and adult females only in *T. minuta* and not in *T. patula* and *T. erecta* 8 weeks after inoculation; there were no differences in number of nematodes found in the roots of the last 2 species. As mentioned earlier Good *et al.* (1965) in the United States found *T. minuta* to be susceptible to *M. hapla* and to *M. arenaria* but not to *M. javanica* and *M. incognita,* We found *T. minuta* a rather suitable host to *M. arenaria,* but not to the other three species. The data about *T. minuta* are therefore conflicting. This could be due to techniques or criteria used in trials. It seems more probable, however, that variation within the plant or the nematode species are at stake. There are great differences between varieties of *T. erecta* in their nematode-suppressing effect and this could also be true of *T. minuta.* It is also known, that biotypes with a different host preference occur within *Meloidogyne* spp. (Triantaphyllou & Sasser, 1960).

The results with *T. minuta* stress, that the *Tagetes* variety must be recorded exactly, and also that the nematode-host studies must not be generalized unless a biological assay confirms the identity of the nematode populations.

3.4. Tylenchorhynchus spp.

The effect of *Tagetes* spp. on *Tylenchorhynchus* populations was studied in tube and field trials. They comprise 5 series of trials (Sections 3.4.1–3.4.4).

3.4.1. *Tube cultures with Tylenchorhynchus spp. as natural populations*

The tube cultures with natural nematode populations described under 3.2.1c, d and e for *Pratylenchus* spp., also yielded results on *Tylenchorhynchus* spp. (Tables 3, 4 and 5). Table 3 shows that *Tyl. dubius* was suppressed by *T. patula, T. erecta* and *T. minuta,* as was *P. crenatus* though to a lesser degree.

Tables 4 and 5 indicate, that no significant suppression of *Tyl. nothus* and *Tyl. brevidens* was realized by the same *Tagetes* spp. in tube cultures with soils where *Pratylenchus* spp. had escaped suppression.

3.4.2. *Effect of different Tagetes spp. and cultivars on a field population with Tyl. dubius.*

In the *Tagetes* variety trial described in Section 3.2.2a *Tyl. dubius* was present alongside *Pratylenchus* and other nematodes. The influence of the different plants on *Tyl. dubius* density can be read from Table 6.

The results indicate that the initial density of 455 *Tyl. dubius* per 100 ml of soil was reduced to 386 in the fallow plots, and to densities between 71 and 201 in the plots grown with the 17 *Tagetes* spp. and varieties. The population in the barley plots increased to 1336. All *Tagetes* varieties suppressed the population significantly better than fallow. This can be read from the untransformed average densities in Table 6, except perhaps for the variety *T. patula Brownie*. Average final density as a percentage of average initial density is also low. Varieties of *T. patula* were not generally more effective than varieties of *T. erecta* or the other *Tagetes* spp., as was true for the suppression of *Pratylenchus* spp. in the same trial. Average final densities as a percentage of average initial density for barley, fallow, *T. erecta* Aurantiaca, *T. minuta*, *T. patula* Harmony and *T. patula* Golden Harmony, were 241 %, 92 %, 33 %, 31 %, 29 % and 18 %, respectively. The differences between barley and fallow and between fallow and the *Tagetes* varieties are significant, whereas differences between the 4 *Tagetes* varieties are not statistically significant.

3.4.3. *Initial and final densities of Tylenchorhynchus populations in 12 different soils after long-term monoculture of T. patula.*

The initial and the final densities of *Tylenchorhynchus* populations, and other sympatric genera, in the *T. patula* monoculture plots of 12 different field trials can be read from Table 7.

Long-term culture of *T. patula* suppressed the generally high *Tylenchorhynchus* densities to a very low level (0–50 specimens per 100 ml soil) in most soils. In 3 of the 12 soils a moderate population was maintained, namely 215 *Tylenchorhynchus* sp. in the heavy clay soil of Field VIII, 110 *Tyl. dubius* in the peaty sand soil of Field II and 105 *Tyl. dubius* in the sand soil of Field IX.

Tyl. dubius is the most generally occurring species of the genus, or maybe of all plant nematodes in Western Europe. It was present in all lighter soils, therefore also in I, III, V, VI, X and XI. It is reasonably certain from these data that *Tyl. dubius* is as a rule effectively suppressed, but that it can maintain a moderate density in some soils or under certain circumstances on *T. patula*.

3.4.4. *Effect of monoculture and biennial rotations of T. patula.*

In a loamy sand at Wageningen (Trial I of Fig. 1 and Table 7) *T. patula* and other crops were grown as a cross trial for 10 years. The initial density of *Tyl. dubius*, was 550 specimens per 100 ml of soil. The populations were determined every year. The densities of the monoculture plots of *T. patula*, fallow, oats and pea are given in Fig. 3A. Also densities of biennial rotations of *Tagetes* alternated with fallow, oats or pea, were available; they are depicted in Fig. 3B.

It appears from Fig. 3A that density is depressed more by *T. patula* than by

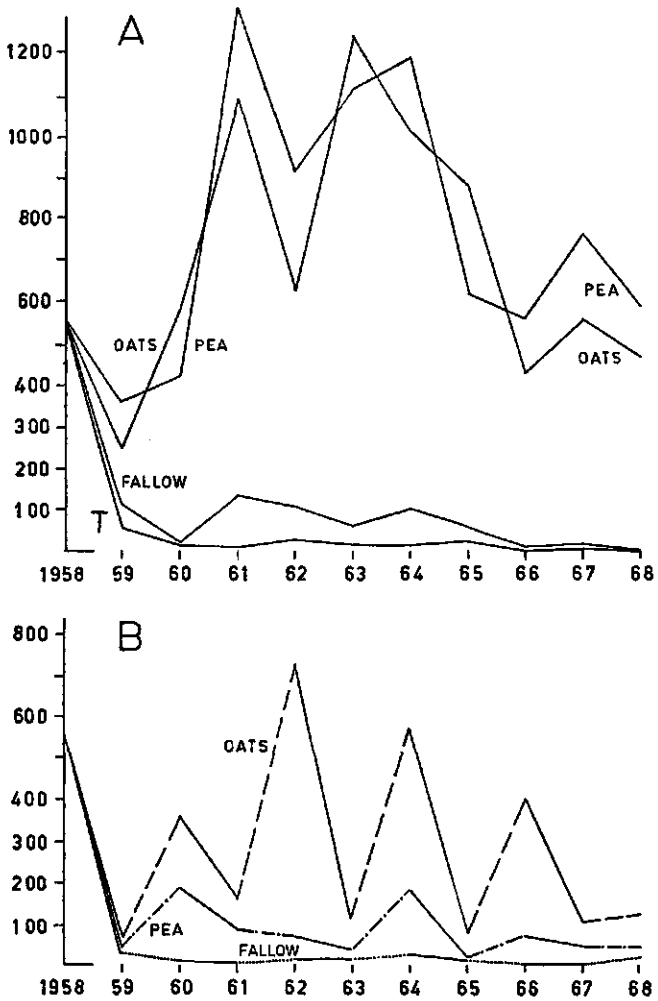


FIG. 3A. Effect of monoculture of *T. patula* (T), fallow and good hosts on *Tyl. dubius* in a field trial on loamy sand (Trial I of Table 7 and Fig. 1).
Ordinate: population densities per 100 ml soil determined in the spring of the years indicated. Abscissa: year of sampling.

B. Effect of two-year rotations in which *T. patula* was grown alternately with a good host or fallow on *Tyl. dubius*. Further explanation for ordinate, abscissa and trial number as in A.

fallow, it falls to 10% of the original density in the first and to 2% in the second year of *Tagetes* cultivation; the figures for the fallow plot were 21% and 4%, respectively. In later years the density on the *Tagetes* plots remained between zero and 6%, on the fallow plots always slightly higher. This difference may be due to the fact that in the fallow plot in practice some weeds always grow or

weed seed germinate or stray roots of plants from adjoining plots penetrate, so that a limited density is maintained there on host plants of this polyphagous nematode. Oats and pea built up a high density of *Tyl. dubius* and maintained it throughout the trial period of 10 years, though populations sometimes fluctuated.

These results are supported by the results of the biennial rotation plots. *Tagetes* alternated with fallow suppresses the population rapidly and permanently to a very low level, between 0 and 20 per 100 ml of soil. It does not seem to reach absolute zero. *Tagetes* alternated with the good host oats and pea maintains moderate populations far above those of monoculture *Tagetes* and far below those of monoculture oats or pea.

The suppressive effect of *Tagetes* is visible each year in both rotations. Oats appears to be a better host than pea in this trial. This was not visible in the monoculture plot, but it is in accordance with data in literature.

Thus *Tagetes* suppressed *Tyl. dubius* rapidly and permanently and to a greater extent than fallow in this trial.

3.4.5. *Review of published results*

Records in literature indicate a suppression of *Tylenchorhynchus* populations, especially *Tyl. dubius*, by one season's cultivation of *T. patula*. Numbers of *Tyl. dubius* found per 100 ml of soil following one year of *T. patula* and after a year of fallow were 13 and 32 (Oostenbrink, Kuiper & s'Jacob, 1957), 55 and 495 (Meijneke & Oostenbrink, 1958), 40 and 95 (Oostenbrink, 1960b), 64 and 79 (Oostenbrink, 1966), respectively.

When *T. patula* was grown for 6 years in succession Oostenbrink (1966) found the following series of *Tyl. dubius* densities per 100 ml of soil;
T. patula: 85 (initial density) – 55 – 10 – 10 – 30 – 20 – 20
Control fallow: 85 (initial density) – 115 – 20 – 140 – 105 – 55 – 100

T. patula clearly suppressed *Tyl. dubius* but this suppression was often weak, so that more than one season of *Tagetes* may be needed to reach the minimum (equilibrium) density: the minimum density was 10–30 per 100 ml of soil and not zero. Fallow plots again maintained a density considerably higher than in *Tagetes* plots which fluctuated between 20 and 140, as in Fig. 3A.

3.4.6. *Discussion*

Tyl. dubius is a polyphagous ectoparasite. A large proportion of the population can survive more than one year in fallow plots, but in the second year its density drops considerably to a low level, which is then maintained. This collapse after 1–2 years indicates that the nematode can survive a long starvation and that it requires higher plants to maintain dense populations. The maintainance of a low residual density is supposed to be due not to extreme longevity, but to moderate reproduction on germinating weeds, algae, fungi or another substrate such as stray roots of crops in the adjoining plots, especially so because this remaining density fluctuates (Oostenbrink, 1966). Our data support this view; ruderal hosts could also explain why the population density under *Tagetes* never

becomes zero. The final population level on the fallow plots may be higher than in the *Tagetes* plots because more weed seedlings or stray roots from adjoining plots can start to grow in fallow soil than in the well covered densely rooted soil of the *Tagetes* plots.

All data together indicate that *Tylenchorhynchus* spp. are suppressed by *T. patula* in different soils (Table 7), that *Tyl. dubius* is suppressed more and more rapidly by *T. patula* than by fallow (Fig. 3A, B; literature data), that *T. erecta* and *T. minuta* are also effective though less than *T. patula* (Table 6).

Comparison with *Pratylenchus* suggests that *T. patula* suppresses *Tyl. dubius* less effectively and less rapidly. This may be due to the difference in parasitic habit between the two nematodes. In contrast to *Pratylenchus*, *Tylenchorhynchus* usually remains outside the host plants; its chances for escape from the effect of *Tagetes* may therefore be greater than that of *Pratylenchus*. Moreover *Tylenchorhynchus* populations have as a rule a higher density on good host plants and reproduce faster than *Pratylenchus* spp. The same percentage reduction by *Tagetes* would leave a higher population density, and the same is true for limited reproduction on incidental weed seedlings and stray roots of other plants.

The generally great difference between the effect of *Tagetes* and fallow and the much more rapid population fall under *Tagetes*, therefore, indicate that there is indeed a distinct effect of *T. patula* on *Tyl. dubius*. The data are, however, not suitable for deciding whether the nematode does reproduce at all on *T. patula* and maintains a low density *ad infinitum* on the plant. They also do not much elucidate the mechanism of the *Tagetes* effect.

3.5. Rotylenchus and Helicotylenchus spp.

The effect of *Tagetes* spp. on *Rotylenchus* and *Helicotylenchus* spp. was studied by field trials only. There were again 4 sets of data and also here most data concern the effect of *T. patula* Harmony, on the most widespread nematode of the group, *R. robustus*. Comparative effects of *Tagetes* varieties, long-term effect of *T. patula* monoculture in different soils, population fluctuations under *T. patula* in comparison to fallow and good hosts and a summary of relevant published results were drawn from the same trials as for *Tylenchorhynchus*.

3.5.1. *Tube culture with R. robustus as a natural population.*

The tube culture described under Section 3.2.1.c (Table 3) indicates, that *T. patula* probably suppressed *R. robustus* slightly in comparison to fallow, but that this was not true for *T. erecta* and *T. minuta*.

3.5.2. *Effect of different Tagetes spp. and cultivars on a field population of R. robustus.*

The *Tagetes* variety trial depicted in Table 6 indicates little about suppression of *R. robustus* because the population density was very low. It indicates, however, that none of the *Tagetes* spp. was a good host plant, because no noticeable reproduction occurred in any plant treatment.

3.5.3. *Rotylenchus and Helicotylenchus populations in 12 different soils during long-term monoculture of T. patula.*

Table 7 shows, that *Rotylenchus* or *Helicotylenchus* were originally present in moderately high densities only in 2 fields, I and V, and that they maintained these population densities of 385 and 510 nematodes here after 10 and 8 years monoculture of *T. patula.* In both cases the species was *R. robustus.* Other species of *Rotylenchus* or *Helicotylenchus* did not occur, though low densities were originally present in most fields.

T. patula, therefore, did not act as a good host in most fields but maintained the 2 rather dense populations of *R. robustus.*

3.5.4. *Effect of T. patula, fallow and oats in a loamy sand*

In Cross Trial I, already mentioned in Fig. 1 and in Section 3.4.4 for *P. crenatus* and *Tyl. dubius,* densities of *R. robustus* were observed for 10 years in the monoculture plots of *T. patula,* fallow and the good host plants oats. The initial density was 274 *R. robustus* per 100 ml of soil (Fig. 4).

The population did not drop to a low level in any of the fields. The population under *Tagetes* was maintained for several years at a density between 200 and 400 and reached a peak in 1966 and 1967. The population under fallow was generally higher and fluctuated more from the start, though it dropped to below the level in the *Tagetes* plots in the years 1966–1968. Oats maintained a population fluctuating at a slightly higher level.

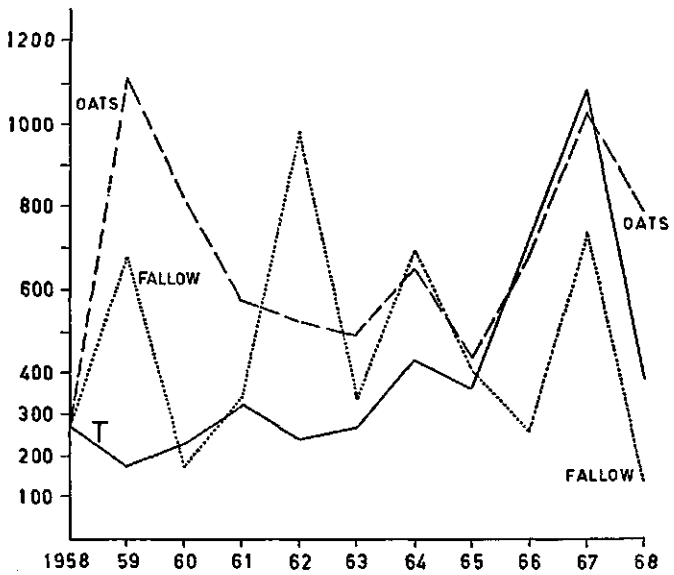


FIG. 4. Effect of monoculture of *T. patula* (T), compared to fallow and oats on *R. robustus* in a field trial on loamy sand (Trial 1 of Table 7 and Fig. 1).
Ordinate: population densities per 100 ml of soil determined in the spring of the years indicated. Abscissa: year of sampling.

It is evident from this graph, that *Tagetes* did not effectively suppress the population in this soil. The unexpected rise in 1966–1967 and the fluctuating high densities in the fallow plots make it very likely, that *R. robustus* can live and reproduce on another food source than the crops grown, perhaps weed seedling or more likely even algae or fungi. This food source must be rich here, because populations are maintained at a high level in the fallow plot. It is therefore not possible to conclude from these data whether *T. patula* was a host of *R. robustus* or not. The lower density under *T. patula* than under fallow or oats in the period of 1958–1965 suggests, that *T. patula* was not a good host plant.

3.5.5. *Review of published results.*

Numbers of *R. robustus* per 100 ml of soil after a year of *T. patula* or a year of fallow were 70 and 102 (Oostenbrink, Kuiper & s'Jacob, 1957), 230 and 955 (Meijneke & Oostenbrink, 1958), 210 and 305 (Oostenbrink, 1960) and 298 and 688 (Oostenbrink, 1966), respectively.

When *T. patula* was grown for 6 years in succession, Oostenbrink (1966) found the following series of *R. robustus* densities per 100 ml soil:
T. patula: 520 (initial density) – 170 – 225 – 320 – 240 – 265 – 430
fallow: 520 (initial density) – 675 – 170 – 340 – 985 – 335 – 695

These data suggest, that *R. robustus* is suppressed by *T. patula* to a moderate density which may be maintained under monocultured *Tagetes* with continuous fluctuations.

3.5.6. *Discussion*

R. robustus is a polyphagous nematode, which lives mainly as an ectoparasite though part of the nematodes may enter the host roots. The species is persistent, like *Tyl. dubius*, and there are unexplained aspects of its behaviour such as maintenance of a high density in fallow soil, unexpected population increases in fallow soil and on *Tagetes* and other aspects (cf. Oostenbrink, 1966).

Originally Oostenbrink, Kuiper & s'Jacob (1957) thought that *R. robustus* was not suppressed by *T. patula*, but in a later publication Oostenbrink (1960b) altered his opinion and stated that high populations of the nematode were suppressed but moderate ones of 50–250 nematodes per 100 ml of soil were not, or even increased. This suggests that *T. patula* is a host of *R. robustus* which sustains moderate population densities.

The data brought together here generally accord with this view. The behaviour of *R. robustus* populations under continuous fallow and the incidental peaks under *Tagetes*, however, stress that factors other than the presence of higher plants may be dominant in the population dynamics of this nematode, and therefore that we cannot state that *T. patula* normally reduces high densities to moderate level, which is generally lower than where soil is kept fallow. Neither does *T. patula* suppress the population effectively or rapidly.

3.6. Hemicycliophora, Paratylenchus, Criconemoides and Trichodorus spp.

Data about the effect of *Tagetes* spp. are drawn from a tube culture with a natural mixture of species from all genera in a marine sandy soil, from the *Tagetes* variety trial already discussed earlier for *Pratylenchus* spp., *T. dubius* and *R. robustus* (Table 6), and from the initial and final populations in long-term monoculture plots of *T. patula* on different trials (Table 7).

3.6.1. *Tube culture with a mixture of species of all four genera in marine sandy soil*

The soil used was a marine sandy polder soil from a plot in Middenmeer in which Kuiper (1963) observed reproduction of some ectoparasitic plant nematodes on *T. patula* in a field trial. This soil harboured significant populations of the ectoparasites *Hemicycliophora conida* Thorne, *Paratylenchus microdorus* Andrassy, *Macroposthonia curvata* (Raski) and *Trichodorus teres* in addition to *Pratylenchus neglectus* and a number of other stylet-bearing species and saprozoic nematodes. Soil was collected from the field and thoroughly mixed. These manipulations were done gently to save *Trichodorus teres*, which is known to be very sensitive to mechanical disturbance of the soil (Bor & Kuiper, 1966). Nine samples of 300 ml each were examined to determine the initial densities of the populations.

Fifty-four plastic tubes of 4 × 4 × 20 cm were each filled with 300 ml of soil. Seven series were planted on 31 October 1964 with 2-weeks-old seedlings of 2 cultivars of *T. patula*, 2 cultivars of *T. erecta* and with *T. minuta*, *T. lucida* and *T. signata*.

Sugar-beet, *Beta vulgaris* L. ssp. *vulgaris* and controls without a plant were also set up. The effect of the different treatments were assessed by determining the nematode populations in the soil of entire tubes 6 months later. Further data about the trial and the results are in Table 13.

The *Pratylenchus neglectus* density dropped in the unplanted control to about 20% of its initial density. The densities in the soil around the *Tagetes* spp. and beet were still lower. The test was not intended to investigate the effect on populations of this endoparasite. Final densities were determined only for nematodes in the soil and not for those in the roots, so that the picture is incomplete. It is likely that beet, which is a suitable host of *P. neglectus* (Oostenbrink, 1953) on lights soils, harboured most of the population in its roots at the end of the trial. This is probably not so in *Tagestes* spp. (Section 3.2.1d). It is therefore likely that all *Tagestes* spp. tested had a moderate reducing effect on population densities of *P. neglectus* in this soil in comparison with fallow. The best effect was obtained with *T. minuta* here, which had formed far more roots than any of the other *Tagetes* spp. The small amount of roots in the other species may have reduced their effects.

The effects on *Hem. conida* were very striking. The population dropped in the fallow soil and under beet. Relatively low initial densities of the nematode, how-

TABLE 13. Effect of seven *Tagetes* spp. and varieties, beet and fallow on a mixed population in marine sandy soils. Greenhouse trial with tubes of 300 ml of soil; 6 replicates per treatment; planting date 31.10.1964. Average nematode figures per 300 ml of soil 6 months after planting; between brackets averages of logarithmically transformed figures and least significant differences (= L.S.D.) at 5% and 1% levels. P. = *Pratylenchus neglectus*, Hem. = *Hemicycliophora conida*, Pa. = *Paratylenchus microdorus*, M. = *Macroposthonia curvata*, Tr. = *Trichodorus teres*, O. = other stylet-bearing nematodes, S. = saprozoic nematodes

	Number of active nematodes per 300 ml of soil:							Fresh weight of roots, in g
	P.	Hem.	Pa.	M.	Tr.	O.	S.	
Initial density	1685	66	72	8	225	282	2033	
Final densities:								
No plant	362 (2.47)	16 (0.65)	34 (1.00)	0 (0)	2 (0.35)	31 (0.98)	8254 (3.76)	
T. patula Golden Harmony	225 (1.93)	12128 (3.83)	2 (0.17)	0 (0)	78 (1.58)	91 (1.62)	6441 (3.78)	3.6
T. patula Harmony	241 (2.30)	8055 (3.67)	5 (0.37)	0 (0)	138 (2.00)	87 (1.90)	7974 (3.87)	2.7
T. erecta Aurantiaca	160 (2.20)	151 (1.37)	23 (1.10)	0 (0)	69 (1.75)	130 (2.02)	5766 (3.75)	5.4
T. erecta Spungold	207 (2.32)	1 (0.13)	6762 (2.88)	0 (0)	57 (1.58)	75 (1.63)	5118 (3.78)	2.4
T. minuta	84 (1.80)	1 (0.13)	181 (1.47)	0 (0)	31 (1.18)	58 (1.73)	8607 (3.93)	21.4
T. lucida	182 (2.12)	951 (1.98)	6294 (2.48)	0 (0)	88 (1.42)	62 (1.32)	4996 (3.60)	1.2
T. signata	137 (2.02)	1559 (2.75)	3906 (3.50)	0 (0)	156 (1.78)	56 (1.62)	9277 (3.85)	1 (dead plants)
Beet	255 (2.37)	4 (0.47)	553 (1.38)	207 (2.17)	174 (1.92)	37 (1.50)	19851 (4.28)	27.2
Least significant differences: 5%	(n.s.)	(0.998)	(1.240)	(0.132)	(0.721)	(n.s.)	(0.358)	
1%	(n.s.)	(1.335)	(1.660)	(0.176)	(0.964)	(n.s.)	(n.s.)	

ever, were built op to extremely high levels by both cultivars of *T. patula* and moderately high by *T. lucida* and *T. signata*. Also *T. erecta* Aurantiaca caused a slight increase. *T. erecta* Spungold and *T. minuta* were not a host at all. Different species or cultivars of *Tagetes*, therefore vary widely or even have opposite effects on populations of *Hem. conida*. Some species may be very good hosts of the nematode, while others are non-hosts, and within the same species one cultivar may be a host and another not.

Marked effects were also measured for *Pa. microdorus*. The population dropped in the fallow soil and increased moderately with beet. Great differences in host status were again displayed by the *Tagetes* treatments, but these differences were not correlated with the effects on *Hem. conida*. The two cultivars of *T. patula* which were good hosts of *Hem. conida* did not bring about any multiplication of *Pa. microdorus*. The reverse is true for *T. erecta* Spungold; this cultivar is not a host for *Hem. conida* but a good host for *Pa microdorus*. *T. lucida* and *T. signata* caused a strong and *T. minuta* a slight increase.

The low initial density of *Ma. curvata* increased about 25-fold under beet, but disappeared from all other treatments. The initial density, however, was too low to measure specific effects of *Tagetes* compared with fallow. The occurrence of reproduction on beet but not on other plants indicates at any rate, that none of the *Tagetes* spp. was a good host. Steiner (1941) found *Tagetes* to be a very good host of the closely related species *Nothocriconema mutabile* (Taylor) (syn. *Criconemoides mutabile* Taylor).

The *Tr. teres* population decreased in all treatments, most in the controls without plants and least under beet, which is known to be a suitable host for this nematode (Kuiper & Loof, 1962). It is probable from trials of Bor & Kuiper 1966) that the original population was strongly reduced by the transport and mixing of the soil. It is, therefore, unknown what the true initial density was, and therefore appropriate to compare final densities in the soil of the planted tubes with those in the controls. It appears then, that populations of *Tr. teres* would practically have disappeared if *Tagetes* and beet had not been grown in this soil, and therefore that all these plants were good hosts. Population was highest under beet, followed by *T. signata* and *T. patula* Harmony. Least suitable hosts were *T. minuta* and the 2 *T. erecta* cultivars.

The other stylet-bearing nematodes were not numerous and do not deserve special discussion; differences were hardly remarkable.

Significant differences occurred in the saprozoic nematodes which in all treatments, including fallow, increased their density. This indicates that the soil itself contained decaying organic material and that the environmental conditions during the trial suited these nematodes. The differences in densities are probably primarily related to the presence of decaying organic material. The highest densities occurred under beet, which plant showed the highest root weight. Next in density were *T. signata*, with very large numbers especially in tubes with a dead plant, and *T. minuta* again with a high root weight. The lower densities under a number of *Tagetes* spp. than in the fallow soil suggest that some of the nematodes in the mixture were reduced or slightly checked.

3.6.2. *Effect of different Tagetes spp. and cultivars on a field population with Paratylenchus spp.*

The *Tagetes* variety test summarized in Table 6 also yielded results about *Paratylenchus* spp. which were present at a low initial density. The density after barley and potato was the same as after fallow. None of the *T. patula* cultivars increased population significantly. *T. lucida, T. signata, T. minuta* and several *T. erecta* cultivars, however, did so and must therefore have allowed *Paratylenchus* spp. to reproduce. As in Table 13, *T. lucida* and *T. erecta* Spungold were most suitable as host plants; *T. erecta* Aurantiaca was less suitable than *T. erecta* Spungold.

3.6.3. *Initial and final densities in 12 different soils after long-term monoculture of T. patula*

Pratylenchus and *Tylenchorhynchus* populations were generally suppressed to low levels by long-term monoculture of *T. patula* and *R. robustus* maintained a moderately high density in the 2 fields where such a density was initially present.

In none of the twelve soils did *Paratylenchus* spp. build up to a considerable population on *T. patula*, as with *Paratylenchus* spp. on some other *Tagetes* spp. in the trials in Sections 3.6.1 and 3.6.2. In the only 2 fields with a measurable initial density the population dropped considerably, from 458 to 165 in Field II and from 43 to 0 in Field IV.

Of the other ectoparasites present only *Trichodorus* ,mainly *Tr. pachydermus* built up or maintained moderate densities on *T. patula* in Fields I, IX and XI. *T. patula* was certainly a less suitable host for these *Paratylenchus* and *Trichodorus* spp. than other crops grown alongside *Tagetes* in the same soil. The same holds for *Criconemoides* spp. which multiplied vigorously on other crops in some of the fields but reached no measurable density under *T. patula. Hemicycliophora* spp. were not originally present in measurable densities and did not come to the fore in any of the 12 fields.

3.6.4. *Discussion*

The criconematoid genera *Hemicycliophora, Paratylenchus* and *Macroposthonia* and the dorylaimoid genus *Trichodorus* all comprise many species. Species of these genera are all ectoparasites as far as is known. Records on suppression of these species by or reproduction on *Tagetes* spp. are scarce and the results are inconsistent. The results available provide some insight into but not a full review of the *Tagetes*-nematode relationships, which evidently show much variation in this group.

Table 7 shows that species of these 4 genera do not occur in significant densities in arable soils. The *Paratylenchus* spp. which occurred in high densities in some fields, were suppressed by *T. patula* and no species, apart perhaps from *Tr. pachydermus*, occurred on any of the monoculture *T. patula* plots.

However, Table 13 shows that in marine sandy soil *Hem. conida* (which evidently is not widespread) reproduced strongly on *T. patula*, whereas *Pa. microdorus* reproduced strongly on *T. lucida, T. signata* and *T. erecta* Spungold, but not

on *T. erecta* Aurantiaca or on *T. patula* cultivars. *Tr. teres* probably reproduced here on all and *Ma. curvata* on none of the *Tagetes* varieties tested. Table 6 shows that *Paratylenchus* sp. (undetermined, but different from *Pa. microdorus*) reproduces well on *T lucida*, *T signata* and *T erecta* Spungold, but not on *T. erecta* Aurantiaca and several *T. patula* cultivars.

Ectoparasites of these genera are thus not suppressed by all *Tagetes* cultivars. Therefore the host-parasite relationships between the nematode species and the *Tagetes* cultivars vary as widely as with other host plants.

3.7. Ditylenchus dipsaci

Three trials studied the reproduction of *D. dipsaci* (Kühn) Filipjev on *Tagetes* spp. and the corresponding symptoms of attack. In the first natural infection of *T. patula* was observed in soil infested with the onion race[1] of *D. dipsaci*. Nematodes of the same population were inoculated onto decapitated *T. patula* plants. In the third trial 4 races of *D. dipsaci* were inoculated onto *T. erecta* and *T. minuta*.

3.7.1. *Natural infection of T. patula grown in infested soil*

Two-weeks-old seedlings of *Vicia faba* L. were planted outdoors on potting soil infested with *D. dipsaci* in April 1965.

In September of that year the heavily infested plants were chopped and worked into the soil. Four weeks later the soil was cleaned of crude debris by sieving and mixed. A clay pot 25 × 25 × 10 cm was filled with this soil which contained about 3500 active specimens of *D. dipsaci* according to analysis of samples. A similar pot was filled with a steam-sterilized portion of this soil. On 15 October 1965 each pot was planted with 25 germinating seeds of *T. patula* at equal distances; both pots were placed in a greenhouse at 15 °C.

All seed germinated further but 5 weeks after sowing only 8 of the 25 plants showed definite symptoms of attack. The first pair of axillary shoots were stunted, and stem and leaf bases were swollen. The leaves were seriously malformed and chlorotic, and flower heads were also affected and distorted. In 2 plants the main shoots were affected and further growth was checked. In the other 6 plants the main shoots were normal, but affected side shoots occurred beneath a healthy canopy at a later stage. When infested plant parts were macerated in water, numerous *D. dipsaci*, evidently at all stages of development, could be seen escaping from the tissue with a dissecting microscope.

3.7.2. *Inoculation of D. dipsaci onto T. patula plants*

D. dipsaci of the same population as used before was inoculated onto decapitated *T. patula* plants. Sixteen seedlings of *T. patula* in the four-leaf stage, about

[1] The species *D. dipsaci*, as well as several other plant nematodes, comprise populations which differ in host affinity without demonstrating morphological differences. These populations are sometimes indicated as races, strains, varieties, biotypes or pathotypes. Oostenbrink (1968) proposed the term trophotypes, which indicates difference in feeding and reproduction.

4 weeks old, were decapitated above the first pair of leaves, leaving about 0.5 cm stem above these leaves. A superficial scratch was cut with a needle along two opposite sides of the stem as far as the base of the axillary buds. A glass tube 1.5 cm long of suitable width was loosely fitted around the stem and filled with loose cotton wool. A hundred fourth-stage larvae of *D. dipsaci* revived from the dry-stored population were added in a few drops of water. The cotton wool was kept wet by adding drops of water when necessary. Sixteen control plants were treated in the same way, except that nematode-free supernatant of the nematode suspension used for the inoculations was added.

A month later, 7 of the 16 inoculated plants showed symptoms of *Ditylenchus*. Axillary buds in these plants had extremely stunted shoots as described in the previous test, whereas control plants and apparently uninfested plants were normal. Three months later healthy shoots had grown through the infested parts in some of the infested plants, while these parts themselves seemed to be recovered from the attack; these stems soon formed lignified tissue with little parenchymatous tissue in which stem nematodes normally live.

Other plants continued to suffer seriously from the attack; the swollen infested stems became yellow-brown and were coated with a spongy layer of tissue with cracks in some places. Axillary buds had grown out but they were also severely infested and stunted. Swollen leaf-bases had brown necrotic areas and the entire shoot looked bushy. Infested parts of plants suffering from such old infestation were teased into small pieces and placed on a cotton-wool filter in a shallow dish with water. Two days later an average of 5377 *D. dipsaci* of all stages were extracted from about 0.8 g of tissue. The results show that development and reproduction of *D. dipsaci* in *T. patula* is possible.

3.7.3. *Inoculation of different races of D. dipsaci onto T. erecta and T. minuta*

T. erecta and *T. minuta* were tested for their host suitability and susceptibility to different races of *D. dipsaci* by decapitation and inoculation as described above.

The different inocula were 2 populations of the onion race (U_6 and U_9), one of tulip race (T_1) and one of rye race (R_9), supplied by Dr J. W. Seinhorst, Wageningen. The nematodes were revived from the dessicated state and of each race 0,5, 50 and 500 nematodes were inoculated onto the plant on 26 and 30 November 1966. There were 6 plants for each rate of inoculation for each *Tagetes* sp.

The plants were examined weekly for symptoms and after 3 months plant parts surrounding and including the site of inoculation or other parts showing symptoms were cut out and processed for nematode extraction by the cotton-wool-filter method. The glass tube with cotton-wool used for inoculation was also included in the extraction.

In some plants of *T. erecta* symptoms similar to young infections of *T. patula* appeared a month after inoculation, although only on plants inoculated with the R_9 and U_9 populations. Stems of infested shoots may still grow rapidly in length in *T. erecta* and may then manifest attack in terminal ends far above the site of inoculation (Plate IA).

Of all 18 *T. erecta* plants inoculated with R_9 only one specimen receiving 500 nematodes developed symptoms. Three months after inoculation 4825 nematodes were extracted from this plant. The total number of nematodes extracted from the other 17 plants inoculated with R_9 was only 108. This may be the remainder of the original inoculum and does not indicate reproduction. Infection and reproduction, therefore, was evident although only on one of the 18 inoculated plants.

Inoculation of *T. erecta* with 5, 50 and 500 nematodes of U_9 race produce symptoms in 1, 3 and 6 plants, respectively. The number of nematodes recovered from all 6 plants of each set were 0, 353 and 14250, respectively. Infection and reproduction, therefore, took place in several plants.

No infection or reproduction on *T. erecta* was achieved by inoculation of the T_1 and U_6 races.

In *T. minuta* none of the inoculations induced symptoms, except in one plant which had been inoculated with 500 U_9 nematodes. Only 275 nematodes were recovered, however, from this plant 3 months after the inoculation. The total number of all other U_9-inoculated *T. minuta* was negligibly small, 22, and was probably again residual inoculated nematodes.

3.7.4. *Discussion*

D. dipsaci is well known as a polyphagous parasite of aerial and underground organs of many plants. Oostenbrink (1961) reported serious damage of *T. patula* through attack by *D. dipsaci* under field conditions; in affected parts of the plant he found no nematodes but suggested that they could have been there before. Thus *T. patula* may be susceptible to damage by stem nematodes but its suitability for reproduction of the nematodes is not clear. Non-hosts or poor hosts of *D. dipsaci* may show swellings and distortions, but unlike good hosts the middle lamellae of cells in affected tissues of such non-hosts are not disintegrated and few nematodes, if any, are found in such plants (Seinhorst, 1956). The possibility that *T. patula* is a good host of *D. dipsaci* in which unfavourable environmental conditions arise in the course of further development of the infected plant can, however, not be excluded; high densities of populations may then build up on young plants and be eliminated later in the growing season.

The results of the trials in Sections 3.7.1. and 3.7.2. leave no doubt, that natural and artificially induced infection with the onion race of *D. dipsaci* caused severe damage and reproduction in some of the *T. patula* plants. Eight of the 25 plants in the first and 7 of the 16 plants of the second trial became noticeably infected. The high percentage of escape indicates that infection does not readily take place. Only young parenchymatous tissue seems to be infested and plants may overgrow the infection by forming healthy shoots with lignified tissue through the infected buds.

Some *T. erecta* plants were also succesfully colonized by 2 of the 4 nematode races tested and showed similar symptoms to *T. patula*, as well as infected and swollen shoot tips.

T. minuta did not allow reproduction or damage from *D. dipsaci* races. The

difference in response between *T. erecta* and *T. minuta* could be related to the anatomy of the stem.

The stem of *T. minuta* seedlings develops much lignified tissue at an early stage; parenchymatous tissue is then restricted to a thin layer of the cortex. In *T. erecta* parenchymatous tissue represents a large part of stems of older plants. *D. dipsaci* parasitizes parenchymatous tissue and the stems of *T. erecta* may be more suitable than those of *T. minuta* to the nematodes. The stem of *T. patula* is much the same as of *T. erecta*.

Perhaps *T. patula* and *T. erecta* may support a *D. dipsaci* population in the soil. Although *T. minuta* proved to be a non-host to all races tested, its suitability for suppressing stem nematodes in the soil may be doubted.

As with the cropping of various non-hosts of *D. dipsaci*, populations of this nematode in soils may not be affected enough by the culture of *T. minuta*. The same holds for the culture of *T. erecta* on soil with populations of the T_1 and U_6 races of *D. dipsaci*. The survival and reproduction of *D. dipsaci* on *Tagetes* spp. makes it doubtful whether the nematicidal principles found in these plants play a role in the elimination of the nematode.

Uhlenbroek & Bijloo (1958) demonstrated a nematicidal effect on *D dipsaci* and other nematodes of concentrates made from *Tagetes*. They also reported that root extracts were more effective than extracts from aerial parts. This difference in activity may be due to differences in concentrations of nematicidal thiophenes between plant parts. In parenchymatous stems and leaves of *T patula* and *T. erecta*, this concentration is at any rate too low to prevent *D. dipsaci* populations from increasing. Whether stems, petioles, veins or leaf tissuecontain the thiophenes is not known; there may be suitable and unsuitable tissues in the *Tagetes* leaf.

3.8. APHELENCHOIDES RITZEMABOSI

Two greenhouse trials were with *A. ritzemabosi* (Schwartz) Steiner to study reproduction on and damage to *T. patula*, *T. erecta*, and *T. minuta*. Seedlings were inoculated through the soil and aerial parts of plants directly.

3.8.1. *Natural infection of three Tagetes spp. in infested soil*

Young seedlings of *T. patula*, *T. erecta* and *T. minuta* with just unfolded cotyledons were transferred on 20 October 1965 to a 25 × 25 × 10 cm clay pot with steam-sterilized potting soil in such a way that cotyledons rested on the soil surface. For each *Tagetes* sp. 25 seedlings were planted in a row. A suspension containing 2500 nematodes was poured evenly along each row. Another pot with seedlings treated with nematode-free supernatant of the inoculum suspension served as control. The pots were then placed on sink trays and covered with a polythene-roofed glass cage. They were aerated by open slits at the top and bottom of the cage. Seedlings were sprayed with water from a mistifier each day.

Four days after transplanting, the cotyledons of all 3 *Tagetes* spp. were clearly infested. A few days later they were uniformly yellowish-brown and examina-

tion under the dissecting microscope revealed they were densely occupied with *A. ritzemabosi.* Four weeks after transplanting, all seedlings, treated and controls, had 2–3 pairs of leaves. The cotyledons of the treated plants had by then died but their dry remains were generally still attached to the plants. In controls cotyledons were still fresh. True leaves of *T. minuta* were entirely free of visible infestation. In *T. patula* and *T. erecta* the first pair of true leaves and occasionally one of the second pair had patches occupied by *A. ritzemabosi.* The growing points, however, were not affected so that growth continued normally. In earlier stages of attack light brown necrotic patches appeared under the leaves. The nematode probably penetrates through stomata, which is the common route of infection by this species. In later stages these patches darkened and were bordered by the larger veins (Plate IB). Numerous nematodes of all stages of development and deposited eggs were found in the mesophyll of these patches. No nematodes were observed to feed externally. Inoculated plants grew as much as controls.

Six weeks after seedlings had been transplanted, 20 plants of each *Tagetes* sp. were cut just above ground level and, including stems and remains of the cotyledons, were processed for nematode extraction with the cotton-wool filter.

Foliar nematodes extracted from 20 plants were 1559 and 1600 on *T. patula* and *T. erecta,* respectively. *T. minuta* yielded 98 nematodes only; since all leaves of this species were healthy, it was assumed that these nematodes originated from the dried remains of the cotyledons. The number of nematodes recovered from the plant material of *T. patula* and *T. erecta* was about 60% of that inoculated into the soil 6 weeks earlier. Nematodes in the leaves, however, undoubtedly formed a breeding population, originating from specimens which successfully established themselves. Survival of the nematodes in the soil grown with *Tagetes* was not assessed.

3.8.2. *Inoculation of three Tagetes spp. with A. ritzemabosi*

Plants of *T. patula, T. erecta* and *T. minuta* with 4 leaves ,about a month old were grown in pots and inoculated with *A. ritzemabosi* on 21 October 1965. On the apical buds was placed a piece of moist cotton wool containing nematodes. The plants were inoculated with 0, 50 or 200 *A. ritzemabosi;* for each *Tagetes* sp. there were 6 plants for each rate of inoculation. The nematodes originated from a culture on *Doronicum caucasicum* Bieb.; they were extracted from leaves a day before inoculation. The pots with plants were placed on sink trays filled with a shallow layer of water, covered with a glass cage roofed with polythene sheet and sprayed with water each day as above.

After 2 weeks inoculated plants of *T. patula* and *T. erecta* had light brown, indistinctly bordered patches on the leaves. Leaves of *T. minuta* remained healthy.

When affected leaves were stained with lactophenol-cottonblue numerous nematodes and deposited eggs were found in these patches .More and larger patches were found on plants inoculated at higher rates .A few days later these patches developed brownish decay, and shoots and affected leaves died rapidly

through secondary rot. Nematodes were not counted through loss of the material.

3.8.3. *Discussion*

Most plant-parasitic species of *Aphelenchoides* are parasites of aerial parts, including leaves and buds, of higher plant. Some species, however, are known to be root parasites of higher plants and it is peculiar that one of them, *A. tagetae* was reported by Steiner (1941) from inside the roots of *Tagetes* sp. *A. besseyi* was reported by Sher (1964) to cause necrotic patches on leaves and flower heads of *Tagetes* sp. in Hawaii; he did not mention whether the nematode actually feeds and reproduces on the *Tagetes*. Our trials were with the well known foliar nematode *A. ritzemabosi*, which may live inside and outside the leaves of many plants. Nothing was known about its relation to *Tagetes* spp.

It is evident from our trials that *A. ritzemabosi* penetrates and reproduces in leaves of *T. patula* and *T. erecta* and causes typical leaf lesions as in other host plants. Reproduction is vigorous in the cotyledons but nematodes are limited in the actual leaves and the plants do finally not suffer much. *T. minuta* is not a good host; feeding and perhaps reproduction is only possible in the short-lived cotyledons which may then suffer; plants beyond the cotyledon stage do not allow reproduction or show symptoms. *T. minuta* may be useful to combat *A. ritzemabosi* on infested soil, though the application of antagonist crops or rotation in general may not be at all necessary or applicable for the control of this nematode.

CHAPTER 4

INTERPRETATION OF THE *TAGETES* EFFECT

4.1. Introduction

The foregoing data leave no doubt that certain species of *Tagetes* suppress certain species of plant nematodes unusually strongly. This can, therefore, be called a special *Tagetes* effect, consisting of a complex of factors which has to be analysed. The results in the previous chapter are diverse and even with published data, cannot completely explain the mechanism or mechanisms.

Therefore an attempt is made to analyse the factors and to demonstrate them by further trials and observations.

The *Tagetes* effect manifested itself most clearly with polyphagous endoparasitic root-infesting nematodes, such as *Pratylenchus* and *Meloidogyne* spp. The following investigations therefore deal mainly with these genera.

The *Tagetes* effect on such nematodes may be visible a) before penetration of the plant, i.e. exophytic or exoradicular effect, b) during penetration at or near the surface of the plant, c) inside the plant after entry, i.e. endophytic or endoradicular. These 3 sites are chronological in any infection process and are therefore comparable to the periods before penetration, during penetration and after penetration by a pathogen.

4.2. Exoradicular effects

Possible exoradicular effects may concern hatching of eggs, motility and tropism of active stages, or damage and kill.

No special observations were made of the influence of *Tagetes* exudates on hatching of nematode eggs. Direct influence of *Tagetes* exudates on the hatching of eggs is possible. The main expression of the *Tagetes* effect, however, is a drastic suppression of the population of active nematodes and our observations were confined to them. There were 3 tests on motility and orientation, and 3 on damage and kill, all with *P. penetrans.*

4.2.1. *Motility and orientation*

a. Observation of *P. penetrans* on agar plates

Four adult females of *P. penetrans* were placed on a Petri dish containing 2% Hoagland nutrient agar equidistant and 10 mm from a small cylindrical cage sunk into the agar and enclosing a growing seedling of *T. patula* (cf. Staar, 1959; Rode & Staar, 1961). Plates with a seedling of wheat and of red clover and unplanted plates were available as controls. The cage, 5 mm wide and of perlon gauze of 40 μ mesh, permitted root exudates to diffuse out but kept plant roots inside. Seedlings a week old were transferred into the agar and nematodes were added one or 2 days later, when the roots had started to grow. The plates were kept at room temperature.

With transparant paper fixed under the dish the positions of the nematodes were determined regularly and transferred to a sheet of graphic paper on which the nematode tracks were reconstructed. Tracks are wavy lines but in the ichnograms obtained these waves were straightened out. The nematodes' positions were determined at 10 minutes intervals for the first hour or 2 and after 24 hours.

Nematodes in the dishes with *T. patula* were constantly active. The direction of movement seemed to be quite random and changed at any moment. The distances covered per ten minutes varied from 3 to 28 mm. No definite relation between speed of movement and distance to the seedlings was noticed; nematodes moved with great speed or very slowly in close proximity to the seedling as well as when they were at a greater distance. Nematodes may approach within 0.5 mm of the cage wall or may even reach it, but may then turn away. Usually, however, once they entered the cage they were detained there. Twenty-four hours after placing the nematodes on the agar, large sections of the surface were densely covered by tracks and nematodes could be traced back while they were still moving. The ichnograms obtained from the dishes with seedlings of wheat and red clover indicate that *P. penetrans* behaves similarly to these good hosts.

These results indicate that *T. patula* lacked effect on the orientation of *P. penetrans*, since the nematode was neither attracted nor deterred by the plant. Motility was also neither increased nor decreased in this experiment.

b. Migration of *P. penetrans* and other nematodes into cylinders of soil planted with *Tagetes*

Glass cylinders, 2.2 cm diameter and 8 cm high filled with nematode-free sandy soil, were placed on a 9 cm deep layer of nematode-infested sandy soil separated only by thin nylon gauze with mesh of about 40 µ. The nematode-free soil was heated before the experiment; it did not harbour any plant parasitic nematodes but a few saprozoites were present. The initial density of the infested soil was per 100 ml: 510 *P. penetrans* + 480 ectoparasitic nematodes (355 *Tyl. dubius* + 115 *R. robustus* + 10 *Trichodorus* sp.) + 1695 saprozoic nematodes.

Series of 15 cylinders were planted with 5-days old seedlings of *T. patula*, of red clover or left unplanted on 6.9. 1968. After 6 and 20 days the 5 cylinders of each plant treatment were examined for nematodes, namely the soil by flotation and the roots by the funnel-spray technique and by staining and microscopy. The results are recorded in Table 14.

Table 14 shows, that the number of nematodes in the cropped cylinders was only slightly higher than in the fallow cylinders. The fallow cylinders contained 7 and 4 *P. penetrans* per 150 ml of soil after 6 and 20 days respectively, whereas the average density of the soil layer underneath was 510 per 100 ml. The numbers in the cropped cylinders were slightly higher than in the fallow ones, but there was no strong concentration effect and soil and roots in the *Tagetes* tubes contained more, rather than less *Pratylenchus* than the red clover tubes. The same is true for the ectoparasites (mainly *Tyl. dubius*) and for the saprozoic nematodes.

TABLE 14. Number of nematodes in soil-filled cylinders without a plant, with *T. patula* or with red clover, placed in contact with a naturally infested soil. Initial density of infested soil per 100 ml: 510 *P. penetrans* (P) + 480 ectoparasitic nematodes (E) + 1695 saprozoic nematodes (S). Planting date 6.10.1968. Total nematode numbers per 5 cylinders, soil + roots, determined 6 and 20 days after planting. Between brackets: number of nematodes in roots only.

Plant treatments	P after:		E after:		S after:	
	6 days	20 days	6 days	20 days	6 days	20 days
No plant	0	7	0	3	58	98
T. patula	1 (1)	26 (16)[1])	0	25	42	267
Red clover	0 (0)	3 (2)[2])	0	0	127	51

[1]) + 0 eggs
[2]) + 22 eggs

c. Aggregation of *P. penetrans* around *Tagetes* growing in infested soil

On 6 September 1968 three seedlings of *T. patula* were planted in a row 8 cm apart in a 9-cm-thick layer of sandy soil infested with *P. penetrans*. There were 3 such rows with *T. patula*. Three similar rows were made in the same soil with seedlings of red clover. After 7 days, 21 days and 41 days cores of soil about 2 cm wide, volume about 36 ml, were taken exactly from the spot where the seedlings were growing, also at distances of 2 and 4 cm from the seedlings, therefore exactly between the seedlings in the row. There were 9 sampling points in each row. Nematodes were counted per core in the soil as well as in the roots if present. The results are in Fig. 5 or described below.

Seven, 21 and 41 days after planting the average level of infestation in the soil of the red clover rows was 260, 236 and 249 *P. penetrans* per 36 ml. There was no noticeable concentration of *P. penetrans* around the red clover roots at the first nor at the last sampling date, as can be seen in Fig. 5A. The corresponding average infestation levels in the soil of the *T. patula* rows were 200, 262 and 178 per 36 ml. Also here no noticeable concentration around the *Tagetes* roots occurred at the first sampling date, whereas at the last sampling date the population around the roots was slightly lower at the site of 2 of the 3 plants than between the plants. It is questionable whether this suppressive effect must be considered significant from these figures, because no effect was noticeable around the third plant. The effect was at any rate not yet marked 41 days after planting *T. patula*.

More instructive are the figures on the population inside the roots. It appears that only a few of the nematodes around the roots penetrate, that original penetration after seven days is not less in *T. patula* than in red clover, but that the population grows and reproduces in red clover whereas it remains stable or drops without reproduction in *T. patula* (see Section 4.3 and 4.4).

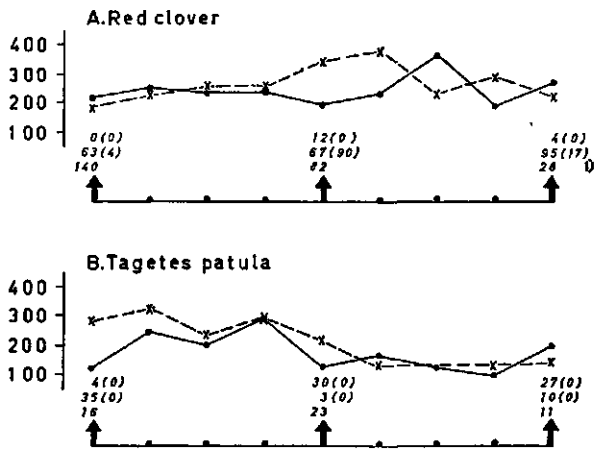


FIG. 5. Concentration of *P. penetrans* in soil around and in the roots of red clover (A) and *T. patula* (B)
Abscissa: linear sketch of row with plants indicated by arrows 8 cm apart and other sampling sites indicated by dots 2 cm apart.
Ordinate: number of *P. penetrans* per core of 36 ml content at different sites and at 2 different times, namely 7 days (broken lines) and 41 days after planting (solid lines). Graphs for 21 days after planting are omitted.
Cursive figures above arrows:
Number of *P. penetrans* in root system of corresponding plant, from top to bottom after 7, 21 and 41 days. Between brackets number of eggs in addition; this was not de termined 41 days after planting. 1) = many finer roots lost for nematode extraction; roots for extraction consisted mainly of tap root and main laterals.

4.2.2. *Damage to or kill of nematodes outside the root by toxic substances*

Root substances can theoretically exert an exoradicular nematicidal effect as soluble or gaseous diffusates or as untransportable remains of excised or shed root tissues. Three experiments traced the possible existence of such an effect: by percolates from *Tagetes* soil, by water cultures of *Tagetes*, and by nematicidal effects in the soil after uprooting *Tagetes*.

a. Effect of percolates from *Tagetes* soil on *P. penetrans* in vitro

Percolates to study possible soluble nematicides were obtained by the well known method for collecting host root diffusates for hatching tests with *Heterodera* cysts (Widdowson, 1958).

Clay pots were filled with about 500 ml washed sand, saturated with Hoagland plant nutrient solution and planted with a two-week-old seedling of *T. patula;* other series were planted with red clover and with apple or left unplanted; finally one series of pots was treated with distilled water instead of Hoagland solution and also left unplanted. To all pots water was regularly added until the sand was just saturated; at two-week intervals a fixed amount of Hoagland

solution was added to all pots which had received it since the beginning.

The pots were placed in a greenhouse at 18°–20°C. Six weeks after planting, percolates were obtained by adding 40 ml of water to the saturated sand of each pot. The water dripping from the perforated pot bottom was collected and percolated again through the same pot twice. The finally caught percolates of all pots of a series were joined in a bottle and stored in a dark room at 2°–3°C. The percolate obtained in this way probably comprised soluble materials in about the same concentration as the soil water *in situ*.

The five different percolates were tested on *P. penetrans* in watch glasses; 2 ml of each percolate was added to a watch glass with 100 adult females of *P. penetrans*, with 3 replicates per percolate. The activity or mortality was determined after 1, 2, 7 and 21 days. There was no difference between the 5 treatments. Percolates from the *T. patula*, red clover, apple and Hoagland solution pots did not have any effect on activity or mortality of *P. penetrans in vitro*, compared with pots of only water, and the figures are therefore not recorded.

b. Effect of *Tagetes* water cultures on inoculated *P. penetrans*

The stem of week-old seedlings of *T. patula*, *T. minuta* and apple were surrounded by cotton wool and fitted into a short glass tube, whereas the roots were placed in tubes with Hoagland nutrient solution for 2 weeks.

Then the root systems of twelve well growing seedlings of each plant were firmly surrounded by a foam plastic stopper which fitted into a perlon gauze bag, and this in turn into a 100 ml bottle. The bottom of the bag was about 2 cm above that of the bottle. The bottles were each filled with 60 ml Hoagland nutrient solution. A week later 100 adult females of *P. penetrans* were inoculated onto the bottom of each bottle thus securing continuous contact of nematodes with fresh root exudates whereas direct contact of nematodes with roots was avoided. There were also control series with Hoagland solution without a plant and with distilled water without a plant. The number of living specimen was determined in 3 bottles of each series after 10, 15, 20 and 25 days and the volume of nutrient solutions in each bottle was adjusted to its original level at the same moments. The results are summarized in Table 15.

TABLE 15. Survival of *P. penetrans* in watercultures of *T. patula*, *T. minuta* and apple. A hundred adult females inoculated in each waterculture. Number of living nematodes as recorded 10, 15, 20 and 25 days after inoculation. Figures are means of 3 replicates.

	Number of days after inoculation			
	10	15	20	25
T. patula	93.7	87.3	86.0	81.3
T. minuta	91.3	87.7	84.0	80.3
Apple	91.0	90.3	87.0	84.0
Control; Hoagland nutrient solution	93.7	85.3	87.7	87.7
Control; distilled water	90.0	89.3	91.3	88.0

In all bottles there was a slight decrease in count of living *P. penetrans* in the course of time. After 25 days numbers in the plant cultures were slightly lower than in the fallow controls. However, these differences were not statistically significant. *Tagetes* suppressed the nematodes too little to explain much of the normal *Tagetes* effect. Moreover the effect does not seem to be specific as apple demonstrates some suppression as well.

c. Nematicidal effects in soil after uprooting *Tagetes*

Ninety tubes were filled with 80 ml of partially sterilized peaty sand. Series of 18 tubes were grown with a seedling of *T. patula*, *T. minuta*, red clover and apple, and one series was left unplanted as control.

Eight weeks after planting all root systems were individually removed and weighed, the soil was sieved to remove remaining root particles as quantitatively as possible and transferred into small jars. On the same day a natural population of 2500 *P. penetrans* comprising different stages was inoculated into each jar. The soil was extracted 10, 20 and 30 days after inoculation and nematodes were counted. The results are given in Table 16.

TABLE 16. Survival of *P. penetrans* inoculated in tubes with peaty sand soil pretreated with planting and subsequent clearing of all roots of *T. patula*, *T. minuta*, red clover and apple. Inoculated number of nematodes: 2500 per tube. Number of nematodes 10, 20 and 30 days after inoculation. Figures are means of 6 replicates.

Soil pretreatment with:	Number of days after inoculation		
	10	20	30
T. patula	1250	1073	1182
T. minuta	1025	1200	[1])
Red clover	1166	1241	1173
Apple	833	902	952
Control (no preplant)	1635	1521	1631

[1]) Extreme low observations due to drying out of soil, rejected.

Statistical analysis shows, that there were no significant differences in numbers of nematodes 10, 20 and 30 days after inoculation. Effects were, therefore, already noticeable after 10 days. From the fallow soil (control) about 65% of the inoculated population was recovered after 10 days and this percentage was the same after 30 days. The soil cultured with *T. patula*, *T. minuta* and red clover suppressed the population significantly more; there were no significant differences between the 3 plants. The percentage survival, however, was significantly lower in the apple soil, namely about 36%. Thus *T. patula* and *T. minuta* suppressed nematodes and the effect was manifested in the soil immediately after removing the plant. The results indicate, however, that this effect is not specific for *Tagetes*, because it was as strong in red clover soil and even stronger in apple soil. So it does not explain the special effects of *Tagetes*.

4.2.3. *Discussion*

The observations and experiments in the last 2 sections make it reasonably certain that the exoradicular effects of *T. patula* on *P. penetrans* are not markedly different from the effects of suitable host plants, and therefore that exoradicular influences do not account for much of the specific *Tagetes* effect.

Whether growing *Tagetes* plants influence the hatching of plant nematode eggs is uncertain. The only data available are from literature. They concern mainly *Heterodera* species. Root exudates of *T. minuta* neither stimulated nor delayed hatch of encysted eggs of *H. rostochiensis* and of *H. schachtii* (Hesling et al, 1961). Omidvar (1961) found that root diffusate of *T. minuta, T. signata* and *T. florida* had neither hatching nor nematicidal effect on encysted eggs of *H. rostochiensis*. Hamlen & Bloom (1968) studied the hatching of amino acid (AA) and non-amino acid (non-AA) fractions of root exudates of widely different plants, including *T. patula* on *M. incognita* and found that, in general, the AA fractions neither stimulated nor inhibited hatch, while the non-AA fractions had an inhibitory effect. Under certain soil-moisture conditions, however, hatch of *M. incognita* eggs in non-AA fractions of *T. patula* was better than in those of other plants. Increased hatch of *Pratylenchus* and *Meloidogyne* eggs owing to *Tagetes* exudates could still be part of the cleaning process to which *Tagetes* sp. treat the soil. The possibility of such stimulation as observed in several host-nematode relationships remains open, but it is at any rate not essential in explaining the specific *Tagetes* effect because the characteristic effect of *Tagetes* is the suppression of population density of active nematodes.

Our observations on motility and direction of movement indicate that if there is any attraction toward plants, *P. penetrans* was at most only slightly attracted or migrated only slightly to any plant, *Tagetes* sp. or suitable hosts, and also that only a small part of the soil population was successful in penetration.

Both points are important aspects of the host-nematode relationship in general and the *Tagetes* effect in particular. Invasion or penetration is dealt with in Section 4.3, motility and hostfinding are scrutinized further here. Literature leaves little doubt, that plant nematodes are generally stimulated by host plants and that they aggregate around growing roots. Diffusable root exudates stimulate nematode activity and perhaps direct nematode movement towards the roots. Such stimuli may be specific, as with potato root exudate which activates encysted larvae of *Heterodera rostochiensis*. In other host-nematode relationships less specific or even unspecific stimuli are noticed, such as unidentified bacterial products (Bergman & Van Duuren, 1959a, b), electric potential (Jones, 1960), and carbondioxide (Klingler, 1959, 1961, 1963; Bird, 1960; Johnson & Viglierchio, 1961; Edmunds & Mai, 1967). But even with marked stimulation, the question is open whether there is a concentration gradient of the stimulating factor in the soil which directs the nematodes towards the roots (Kämpfe, 1960; Chen & Rich, 1963), or whether the activated nematodes move at random until they meet a rootlet and are then detained there (Kühn, 1959; Sandstedt *et al.*' 1961; Staar, 1959). Stimulation without further direction of the nematodes' movement, therefore without attraction, may also lead to aggregation of nema-

todes around the roots, though probably at a slower rate. Literature, therefore, is not pertinent about the exact mechanism of host finding by nematodes.

Observations of *P. penetrans* in agar with growing *T. patula* and red clover did not reveal any influence of the plants on motility and direction of movement. Table 14 indicates, that *P. penetrans* as well as ectoparasites and saprozoic nematodes were probably attracted by or, more likely, detained around or on roots of *T. patula* and red clover, though only slightly in both cases. This is further stressed by the data of Fig. 5, which indicate that both plants are originally infested to the same degree but that the bulk of the population remains undisturbed outside the roots for at least 41 days.

In earlier trials, described in Section 3.2. effective *Tagetes* treatments were observed on *P. penetrans* and *P. crenatus* in sandy soils (Tables 1, 2 and 3) but also uneffective treatments on *P. neglectus* and *P. thornei* in clay soils (Tables 4 and 5). In field trials, however, *P. neglectus* and *P. thornei* were suppressed, even in clay soils, though less effectively and more slowly. Ineffective *Tagetes* treatments on some clay soils with otherwise susceptible nematodes may be due to a failing communication between the *Tagetes* and the nematodes. Effective *Tagetes* treatments finally remove the nematodes from the soil whereas uneffective treatments leave the populations completely undisturbed though active. This may indicate a mechanism which reaches the nematodes outside or on the surface of the roots in the effective treatments, a mechanism which fails in the ineffective treatments, probably due to environmental conditions, and which may be identical with the CO_2 concentration or gradient mentioned before.

The above possible explanation relates to a failure in a host finding mechanism. Another possibility may be a matter of host selection once the nematode, by whatever mechanism or mechanisms – arrives at the plant surface. It can be speculated that a certain nematodes species is either selective or unselective with regard to penetrating prospective host plants. In this context, the data in Tables 2, 3, 4 and 5 suggest a possible lack of selective ability of *P. penetrans* and *P. crenatus* with regard to ingress into *Tagetes* spp. or a good host, wheat, whereas *P. neglectus* and *P. thornei* showed a distinct preference in invading wheat above *Tagetes* spp. Indiscriminate penetration into plants may lead *P. penetrans* and *P. crenatus* to subjection to adverse effects within *Tagetes* root tissues or thrive within wheat roots, whereas as a consequence of discriminate behaviour in the host selection, *P. neglectus* and *P. thornei* may largely escape kill within *Tagetes* roots.

These possibilities were not specially further investigated and remain, hence, speculative.

Damage or kill of nematodes outside the root by toxic substances does not seem to be the most essential part of the *Tagetes* effect, though it may play a role. Experiments by Oostenbrink, Kuiper & 'sJacob, (1957) and by ourselves (Chapter 3) showed that *Pratylenchus* densities in the roots of *Tagetes* as well as in surrounding soil was exceptionally low after some time. Several authors (Wallace, 1963; Rohde, 1965) concluded that *Tagetes* exudes nematicidal substances into the soil, but this need not be so. Perhaps the nematodes penetrate

the roots of *Tagetes* and are killed inside, so that the plant rids the soil gradually of the nematode population. Another possibility is, that Tagetes as well as other host plants exert stimuli which activate the whole soil population until exhaustion whereas only few nematodes succeed in penetration. This would mean: unspecific indirect suppression of the soil borne population combined with specific suppression of the penetrated specimens (Sections 4.3. and 4.4.). Our data do not indicate a strong specific exoradicular effect. Percolates from *T. patula* soil did not kill *P. penetrans in vitro*; this accords with Omidvar's (1961) observation about the non-effect of percolates from other *Tagetes* spp. on *H. rostochiensis* .*Tagetes* water cultures demonstrated only a very slight nematicidal effect on inoculated *P. penetrans* in comparison to unplanted cultures (Table 15). *Tagetes* soil does exert a distinct nematicidal effect for some days after the roots have been removed (Table 16). But this effect is also not specific and not very strong, for red clover was as effective as and apple even more effective than *T. patula* and *T. minuta*. An essential difference between *Tagetes* spp. and good host plants is that in the latter plants this suppression is compensated by nematode reproduction. The suppressive effect seems to be general and could be associated with decay in general of organic plant remains in the soil and not with diffusates of living roots. It could also be of general importance. It could perhaps be related to the unexplained fall in population after maturing of the host crop as demonstrated by Oostenbrink (1966) and by Sharma (in preparation) for *Tylenchorhynchus dubius* and to the suppressing effect of organic additives of plant origin on plant nematode populations (Linford *et al*, 1938; v. d. Laan, 1956; Mankau, 1962; Johnson *et al*, 1967; Singh & Sitaramaiah, 1967).

It could be of significance in explaining part of the *Tagetes* effect, because this exoradicular suppression, specific or not, adds to the decline of the population. But the fact, that some ectoparasitic and most saprozoic nematodes survive and thrive in *Tagetes* soils indicates that this effect is not determinant for the survival and thrift of a nematode population.

In summary, exoradicular effects of *Tagetes* on hatching of eggs are unknown but possible, aggregation of *Pratylenchus* spp. around the roots is likely to occur and *Tagetes* induces an unspecific weak nematicidal principle into the soil which remains effective for some days after the growing plants have been removed. Exoradicular effects are apparently not the main part of the specific *Tagetes* effect. Nematodes penetrate *Tagetes* roots as well as roots of a suitable host, though development inside the roots is quite different. Penetration of and development in *Tagetes* roots will be studied more extensively in the following Sections 4.3. and 4.4.

4.3. Effects on penetration

Specific *Tagetes* effects on penetration of plant nematodes could be due to an accumulation of insoluble or undiffusable chemicals, to a special biosphere on the roots, or to characters of the epidermis and cortex which restrict penetration. None of these mechanisms have been shown to be specific in *Tagetes*. The overall

effect on penetration is measured in trials by the degree of penetration of nematodes, in this case *P. penetrans* and *M. hapla*.

General knowledge about population dynamics and introductory observations showed that several factors influence the numbers of nematodes in plant roots, apart from specific characters of the nematode and the plant. They have to be taken into account when nematode densities in roots are used to measure the degree of penetration, and are listed as follows:

a. The developmental stage of the inoculum. In *Pratylenchus* and other migratory species several stages can penetrate roots, though one may be more effective than the other. Gravid females may deposit eggs soon after penetrating the roots and larvae hatched from such eggs may complicate the picture. These difficulties do not exist if *Meloidogyne* spp. are studied, because only the vermiform second-stage larvae are infective.
b. The amount of active inoculum or the population density in the soil. Penetration may be density-dependent.
c. The amount of roots or potential penetration sites, and the damage caused by the nematodes to the growing roots.
d. The exoradicular influence of the roots on the population around the roots.
e. Soil type, moisture conditions, temperature and other environmental factors.

4.3.1. *Trials with M. hapla and P. penetrans*

Penetration of *M. hapla* and of *P. penetrans* larvae into 3 *Tagetes* spp. and a control plant (tomato for *M. hapla* and red clover for *P. penetrans*) was measured under set conditions with one nematode density. The same trials also studied the

TABLE 17. Average numbers of penetrated larvae in well established root systems of three *Tagetes* spp. and of control plants grown in tubes with 8 ml washed sand or sand-perlite mixture 1 and 2 weeks after inoculation. Between brackets number of larvae recovered from the sand or mixture around the roots.

1	2	3	4	5	6	7
	1000 *M. hapla* larvae per tube; mixture; 6 replicates (cf. Table 18)		500 *P. penetrans* larvae per tube; sand; 4 replicates (cf. Table 19)		250 *P. penetrans* larvae per tube; mixture; 3 replicates (cfl. Fig. 6A)	
Plant	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks [1])
T. patula	66 (263)	53	7 (354)	6	84	51
T. erecta	25 (409)	98	71 (188)	76	217	117
T. minuta	34 (276)	44	19 (224)	47	170	133
Red clover			40 (154)	93	215	178
Tomato	51 (392)	47				
No plant	– (545)	–				

[1]) This series was transplanted into nematode-free soil 1 week after inoculation.

development of penetrated larvae with time and they are fully described in Section 4.4. The number of penetrated larvae 1 week and 2 weeks after inoculation for all 3 trials are summarized in Table 17.

4.3.2. *Discussion*

Table 17 indicates that the penetration of *M. hapla* into its good host, tomato, is not more than into the *Tagetes* spp. It appears also that only a small proportion of the larvae inoculated around the roots succeeds in penetrating. Table 18 shows that nearly all the other larvae disappeared from the soil within 2 weeks and that the penetrators started developing successfully in tomato, unlike in *Tagetes*.

A similar situation is found for *P. penetrans* on red clover and the *Tagetes* spp. The percentage penetration is higher, though varying between experiments. The only significant difference between plant species is here, that the number of penetrators in *T. patula* is lower than in the other *Tagetes* spp. and red clover. This was not found in earlier experiments (cf. Fig. 5 and Table 14), but it may be a real difference because *T. patula* suppresses populations of *P. penetrans* more than the other *Tagetes* spp.

Degree of penetration, therefore, may play a role in the effectiveness of *Tagetes* spp. with respect to *P. penetrans*, but it does not explain the *Tagetes* effect in general. *P. penetrans* enters *T. erecta* and *T. minuta* to the same degree as red clover. *M. hapla* enters all three *Tagetes* spp. as effectively as tomato.

The varying and often low percentage of penetrating larvae could be due to the experimental conditions and the inoculum density. The only experiment with a high percentage penetration is recorded in Table 17 (Cols 6 and 7): 33, 89, 70 and 88 for *T. patula*, *T. erecta*, *T. minuta* and red clover, respectively. It is, however, probable from other trials and from experience with other endoparasitic nematodes, that the percentage penetration in natural infestations is also often low.

4.4. Endoradicular effects

Endoradicular effects were studied with *M. hapla* and *P. penetrans*. Observations were on survival and development of the nematodes, on the presence of nematicidal principals in *Tagetes* and on histological reactions in *Tagetes* tissues.

4.4.1. *Survival and development of M. hapla in the roots*

One-week-old seedlings of *T. patula*, *T. erecta*, *T. minuta* and tomato were planted in small tubes filled with 8 ml of a 1:1 perlite-sand mixture saturated to holding capacity with Hoagland nutrient solution. Until inoculation with nematodes, 1 ml nutrient solution was added weekly per tube and water was supplied several times per week whenever necessary to maintain moisture at about the original level.

Seven weeks after planting the seedlings, all tubes were inoculated with about 1000 infective second-stage larvae of *M. hapla* that were freshly extracted from

galled roots of carrot, *Daucus carota* L., and suspended in tap water free from carrot root sap. After 1, 2, 3 and 4 weeks, 6 tubes of each plant were processed to determine the number of larvae left in the growth medium and the number and developmental stage of rootborne nematodes. For this purpose the root systems were washed free from adhering particles of the medium, fixed in FAA and stained in 0.05% cottonblue-lactophenol; nematodes in the growth medium were extracted by the cottonwool filter method and collected after 48 hours. Whole counts were made of nematode numbers. Also the number of root tips and the number of nematodes in infected root tips were determined. For comparison, tubes without plants were included in the test. The results are summarized in Table 18.

TABLE 18. Entry, survival and development of *M. hapla* larvae in three *Tagetes* spp. and tomato over four weeks.
Plants grown 7 weeks in tubes with 8 ml perlite-sand mixture and inoculated with 1000 larvae per tube. Nematodes in the roots and in the growth medium counted after 1, 2, 3 and 4 weeks and classified according to development; average numbers from 6 replicates.
L = undeveloped second-stage larvae; D = sexually undetermined larvae developed beyond the infective stage; ♂ = males within larval skin; ♀ = mature females; × = observation lacking.

1	2	3	4	5	6	7	8	9	10
Plant	weeks after inoculation	nematodes in or attached to roots					nematodes in the growth medium	number of infested root tips	nematodes per infested roottip
		L	D	♂	♀	total of all stages			
T. patula	1	66				66	263	22.5	2.9
	2	53				53	×	25.4	2.1
	3	50				50	7	25.2	2.0
	4	48				48	6	18.3	2.6
T. erecta	1	25				25	409	6.2	4.1
	2	98	2.0			100	×	18.5	5.4
	3	63	4.3			67	28	16.8	4.0
	4	65	2.8	0.2		70	7	13.8	5.1
T. minuts	1	34				34	276	7.8	4.3
	2	44				44	×	12.7	3.5
	3	22				22	72	7.2	3.0
	4	41	0.3			41	26	15.0	2.7
Tomato	1	51	0.2			51	392	6.7	7.6
	2	47	6.5	0.3		54	×	10.0	5.4
	3	42	31.0	2.0	1.2	76	7	13.6	5.6
	4	32	14.0	4.2	1.8	52	5	8.7	5.9
Control (no plant)	1						545		
	2						×		
	3						163		
	4						121		

The total numbers of nematodes (Col. 7) per root system of each plant species was not significantly different at the 4 times of observation. The average number throughout the observation period per root system of *T. patula*, *T. erecta*, *T. minuta* and tomato was 54, 66, 35 and 57, respectively.

Analysis of data from Columns 9 and 10 showed that there were also no significant differences in the number of infected root tips and the number of nematodes per infected root tip at the different moments of evaluation within each plant species. However, the average number of infected root tips per root systems in *T. patula* (22.8) was significantly higher at the 1% level than in the other plant species tested; no significant differences were found between average numbers in *T. erecta* (13.8), *T. minuta* (10.7) and tomato (9.6). The numbers of nematodes per infested root tip as a mean of all 24 root systems also differed between plant species. For *T. patula* this number (2.4) was significantly lower at the 1% level than for *T. erecta* and tomato, and for tomato (6.1) it was significantly higher than for *T. erecta* (4.7) and *T. minuta* (3.4); differences between the last two numbers were not significant. Thus, more nematodes had penetrated per infested root tip in tomato than in the *Tagetes* spp., though the total numbers per plant were not different. In tomato, however, larvae developed readily after entry and after 3 weeks females bearing egg mass could be detected. Development was practically blocked in *T. patula* and *T. minuta* and distinctly retarded in *T. erecta;* sexually differentiated larvae were present 3 weeks after inoculation in *T. erecta;* these were all males.

The number of larvae remaining in the growth medium (Col. 8) declined rapidly in all tubes. In the tubes without plants, however, their number remained higher and decreased much slower. When logarithmically transformed values of these numbers are plotted against time, the inclination of lines indicates no significant differences in the rate of fall of populations under *T. patula*, *T. erecta* and tomato. The respective linear equations for *T. erecta*, *T. minuta* and the unplanted controls were found to be: $\hat{y} = 3.1986–0.5857X$; $\hat{y} = 2.7986–0.3357X$ and $\hat{y} = 2.9471–0.2264X$. The decline with *T. erecta* was significantly greater than with *T. minuta* at the 5% level and with the control at the 1% level. The difference found between rate of decrease in the *T. minuta* tubes and in the unplanted tubes was not significant.

Healthy root tips were present in excess in all plant species tested, so availability of suitable sites of entry was not a limitting factor in the larval invasion. Soon after invasion, root tips generally responded with a light-brown discoloration of infested tissue in *Tagetes* spp. as well as in tomato. A week after inoculation syncytia and slight swelling of infected root tips could be detected, except in *T. patula* which may possibly show them after the second week or later. In all *Tagetes* spp., however, syncytia if present were much smaller and swelling of the infected root tip was less pronounced than in tomato. No root tips were found which showed symptoms of infestation without larvae inside.

The above results indicate that *M. hapla* penetrated *Tagetes* spp. and tomato only within the first week after inoculation, and that all larvae which had made successful entry survived throughout the 4 weeks period in roots of these plants.

The rate of entry into *Tagetes* was the same as into tomato. The number of root tips infected and the number of nematodes per root tip differs between plant species. Differences in host suitability, however, is convincingly demonstrated by the development of penetrated larvae. In tomato, adult males were present after 2 and adult females after 3 weeks. In *T. erecta* no females were formed, but adult males were present after 4 weeks. No development was noticed in *T. patula* and hardly any in *T. minuta*. The results agree with those of earlier experiments (Tables 8 and 9) in which *T. erecta* was the only *Tagetes* sp. in which *M. hapla* multiplied at all.

At an inoculum density of about 1000 larvae of *M. hapla* under the experimental conditions only about 5% of the number of inoculated larvae entered the plants despite the abundance of active larvae around the roots during the first and perhaps the second week after inoculation and also despite the abundance of suitable sites of entry. Thus, the majority of nematodes seemed unsuccessful in penetrating the roots and lost their activity and infectivity within one or two weeks. This is in agreement with observations by Thomason *et al.* (1964) on *M. javanica;* they found that larvae lost their infectivity rapidly after four days when kept in water at 27 °C, while mobility was lost after eight days. Rapid loss of infectivity also was reported by Wallace (1966) with *M. javanica* in soil; he suggested that this was a consequence of depletion of food reserves, therefore sources of energy, during migration of the larvae in the medium, the rate of depletion being dependent on the activity of the nematodes. This hypothesis may be generally valid for *Meloidogyne* spp. and possibly also for species of other endoparasitic nematodes. Our trials suggest that loss of motility and infectivity of *Meloidogyne* larvae is much more rapid in the presence of plants, *Tagetes* spp. as well as tomato, than in unplanted soil. Numbers of active larvae extracted from the medium in the plant tubes were lower, especially 3 and 4 weeks after inoculation, than those from the unplanted tubes. This could be due to increased activity in the presence of the growing plant, either more migration in the growth medium or more energy consuming attempts to enter roots, or both. It could, to a small extent, also be due to adverse effects of micro-organisms or their metabolites (Walker *et al.*, 1965), or nematicidal products of decomposing plant material (Sayre *et al.*, 1965; Hollis & Rodriguez Kabana, 1966). In *M. hapla* and possibly other *Meloidogyne* spp. populations of active larvae remaining in a plant grown and in a fallow medium may both lose their infectivity in the course of one growing season and may therefore be of no importance for future infections. With populations of more persistent nematodes, however, these differences may be important: the infectivity of the nematodes around the roots may decrease rapidly under growing plants and be conserved wholly or to a large extent in unplanted soil for considerable periods.

4.4.2. *Survival and development of P. penetrans in the roots*

Three trials studied survival and development of P. penetrans in *Tagetes* spp. in comparison to known host plants.

a. *P. penetrans* inoculated onto growing *Tagetes* spp. and red clover

Nematodes for inoculation were collected from infested roots of *Digitalis purpurea* L. by the blender cotton-wool method. The suspension comprised 4 stages of different size which could be recognized under the dissecting microscope: sexually undifferentiated larvae (average length 230 μ), preadult females (av. 391 μ), adult females (av. 552 μ) and males (av. 464 μ). The 3 larger stages were separated by washing the suspension repeatedly through a pile of 7 sieves of 50 μ mesh diameter (cf. Seinhorst, 1956). The final suspension comprised only undifferentiated larvae when the few juvenile females, mature females and males left were removed by handsorting.

Seedlings of *T. patula, T. minuta, T. erecta* and red clover were grown for 3 weeks in small glass tubes with 8 ml of washed sand. Hoagland nutrient solution and water had been added carefully at intervals to maintain favourable growing conditions and the tubes were well rooted. Then each tube was inoculated with 500 larvae of *P. penetrans*. Four replicate plants from each plant species were evaluated after 1, 2, 3 and 4 weeks. The plants were washed free from soil, fixed in FAA and differentially stained with lactophenol-cottonblue to determine the number of penetrated larvae. The active larvae in the growth medium were counted by washing the sand and the tube and root soakings onto a cottonwool filter and making nematode counts in the suspension collected from under the filter the next day.

The *Tagetes* spp. maintained an apparently healthy condition after inoculation. The red clover plants, however, never throve; the roots decayed and the plants declined or died even from the first week after inoculation. Up to 3 of the 4 red clover plants were dead or practically so on each date. Data obtained from the remaining plants were not included in the statistical analysis, but are nevertheless recorded in Table 19. The decline of red clover may be due to the damage caused by the nematodes, because the inoculated density was many times the equilibrium density of *P. penetrans* on red clover which plant is susceptible to damage (Oostenbrink, 1961). From the second week on the nematode population in the red clover roots dropped suddenly.

The numerical results of this experiment are summarized in Table 19. The overall recovery of the inoculated nematodes was 53%, 26%, 14% and 10% one, 2, 3 and 4 weeks after inoculation; 46%, 14%, 5% and 2% of the nematodes were in the soil and 6%, 11%, 9% and 8% were in the roots of the various plant species at these dates respectively. This shows a rapid decline of the inoculated nematodes in the soil as far as they did not enter the roots, whereas the percentage penetration was generally low. Both facts may be due to the damage, weakening or disturbance which the nematodes suffer in the process of inoculation as was found earlier. This is not a specific *Tagetes* effect, because it does occur in tubes with good hosts as well.

The percentage penetration, therefore, was low in general. The figures, however, indicate significant differences which are supported by results of microscopical observation of the roots. *T. patula* harboured fewest nematodes throughout the four-weeks observation period: 7, 6, 6 and 8 in the consecutive weeks.

TABLE 19. Penetration of *P. penetrans* larvae in well established root systems of three *Tagetes* spp. and red clover grown in tubes with washed sand.
Five hundred larvae inoculated per tube with 8 ml sand. Number of larvae in the roots and, between brackets, in the surrounding sand, as recovered 1, 2, 3 and 4 weeks after inoculation. All figures are means of 4 replicates, except for number in red clover roots 1, 2, 3 and 4 weeks after inoculation which were averages from 1, 2, 2 and 3 replicate plants respectively (see also text).

Plant	Number of *P. penetrans*			
	1	2	3	4
	weeks after inoculation			
T. patula	7 (354)	6 (97)	6 (35)	8 (15)
T. erecta	71 (188)	76 (83)	78 (17)	61 (12)
T. minuta	19 (224)	47 (70)	43 (26)	69 (7)
Red Clover	40 (154)	93 (39)	54 (22)[1])	16 (5)[1])

[1]) partial decay of rootsystems

There were small superficial necrotic patches in the roots, most of them with 1 or 2 nematodes. Roots of *T. erecta* contained about ten times as many larvae as *T. patula*, throughout the 4 weeks without much variation.

In *T. minuta* roots the number of larvae was lower than in *T. erecta* after 1 week, but was about as high after 2 and 3 weeks and was, contrary to the other *Tagetes* spp., rising in the fourth week. This is probably due to some nematode reproduction in *T. minuta* after the third week: there were several lesions with some deposited nematode eggs comprising viable larvae at the time (cf. also Tables 1 and 2, indicating that *T. minuta* was less suppressive than *T. patula* and *T. erecta*). In red clover the rate of penetration was not much different from *T. minuta*. In the first week it was slightly lower, in the second week it was higher. The main difference between a suitable host, as red clover, and *Tagetes* spp. seems to be, as in earlier experiments, that in the first plant reproduction takes place and in the second decline or maintenance of a low equilibrium.

Comparisons were made of rates of penetration into the 3 *Tagetes* spp. 2 weeks after inoculation. For this purpose numbers penetrating the roots were expressed as percentage of the inoculated number and subjected to arcsine transformation. The transformed values were 0,215, 0.785 and 0.592 for *T. patula*, *T. erecta* and *T. minuta*. Further analysis showed that the critical value at the 5% level was 0.419. Thus, the rate of penetration into *T. patula* was significantly lower than into *T. erecta* and that there is no difference between *T. erecta* and *T. minuta*

b. Transplanting *Tagetes* spp. and red clover with established infections of *P. penetrans*

The nematodes for inoculation were collected in the same way as described under a, from infested roots of apple.

One-week-old seedlings of *T. patula, T. minuta, T. erecta* and red clover were grown in small glass tubes with 8 ml of the same 1:1 perlite-sand mixture mentioned before. Hoagland nutrient solution and water had been added carefully at intervals to maintain favourable growing conditions. After 3 weeks when the tubes were well rooted, each was inoculated with 250 larvae of *P. penetrans*. Three replicate plants of each species were examined for rootborne nematodes after one week; their root systems were washed free from adhering particles, fixed in FAA and differentially stained in lactophenol-cottonblue. On the same day 9 other plants of each species were also separated from particles of the growth medium in a careful way so as to leave the entire plant intact, and then transplanted in tubes with fresh uninfested growth medium. Three replicate plants of each species were then examined for rootborne nematodes 1, 2 and 3 weeks after transfer into nematode-free medium.

The numbers and percentages of *P. penetrans* that penetrate the different plant species in this trial are already recorded in Table 17.

The number of *P. penetrans* found a week after inoculation in the plants depicted before in Col. 6, Table 16, can be regarded as the initial number of rootborne nematodes in the plants transferred to new tubes with nematodes-free growth medium. In Fig. 6A for each plant species the mean "initial" numbers

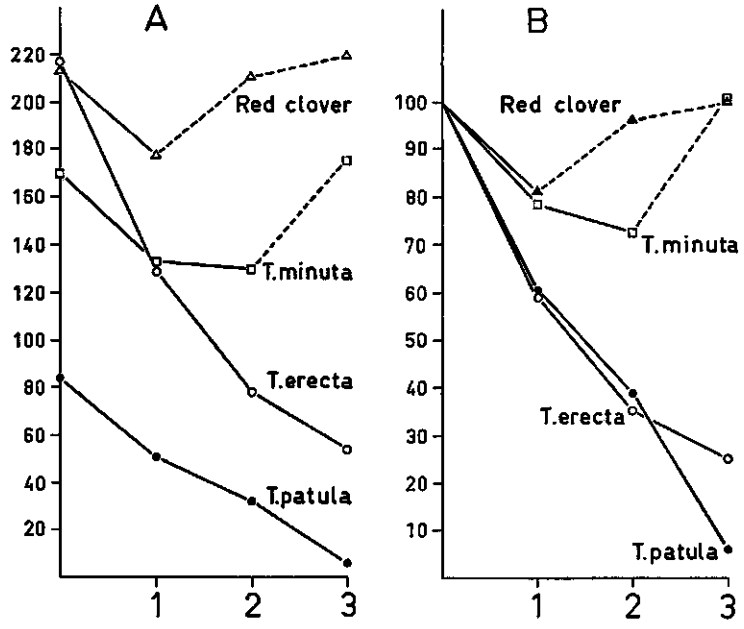


FIG. 6. Survival of *P. penetrans* within roots of *Tagetes* spp. and red clover.
Abscissa of individual graphs: number of weeks after transplantation of intested plants into nematode-free growth medium. Ordinate of individual graphs: number of *P. penetrans* within rootsystems (A) and as percentage of numbers at the moment of transplantation (B). Broken lines indicate occurrence of reproduction; number of newly formed larvae are added up to that of surviving nematodes.

of nematodes in the roots and the numbers found 1, 2 and 3 weeks after transplantation form a graph representing the population change with time. In Fig. 6B the numbers were expressed in percentages relative to the corresponding 'initial' populations.

For comparison of rates of penetration into the 4 plant species initial numbers of nematodes in the roots were expressed as percentage of the number inoculated and than arcsine transformed.

Average values of 1.25, 2.45, 1.97 and 2.42 were found for *T. patula, T. erecta, T. minuta* and red clover. Analysis of variance showed that critical difference at the 5% level was 0.837. The rate of penetration into *T. patula,* thus was smaller than into the 3 other plant species, but this was significant only with respect to *T. erecta* and red clover. Differences between *T. erecta, T. minuta* and red clover were not significant.

Larvae of *P. penetrans* which made successful entry into the roots evidently showed a decrease in number in the first week with all plant species tested. This may be partially due to loss of small infected roots during transplantation or during establishment of the plants in the new growth medium. No apparent loss of roots has been noticed, however, during subsequent weeks when the declining trend appeared to continue with *T. patula* and *T. erecta* but not with *T. minuta* and red clover. *P. penetrans* was evidently killed in roots of *T. patula* and *T. erecta,* whereas in *T. minuta* they survived and may even develop and multiply, though at a rate slower than in red clover.

P. penetrans in roots of *T. patula* and *T. erecta* were not associated with the presence of eggs in the infested tissues, whereas in *T. minuta* some eggs were found 2 weeks after transplantation and newly hatched larvae (considerably smaller than all larvae found within roots in previous weeks) were found 3 weeks after transplantation.

If allowed to parasitize *T. erecta* for more than 4 weeks perhaps part of the rootborne nematodes may survive and succeed in developing into reproductive adults (cf. Table 2).

Continued decline of numbers occurred with *T. patula* and *T. erecta.* There was no significant difference in the rate of decline between the two species. It should be noted, however, that significantly lower numbers were found in *T. patula* than in *T. erecta* upon transplantation (i.e. after one week) and that the penetrated larvae dropped to 7% in *T. patula* against 25% in *T. erecta.*

The above results therefore emphasize that the degree of penetration of *P. penetrans* into the roots of *T. patula* is lower than of *T. erecta, T. minuta* and red clover; *T. patula* may show resistance to penetration by this nematode.

The results also emphasize that, once *P. penetrans* enter roots, they are gradually largely eradicated in *T. patula* and *T. erecta* and survive or reproduce in *T. minuta* and red clover.

c. Analysis of a developing *P. penetrans* population inoculated to *Tagetes* spp. and clover

Seedlings of *T. patula, T. minuta* and red clover were grown for 4 weeks in

glass tubes with about 80 ml nematode-free peaty sand and controls were of fallow tubes. Each tube was inoculated with a specially prepared suspension comprising on average 1000 *P. penetrans*, namely 50 juvenile females and 950 undifferentiated larvae. Six replicate plants of each species were evaluated 5, 10, 15 and 20 weeks after inoculation. The whole root systems were macerated and the total soil contents were extracted to determine number and developmental stage of the nematodes. The results are summarized in Figs 7 and 8 and Table 20.

Fig. 7 demonstrates great and essential differences between the 4 treatments.

In fallow soil the total number of inoculated nematodes had dropped to about 20% after 5 weeks, but the population remained alive and evidently unaltered

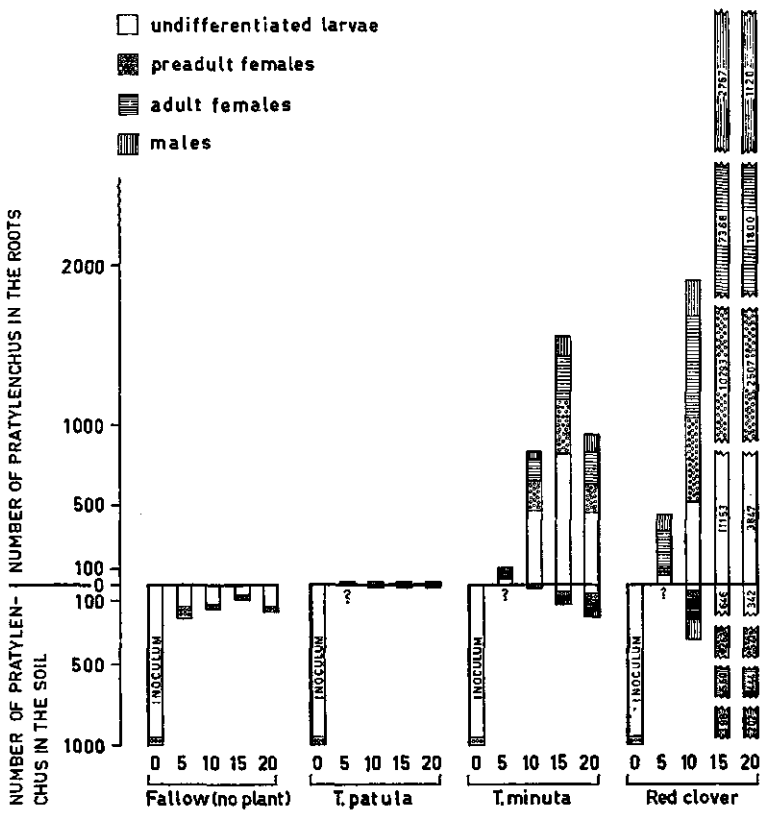


FIG. 7. Development of a *P. penetrans* population in tubes grown with *T. patula*, *T. minuta* and red clover compared to fallow tubes.
Inoculation of 1000 nematodes, namely 50 immature females and 950 sexually undifferentiated larvae per tube with 80 ml of soil.
Abscissa: plant names and evaluation dates (0, 5, 10 and 20 weeks after inoculation).
Ordinate: numbers of nematodes in the soil and in the plant roots; mean of 6 replicates.
Numbers of soil borne nematodes 5 weeks after inoculation were not determined in the planted soils and are indicated by question marks in the figure.

for the next 15 weeks. The initial decrease may be due to the process of inoculation and therefore be an artefact, as was suggested earlier. It is remarkable that the decrease concerns only or largely the undifferentiated larvae whereas the young females survived much better. The figures indicate, that undifferentiated larvae did not develop into adults in fallow soil during the 20 weeks of observation.

In the *T. patula* tubes the total population was suppressed to a very low level and remained low until between the 15th and 20th week very slight reproduction occurred (Table 20). After 5, 10, 15 and 20 weeks the roots harboured on an average per tube in this order a total of 12.0, 1.8, 0.5 and 15.8 nematodes and the soil no value (undetermined), 0.5, 0.2 and 0.2. The number of adult specimens in these populations was, again in this order, 2.7, 0.1, 0.2 and 9.8 in the roots and no value, 0.2, 0 and 0.2 in the soil. It appears that *T. patula* initially almost completely eradicated *P. penetrans* larvae as well as young females, and therefore markedly suppressed the population in comparison to fallow. It is also clear that eventually, after 15–20 weeks, a very limited reproduction had occurred, and therefore that the equilibrium density of *P. penetrans* under *T. patula* is low, but not zero, when the plant had grown sufficiently. This confirms results obtained in long-term field trials (Oostenbrink, 1966).

The population was also kept low in the tubes with *T. minuta*, though less effectively than with *T. patula*. The total nematode numbers in the roots were 103, 839, 1556, 943 and in the soil no value, 15, 116, 195 after 5, 10, 15, 20 weeks, respectively. This means that reproduction to above the initially inoculated number occurred in the tubes. The fact that nematodes matured and reproduced to a moderate extent is evident from the total numbers as well as from the numbers of adults (Fig. 7, Table 20). The inoculated population in the soil evidently dropped initially much more than in the fallow tubes, but the soil population was replaced by a mixture of developing specimens from the 15th week. These are probably specimens from the infested roots. The population in the red clover tubes developed rapidly and reached a considerable density, as could be anticipated because red clover is known as a suitable host plant. The inoculated population probably dropped initially as well as in the other tubes, but this is not clearly demonstrated because the soil was not examined after 5 weeks, whereas after 10 weeks a mixed population probably originating from the rootborne population already existed in the soil. The total nematode numbers in the roots were 435, 1895, 31581 and 9073 and in the soil no value, 339 3866 and 1559, after 5, 10, 15 and 20 weeks, respectively. The number of nematodes in roots was 9000 per g root tissue after 15 weeks, which is a very high density. The densities, therefore rose quickly and dropped again at the end of the season, as was found with other nematodes (Oostenbrink, 1966). This may be connected with ageing or decline of the roots; the average root weights dropped from 3.5 g after 15 weeks to 2.3 g after 20 weeks which may even be a consequence of damage caused by the nematodes. It is probable that also in the red clover tubes the inoculated population dropped initially to a low level, that the penetrated nematodes already began to reproduce after 5 weeks and that the soil popula-

tion at later dates was again a mixture of stages reflecting the density and composition of the population present in the roots. Fig. 7 and Table 20, therefore, indicate that only a little of the inoculum reproduced, and that the 3 plant species represent degrees of host efficiency varying from very high (red clover) through low *(T. minuta)* to nearly zero *(T. patula)* as expressed in final nematode densities in roots and in the soil.

Two other criteria of host efficiency, namely speed of nematode reproduction and the presence of adults and of a variable sex ratio, are considered further in Table 20 and Fig. 8.

The speed of reproduction is measured by the time necessary to form new adults or new larvae. About 50 immature females were incorporated in the inoculum of each tube. These preadult females survived in the fallow soil to a large extent throughout the observation period of 20 weeks. This was, however, not so in the planted tubes. Under *T. patula* the population was reduced almost to zero, and some new adults and new undifferentiated larvae appeared only after 20 weeks. This means that fully mature females and males and also new larvae were not formed until 15–20 weeks after inoculation. Under *T. minuta* a few fully mature females and males were already present after 5 weeks. Fig. 8, illustrating distribution of larval size in the populations from the fallow and the planted tubes 5 weeks after inoculation, indicates that the *T. minuta* tubes did not yet harbour small new larvae. Small larvae were present in the fallow soil and in the *T. patula* tubes (indicating very little or no growth of the inoculated larvae). Only larger larvae were found in the *T. minuta* tubes (indicating growth of the inoculated larvae, but not yet formation of new larvae). In the red clover tubes large and small larvae were numerous (indicating growth of inoculated larvae and formation of new generation larvae). From the numbers of mature females, males and larvae, and from the larval sizes, reproduction had clearly taken place already after 5 weeks.

The percentage of males among the sexually differentiated nematodes was generally between 15 and 25%.

TABLE 20. Number of sexually differentiated *P. penetrans* and, between brackets, the percentage of males at 4 different times after inoculation of 1000 nematodes (950 undifferentiated larvae + about 50 immature females) to fallow tubes (no plant) and tubes planted with *T. patula*, *T. minuta* or red clover.
Mean figures of 6 replicates.

Weeks after inoculation:		5	10	15	20
No plant	soil :	68 (0%)	21 (0%)	22 (0%)	25 (0%)
T. patula	roots:	2.7 (0%)	0.1 (0%)	0.2 (0%)	9.8 (9%)
	soil :	–	0.2 (0%)	0 (0%)	0.2 (0%)
T. minuta	roots:	63 (20%)	364 (15%)	724 (17%)	491 (22%)
	soil :	–	11 (17%)	67 (24%)	133 (21%)
Red clover	roots:	370 (26%)	1370 (16%)	20428 (14%)	5427 (21%)
	soil :	–	313 (44%)	3220 (12%)	1217 (17%)

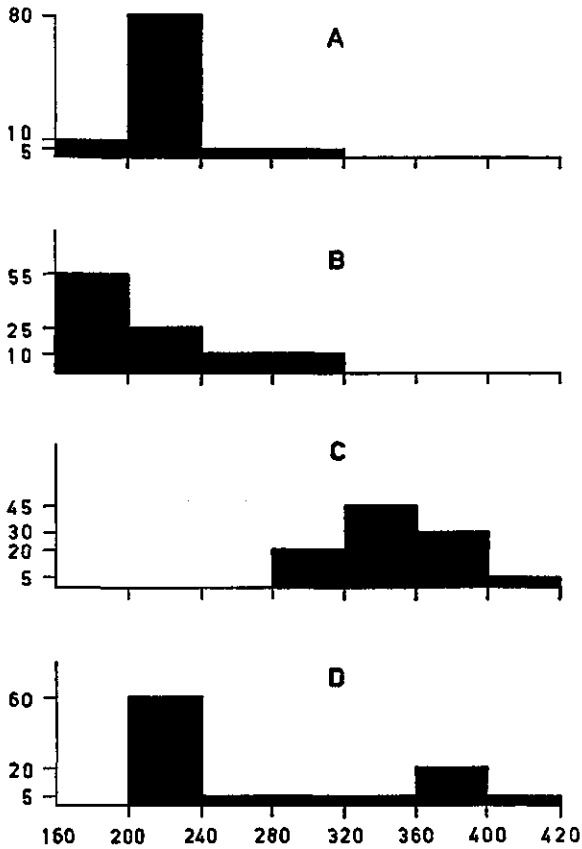


FIG. 8. Frequency distribution of sexually undifferentiated larvae of *P. penetrans* of different sizes in fallow tubes (A) and in rootborne populations from tubes with *T. patula* (B), *T. minuta* (C) and red clover (D), 5 weeks after inoculation. Percentage nematodes of different bodylength categories determined from 20 sexually undifferentiated larvae from each population.
Abscissa: size categories in μ (160–199, 200–239, and so on). Ordinate: percentages nematodes.

It is remarkable, that populations which just reached maturity, comprised a lower percentage males in the very resistant *T. patula* (9%, 20 weeks after inoculation) than in the moderately resistant *T. minuta* and the very suitable host red clover (20% and 26%, 5 weeks after inoculation). This is contrary to experiences with resistance to *Heterodera rostochiensis* in potato and to the widely accepted opinion that relatively more males are formed when plants are resistant to nematodes. There is also no indication that the percentage males increases as the host plant ages. The indication that the percentage males among the full-grown nematodes is slightly higher around the roots than in the roots of well

growing infested plants (red clover, 10 weeks after inoculation; *T. minuta* 10 and 15 weeks after inoculation), may indicate that males migrate probably slightly more than females. The only specific effect in *Tagetes* spp. on the sex ratio is therefore probably the low percentage of males in the slowly developing, sparse population in the roots of *T. patula*. This conclusion is not biased by the fact that young females were inoculated because *T. patula* is the only plant where all those females were excluded in the course of the growing period.

d. Survival of *P. penetrans* escaping from *Tagetes* roots

It is possible that part of the rootborne nematodes migrate into the surrounding soil, especially with *P. penetrans* (cf. Tables 1, 20). It holds for good hosts, but also for *Tagetes* spp (Table 20). The activity of *P. penetrans* emerging from *Tagetes* roots into the soil was compared with that of other plants as follows.

Fifteen single seedlings of *T. patula, T. minuta,* red clover and apple were planted in tubes with peaty sand, comprising a natural density of 352 plus an inoculated density of 1500 *P. penetrans* per tube. After 5 weeks 3 root systems of each plant were lifted, washed free from soil, weighed and processed with the blender-cotton wool filter method to assess the number of *Pratylenchus* and their activity in the suspension. The other root systems were also lifted, washed and weighed and then placed one by one into a jar with 100 ml of uninfested peaty sand; 6 root systems per plant were incubated as a whole and 6 were cut up into lengths of about 1 cm and uniformly mixed with soil. The soil was kept moist and at room temperature. Four weeks later the soil of each jar with the now disintegrated roots was elutriated, the catch was placed on a cotton wool filter and the nematodes which passed the filter were counted and examined to assess their activity.

Table 21 summarizes the number of nematodes in the different plant roots destined for the incubation test and indicates the percentage adults as well as the activity of the nematodes in the water with macerated root tissue and nematodes immediately upon blending the roots.

TABLE 21. Plant roots grown for incubation (cf. Table 22). Number of *P. penetrans* in the roots of different species 5 weeks after planting in tubes with infested peaty sand, and condition of the nematodes after separating them from the roots by blending. Numbers are totals per 3 plants.

Plant	Fresh root weight, in mg	*P. penetrans*		
		Number	Percentage adults	Percentage inactive or dead in suspension
T. patula	3615	220	5%	98%
T. minuta	4190	2638	30%	19%
Red clover	6445	16524	36%	10%
Apple	610	2158	48%	12%

T. patula harboured also in this case very few nematodes which were generally inactive in the root tissue suspension and of which only few were adults. The few nematodes and the low percentage of adults confirms the unsuitability of *T. patula* as a host, as established earlier; the few adults may have penetrated as such because the intital population comprised full-grown specimens alongside larvae. The high percentage inactive nematodes in the root suspension confirms the nematicidal effect of the *T. patula* root tissue or cell saps (cf. Section 4.4.3.). The inactive specimens did not recover when they were transferred to clean water for some days. *T. minuta* harboured more nematodes and was evidently less unsuitable as a host and had less toxic tissues than *T. patula.* Red clover seems a very suitable host, and so does apple when the low root weight is taken into account.

Table 22 illustrates the escape of *P. penetrans* from roots of the different plants into soil and the condition of the nematodes. It can be calculated from the table that of the nematodes inoculated with whole root systems of *T. patula, T. minuta,* red clover and apple about 3%, 38%, 44% and 46%, respectively, were extracted as active specimens from soil after four weeks' incubation. These percentages were 8, 29, 15 and 19, respectively, for cut roots. This shows that *T. patula* roots allow very little penetration or development and also very little escape or survival of the nematodes once they are inside the root.

TABLE 22. *P. penetrans* escaped into soil from roots of different plants incubated in the soil for 4 weeks.
Weights and numbers are totals per 6 plants.

Incubated root material	*P. penetrans*			
	Number initially inoculated with the roots, according to Table 21	Finally extracted from soil: Number	Percentage adults	Percentage inactive or dead in suspension
T. patula:				
Whole roots 7146 mg	435	13	85%	15%
Cut roots 6832 mg	416	33	46%	61%
T. minuta				
Whole roots 8783 mg	5533	2115	52%	12%
Cut roots 8770 mg	5525	1602	47%	7%
Red clover:				
Whole roots 12082 mg	30978	13506	41%	16%
Cut roots 13686 mg	35091	5364	33%	9%
Apple:				
Whole roots 1277 mg	4517	2060	52%	19%
Cut roots 1657 mg	5861	1095	56%	6%

T. minuta does not differ significantly from the good host red clover and apple for escape; some 40% recovery of the population inoculated with roots 4 weeks before was reached with all 3 plants. This indicates that *T. minuta* tissue shows little or no direct nematicidal effect in comparison with red clover and apple. It is remarkable that the percentage escape or survival from roots cut to pieces is lower than from whole root systems in *T. minuta*, red clover and apple, whereas the reverse is true with *T. patula*.

Cutting of roots may enhance the general nematicidal effect of decaying roots of normal plants as described under Section 4.2.2c; in the case of *T. patula* the direct effect of specific nematicides may predominate. In that case cutting of roots may allow some rapid escape of larvae which would otherwise be retained in whole roots, but which may be weakened despite their liberation and therefore be inactivated sooner in the final suspension (Col. 5). Adults of *P. penetrans* evidently have a better chance of escape from *T. patula* roots than larvae; the inoculated whole roots harboured about 20 adults and 415 larvae, whereas 11 adults and 2 larvae were recovered from the soil 4 weeks later. With cut roots about 20 adults and 396 larvae were added, whereas 15 adults and 18 larvae were recovered later. Selection towards adults during the incubation of roots occurred to a lesser degree for *T. minuta*, and not or hardly in the other plants.

It appears from the experiment that *T. patula* allows very little escape or survival of *P. penetrans* as soon as the nematodes have entered the roots, whereas the population in *T. minuta* roots does escape and may be infective as for suitable host plants.

4.4.3. *Nematicidal substances in Tagetes roots*

The presence, constitution and characteristics of nematicidal substances in *Tagetes* were extensively studied by Uhlenbroek & Bijloo (1958, 1959). They isolated α-terthienyl and 5-(3-buten-l-ynyl) -2,2'-bithienyl from *Tagetes* roots, and noticed their strong nematicidal effect (mean lethal dosage 0.1–5 p.p.m) on several nematodes, also species which are not suppressed in soil by growing *Tagetes* plants. These chemicals were shown to be insoluble in water.

In our experiments we wanted to determine or to confirm, without further chemical analyses, whether *Tagetes* saps comprised a nematicidal principle in such concentrations that it affected nematodes and whether extracts from different *Tagetes* spp. differed in their activity. Swarup & Sharma (1967) showed that aqueous extracts of the roots of *T. erecta* are lethal or inhibitory to the hatch of eggs of *Meloidogyne* spp., but also that the active principle in the roots does not seem to be in high concentrations as dilutions rapidly decreased or destroyed their potency. Four experiments were made to study the effect of root extracts of *Tagetes* spp. on *P. penetrans*.

a. Survival of *P. penetrans* in expressed root juices of *T. patula*, *T. minuta*, potato and in antibiotics solution

After some preliminary studies the following method was applied in this experiment. Clean roots of *Tagetes* plants of about 2 months old were ground in

a mortar and put in cheesecloth. The sap was squeezed out, centrifuged for some hours and decanted. After a day precipitated substances were removed by centrifuging again and ethoxy-ethylene mercurichloride and streptomycin sulphate were added to a concentration of 4 and 1000 p.p.m., respectively, to prevent fungal and bacterial development (cf. Mountain & Patrick, 1959). The same procedure was followed for roots of a good host, potato. The saps were kept in brown glass bottles at 4 °C or frozen. Fifty adult female *P. penetrans* from a culture on red clover were placed in a shallow layer of the saps and of the antibiotics solution in water, in watch glasses and kept in moist Petri dishes at room temperature. Surviving nematodes, i.e. specimens which moved or did at least not show disintegration of body contents or of the stylet shaft, were counted every day and transferred into fresh liquids.

The results are graphically represented in Fig. 9A. The percentage survival drops during the third day in all treatments, but from then on the series antibiotics-potato-*T. minuta*-*T. patula* shows increasing mortality. Affer 10 days the percentage survival is 62, 42, 14 and 0 in that order.

b. Survival of *P.penetrans* in root extracts of *T.patula*, potato and in ascorbic acid-antibiotic solution

The method described under a. was followed with some modifications. Besides antibiotics, 1 % ascorbic acid was added to the saps and to the control solution. Ascorbic acid reduces browning of the roots and keeps the sap clear. In a preliminary trial 1 % ascorbic acid solution did not cause higher mortality than 0.1 % and 0.01 % solutions or than the antibiotics solution used as a control under a. The saps were, in these trials, filtered through 0.6 μ bacterium filter and their pH was determined. The pH appeared to be 4.2. in the *T. patula* rootsap and 3.9 in the potato rootsap. *T. minuta* was not included in this test. The nematode assay was performed as under a, but with 100 nematodes per watch glass.

Fig. 9B represents the results. Some mortality occurred already after 1 day. The percentage survival after 11 days in the control solution, potato rootsap and *T. patula* rootsap was 64, 51 and 11, respectively.

c. Survival of *P. penetrans* in root extract of *T.patula*, *T.minuta* and potato

This experiment was set up as under b, but 0.05 % mercapto-ethanol, an effective reducing agent, was added to the root saps as well as antibiotics. A preliminary test indicated that very little mortality occurred in a 0.05 % solution of mercapto-ethanol in water, but that higher concentrations were nematicidal. Solutions were not filtered through a bacterial filter. The control solution of antibiotics was omitted. The nematode assay was performed as before, with 100 nematodes per watch glass and the observation period was only 7 days.

Fig. 9C represents the results. There was again some mortality from the first day on and the final percentage survival was 60, 24 and 23 for potato, *T. minuta* and *T. patula*, respectively.

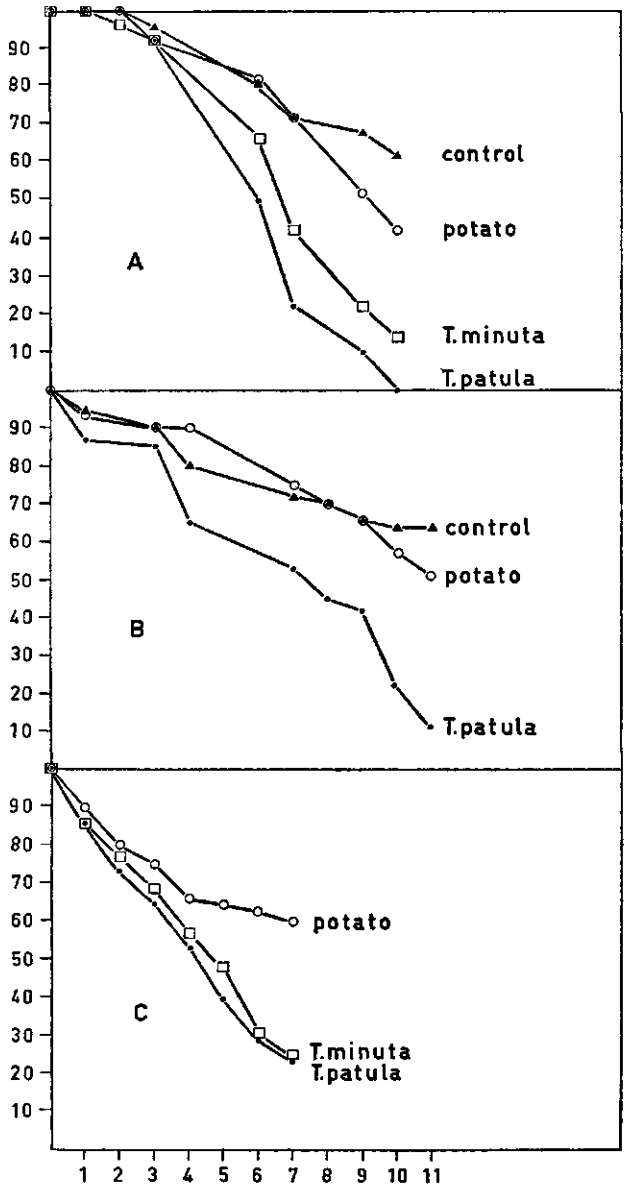


FIG. 9. Survival of *P. penetrans* immersed in root extracts of *T. patula*, *T. minuta* and potato in comparison to control solutions of antibiotics and reducing agents (see text), in 3 experiments A, B and C.
Abscissa: duration of immersion in days. Ordinate: percentage survival.

d. Survival of *P.penetrans* in different fractions of root extracts of *T.patula*

Root extracts of *T. patula*, prepared according to the method described under Section 4.4.3C, were fractionated over a column of a dextran gel, Sephadex G-75 (Pharmacia, Uppsala, Sweden) in a joint experiment with F. J. Gommers. Only the protein concentration of the fractions was determined by means of the combined Biuret and Folin-Ciocalteu method (Lowry *et al.*, 1951). The effect of the dissolved fractions on mortality of *P. penetrans* was determined by the assay method recorded under a-c. The first 2 fractions caused a high nematode mortality due to an artefact. Fractionation over Sephadex, namely, must be done at pH 7 by pretreatment of Sephadex with 0.01 M phosphate buffer. The phosphate buffer, which is collected in the first 2 fractions, seemed nematicidal by itself.

Two experiments are recorded here, with the number of fractions given according to the order of eluation. In the first experiment Fraction 8, in a series of 10, caused 70% mortality to 46% or less in the other fractions. In the second experiment Fraction 10 in a series of 12, caused 58% mortality against 40% or less in the other fractions. A relatively high mortality, therefore occurred in one of later fractions. No further analysis was made to identify the active principle(s). High mortality was not correlated with the total protein concentration, because this was highest in fractions 5 and 6 (1.2 and 1.7 mg/ml, respectively) and quite normal in Fraction 10 of the second test (0.05 mg/ml.)

The experiments leave no doubt that the root extracts tested in comparison to control solutions comprise a nematicidal effect against *P. penetrans* which appears from the third day on. *T. patula* is more effective than *T. minuta;* both are more effective than potato. It is likely that this effect *in vitro* is related to the *Tagetes* effect on nematodes in soil and that it is caused by the nematicides isolated from *Tagetes* roots by Uhlenbroek & Bijloo. This was, however, not explicitly proved by our experiments because we only showed that the effect occurred, that it was strongest in the most effective species, and also that the most effective fraction of the *T. patula* root sap was not the fraction with high protein content.

Two further points are noteworthy. First: There is a slow but steady kill of nematodes in the control solutions, wherever soil nematodes are kept in water or aqueous solutions. Second: The nematode kill in potato root extracts, distinctly lower than in *Tagetes* extracts, is significantly higher than in the control solutions. This indicates the presence of a nematicidal principle in potato root extracts too. It is rather weak, but it supports the indication found earlier that plants can establish in general an 'antinemic potential' by means of their substances in water or soil. The specific *Tagetes* effect, therefore, is probably different and must at any rate be measured in comparison to the effect of potato or other plants. Also then the nematicidal effect of root saps of *Tagetes* spp., especially *T. patula*, is marked.

4.4.4. *Histological reactions in Tagetes tissues*

In any plant-nematode relationship the phenomena of host suitability or efficiency (measured in terms of nematode reproduction or post plant density)

and of susceptibility to attack (measured in terms of plant symptoms or damage) must be considered and evaluated separately. The marked effect of *Tagetes* on certain plant nematodes is a question of host suitability and was measured as such by studying the nematodes. Observations on the plant reaction, however, may contribute to explain the mechanism of the *Tagetes* effect and is considered here.

Infestation of *Tagetes* spp. by stem and leaf nematodes may cause typical swellings, distortions, malformations or leaf blotches as in other plants (Plate 1A and B), heavy infestation by *Trichodorus teres* may apparently cause root damage and poor growth. Long-term field trials with different monocultures, however, have shown that *T. patula* generally maintains good growth whereas other monocultures often decline, and also that *Tagetes* normally has a well developed rootsystem without discolorations contrary to most other plants. Despite this healthy appearance *Tagetes* roots may show histological reactions upon nematode infestations (Christie, 1949; Seinhorst & Klinkenberg, 1963). *Tagetes* is especially effective against endoparasites, such as *Meloidogyne* and *Pratylenchus* spp. Our observations were therefore restricted to the early symptoms in and on roots of *T. patula* and *T. minuta* by *M. hapla* and *P. penetrans*. Comparisons were also made with tomato and red clover roottisue attacked by the first and last mentioned nematode species respectively.

a. Anatomy of healthy roots

The anatomy of four-weeks-old *Tagetes*, tomato and red clover plants, grown in steam-sterilized soil is described. Roots were fixed in FAA and selected pieces were dehydraded in a series of tertiary butylalcohol and embedded in paraffin. Transverse, tangential and longitudinal sections 10 μ thick were made with a rotary microtome; the sections were stained with Johansen's quadruple stain and mounted in Canada balsam (Johansen, 1940). No differences were noticed in the anatomy of roots of *T. patula*, *T. erecta* and *T. minuta*, and *T. patula* is therefore considered representative for these 3 species.

All plant species mentioned have a tap root from which principal lateral roots arise which branch into finer laterals. Primary roots of all these vascular plants consist of 3 concentric parts: a uniseriate epidermis, a multiseriate cortex of which the innermost layer is the endodermis, and the stele. Primary roots of *T. patula* are about 1/4 mm in diameter; the cortex is made up of 5–6 layers of cells with large intercellular spaces. In tomato the cortex is 4 layers thick and in red clover 4 to 5 layers; intercellulars are common also. In *T. patula* and in tomato the stele comprises an outermost uniseriate pericycle, a diarch primary xylem and 2 groups of phloem. With red clover the pericycle is uniseriate also but may be 2 cell layers thick opposite the protoxylem points; the protostele is of the triarch type, 3 phloem strands alternating with 3 exarch xylem rays.

Secondary roots are initiated by the formation of cambial strands between the protoxylem poles and central to the phloem. As is typical for roots of Dicotyledoneae secondary phloem is formed centrifugally and secondary xylem centripetally. In small secondary stage roots of *T. patula* the epidermis and cortex

remain as in the primary stage; in older laterals of about 1.5 mm diameter the cortex is 2–3 layers thick and surrounded by a multiseriate periderm; the xylem comprises about 3/4 of the diameter of these roots. Secondary roots of tomato are commonly tetrarch; 4 wedgeshaped sectors are formed separated by pericyclic rays, each sector formed by secondary phloem, a cambial strand and secondary xylem.

The cortex keeps up secondary thickening by radial cell division and tangential growth of the cells. With red clover the cambium develops laterally to form a closed ring with secondary phloem and xylem on either side. The pericycle in this stage forms a two layered ring; sections of older roots indicate that this forms a cork cambium; periderm development by this layer leads to rupturing and shedding of the cortex and epidermis. The anatomy of bacterial nodules is not described here as they seem not essential in these studies.

b. *M.hapla* in *T.patula* and tomato

Four-weeks-old seedlings of *T. patula* and tomato were carefully transplanted into soil mixed previously with chopped roots of *M. hapla*-infested tomato plants. Two, 4 and 7 days later rootsystems were lifted, cleaned in water and fixed in FAA. For comparison roots of plants grown for 10 weeks and for 3 months in infested soil were also fixed. Attacked root apices were selected under the dissecting microscope and sectioned and stained as indicated in Section 4.4.4.a.

No attacked root apices were obtained from rootsystems fixed 2 and 4 days after transfer into infested soil; this was due largely to dying of fine roots. The material fixed 7 days after exposure to infection yielded much material for sectioning.

Tomato plants exposed one week to infection with *M. hapla* yielded roots in different stages of the infection process. Infected roots varied in appearance from having a stunted apex almost without any thickening to roots with a conspicuously large gall; all infested roots showed a dark yellowish discoloration. Longitudinal sections show that larvae penetrate through the rootcap or just behind it and move in the direction of the apical meristem and penetrate it. They migrate further in the central cylinder and generally take a permanent position in the region near the zone of cell elongation, although some move further. Migration is mainly intercellular and causes no or little damage to the cells. Frequently 3 or more larvae penetrated the same rootapex; invasion by a large number of nematodes may cause necrosis of tissue at the site of entrance. Activity of the apical meristem may be abruptly ceased; there is an immediate hypertrophy of cells of the pericycle along the nematodes' path and especially in the immediate vicinity of the larval head. Transverse and longitudinal sections of infected and slightly swollen roots show that cells of the vascular systems near the nematode's head showed hyperplasia and form a distinct syncytium.[1] Adjacent stelar tissue consisted of undifferentiated and unelongated cells, in con-

[1] The term syncytium is used to indicate the coherent cluster of giant cells, each of which may perhaps be considered a syncytium.

trast to cells at a larger distance from the syncytium which were differentiated into elongated vessel elements. There is an increased division of cells in the pericycle and in some sections lateral roots are being formed. These galls were associated with larval development to a sausage-shaped stage. Sections from galls of tomato plants grown ten weeks in infested soils showed mature syncytia associated with egg-mass bearing females. The syncytia contain 4–6 giant cells with thick walls and bright yellow-orange staining cytoplasma; each giant cell contains a number of giant nuclei in different stages of nuclear wall disintegration and red staining nucleoli. These groups of giant cells were distinctly separated from the adjacent gall tissue. In longitudinal direction gall tissue consists of tracheidal and vessel elements, and in radial direction it consists of parenchymatous cells of pericyclic origin. In older galls syncytia may be surrounded entirely by lignified xylem elements; tissue adjacent to the nematode body is not differentiated into xylem.

With *T. patula* the infected root apices are characterized by their stunted appearance without thickening and a dark yellowish discoloration. Most larvae were found in the process of penetration, half or less of the bodylength protruding from the rootsurface. Most larvae enter through the rootcap; sometimes penetration is via the region just behind the rootcap. At any rate, they commonly migrate first to the region of the apical meristem and penetrate further basipetally within the central cylinder or the cortex. Usually root apices were invaded by one or two larvae. They move intercellularly, occasionally also intracellularly, without causing necrosis. In one instance 13 larvae were found crowded together with their bodies halfway in one root tip which was necrotic in this case and which had formed a lateral immediately behind the damaged portion. Larvae usually stop to migrate when reaching the region between the apical meristem and the zone of cell elongation; they usually then were embedded for a full bodylength in the roottissue. Exceptionally, larvae were found at greater distances from the apex. The immediate reaction of the root to penetration of the nematode is a cessation of division of the apical meristem; calyptral cells frequently were more or less sloughed off and in some instances the root apex was entirely exposed giving the root a blunt appearance. Stelar cells adjacent to the larval head were enlarged and each contained a prominent nucleus; in some instances they show a stimulated division. There was, however, as a rule no indication of hypertrophy nor hyperplasia of pericyclic or cortical cells at any level along the larval body and near the oral end. Consequently there is no increase in diameter of such infected roots.

Ten weeks after exposure to infection *T. patula* yielded only few infected root apices. In one instance, transverse section showed a small syncytium consisting of 2 giant cells with 2 and 6 large nuclei respectively, each with brightly staining (red) nucleoli; the walls of some nuclei were partly disintegrated. The giant cell cytoplasm was stained reddish brown. The syncytium is surrounded by a layer of irregularly shaped, unlignified tracheidal and vessel elements; the pericycle is entirely flattened against the cortex. There seems to be no gall tissue of pericyclic origin, and no or little increase in diameter of the root. In another instance

transverse section shows a similar picture but the syncytium is degenerated into a reddish brown, amorphous mass which appeared shrunken and loosened from the adjacent plant tissue; it lies in a cavity within the root. Occasionally syncytia are more developed and in such cases a larva of advanced stage of development is normally found associated with the syncytium .In one instance a small gall was found which in longitudinal section shows 6 giant cells bordering each other, with a number of smaller ones around or sometimes between adjacent large, giant cells. Judged from the numbers of nuclei the small giant cells seem to have originated from the coalescence of 2–4 cells. There seems to be an incomplete or slow coalescence of cells and a degeneration of their contents (Plate IC). Again a small layer of severely compressed cells was visible between syncytia and the cortex; cortical cells were also flattened, apparently because dilatation due to formation of syncytia is not kept up by radial divisions and enlargement of the cells. Exceptionally a distinct gall was formed, giving the root apex a pendulum-like appearance. A longitudinal section of such a gall shows 2 large giant cells and a few adjacent smaller ones in an advanced degenerated state; they were massive lumps of brown amorphous substance. The gall tissue surrounding them consists of tracheidal or vessel elements mostly arranged in longitudinal rows (Plate 1D).

These observations show that there is no difference in the mode of penetration of *M. hapla* larvae into *T. patula* and into tomato. The immediate reaction of the planttissue during the penetration and migration process is largely the same, except that hypertrophy occurs readily in tomato. A week after exposure to infection, attacked tomato roots may show distinct galling and formation of syncytia. Infected roots of *T. patula* only showed light hypertrophy or some hyperplasia of cells adjacent to the larval head in the central cylinder. Formation of giant cells occurs to a little extent and only occasionally in *T. patula;* this proceeds at a strongly retarded rate and syncytia always were found in a degenerated condition: the cytoplasm staining a reddish brown colour, in contrast to the bright yellow-orange one with tomato. Syncytia formed in *T. patula* may be aborted. In contrast with tomato, usually there is no or slight formation of gall tissue with *T. patula.*

c. *P.penetrans* in *T.patula, T.minuta* and red clover

Four-weeks-old seedlings of *T. patula* and *T. minuta* were carefully transplanted into soil with a dense, established population of *P. penetrans.* Two, 4 and 7 days later root systems were lifted, cleaned in water and fixed in FAA. For comparison roots of 3-months-old plants of these species and red clover grown in infested soil were also fixed. Root pieces were selected under the dissecting microscope and sectioned, stained and mounted as described under Section 4.4.4.a. Further material for general observation, staining and sectioning was collected from the inoculation and infestation trials described earlier.

Observation of infested roots at low magnification under the dissecting microscope already shows that larvae penetrate the young roots at random sites of their surface, except at the apex, and that lesions are caused in all 3 plants

(Plate 1E). The lesions were necrotic, small and rather superficially located in *T. patula*, and slightly necrotic or free of necrosis and covering large areas in red clover. In *T. minuta* lesions may consist of necrotic tissue, but those of the type in red clover were found frequently also. In *T. minuta* and red clover lesions and adjacent tissue harboured varying numbers of nematodes. Incidentally larvae were found with their anterior part inside and posterior part outside the roots, but larvae normally penetrate within 2 days and take up a longitudinal position in the first, second or third cell layer of the root cortex which is 4–5 cell layers thick apart from the one cell thick epidermis. Sometimes they are found in the epidermis but never in the stele. Nematodes and lesions are therefore practically confined to the cortex and the epidermis. Penetration and cell discoloration or necrosis may already be visible 2 days after inoculation.

Young lesions in *T. patula* normally harbour 1–3 nematodes intracellularly situated in one cell or two cells in a longitudinal row. Such cells have a reddish brown layer packed inside along the walls and sometimes some brown granular contents and dark coloured nucleus (Plate 1F). One or two adjoining cells in each direction may show a similar necrosis, though the reaction is often less marked and the nucleus may seem normal. The intercellular spaces in a lesion are normally filled with a red brown substance. Four weeks after infection young and apparantly older lesions occur side by side. The cells of older lesions have maintained their original shape despite necrotisation and do not show hypertrophy or hyperplasia. Infected roots, therefore, maintain their shape and size. Cells at the inner side of a lesion, however, may be slightly swollen. Necrotic lesions are not aborted, and remain intimately connected with the rest of the cortex. This holds for lesions which border the root surface, though they may be collapsed in this case due to break-down of the radial cell walls. Lesions are often connected with the root surface by a radial row of 2–3 necrotic cells which is apparently the track of penetration. Longitudinal tracks of necrotic cells extending from the lesion are not found in *T. patula*, which indicates low motility of penetrated larvae. Most larvae occupy 2 cells in a longitudinal row, and are then reflexed at head and tail ends. Young lesions normally comprise a few nematodes, old ones may comprise nematodes or be empty. In old lesions most larvae are discoloured and shrunken and therefore apparantly dead, though some of the specimens looked healthy. Larvae with a normal appearance were incidentally noticed outside necrotic areas too.

In *T. minuta* necrotic lesions with dead nematodes were found as in *T. patula*, but they were generally more extended, less intensively coloured and evidently appeared slower. Necrotic cells often had slightly brown-coloured walls and a normal nucleus. A week after inoculation some lesions harboured 6–38 larvae; larvae often occurred outside necrotic areas. Here longitudinal tracks of several weakly necrotic cells, sometimes with deposited nematodes eggs, were found.

In red clover penetration into and movement through the cortex caused no direct or only slight necrosis. Later large lesions with many nematodes may be formed.

4.4.5. *Discussion*

The processes inside the root, more than exoradicular and penetration phenomena, appear to be instrumental in the nematode-suppressing effect of *Tagetes* spp.; the degree of effectiveness is evidently influenced by the *Tagetes* spp. and by the nematode species. *Tagetes* root tissue contains a strong nematicidal principle (Fig. 9A, B and C). It is possible that components vary according to the *Tagetes* sp. Uhlenbroek & Bijloo (1959) already recorded α-terthienyl and a certain bithienyl compound from *T. erecta*, and there may be more. Some other Compositae, viz. *Eriophyllum caespitosum*, *Echinops ritro*, *Gaillardia* hybrid *Burgunder* and several hybrids of *Helenium* were shown to suppress *P. penetrans* effectively also (Hijink & Winoto S. 1967); *Milleria quinqueflora* L., a widely occurring weed in Panama suppressed *M. incognita*. *M. javanica* and *M. arenaria* in addition, but not *M. hapla* (Winoto S., in litt.). Acetylenic thiophenes seem to be specific for many Compositae and species of *Echinops*, *Gaillardia*, *Flaveria* and *Bherkeya* are recorded in literature (cf. Swain, 1963; Hegnauer, 1964) as possessing quite the same thiophene(s) found by Uhlenbroek & Bijloo (1958, 1959) in *T. erecta*. The nematicidal principle from *Helenium* is hitherto unidentified.

M. hapla and *P. penetrans* penetrate the roots of *Tagetes* spp. and good hosts to a limited degree and loose the rest of their soil population by a natural decline in all cases. Invading nematodes, however, reproduce in good hosts and are suppressed in *Tagetes* spp. This holds for *M. hapla* and *P. penetrans*.

With *M. hapla* invaded root tips of *Tagetes* spp. show little damage. Occasionally a light brown discoloration, formation of small syncytia and some swelling occurs as in the good host tomato though less marked. There were no marked differences between the *Tagetes* spp.

With *P. penetrans*, however, *T. patula* was distinctly more effective than *T. erecta* and *T. minuta*. *P. penetrans* causes epidermal and cortical lesions in the *Tagetes* spp. The lesions are small and they do not influence the healthy appearance of the rootsystems as a whole. It is known that lesions are formed in red clover and many other host plants. This is in fact a characteristic of *Pratylenchus* infestations which gave the genus its vernacular name, root lesion nematodes, and is explained as the reaction of plant substances on enzymatic emanations from the nematode body (Mountain, 1961, 1965). The special point in this case is, that the nematodes in the *Tagetes* root generally die within the lesions and that the noxious effect appears to be greater in *T. patula* than in the other *Tagetes* spp. Root extracts of *T. patula* were more nematicidal against *P. penetrans* than those of *T. minuta*. In *T. patula* necroses appear earlier and more conspicuous than in *T. minuta*. The poor penetration of *P. penetrans* and the strong effect of *T. patula*, compared to the other *Tagetes* spp. on this particular nematode is evidently correlated with this strong necrotic reaction of the plant and may be due to it. Formation of necrotic lesions does not explain the *Tagetes* effect against endoparasites in general, because it does not occur with *Meloidogyne* spp. It is possible, however, that strong and rapid appearance of this necrosis is a consequence of nematodes dying from intoxication, as in the root sap, and releasing their enzymes which start necrosis.

It is conceivable that the nematicidal effects of *Tagetes* spp. have to be incorporated into, or superimposed upon plant nematodes relationships which exist between particular *Tagetes* spp. and particular nematode spp.

The nematicidal effect differs with the *Tagetes* spp. and is in none of the species lethal enough to suppress populations of *Meloidogyne* and *Pratylenchus* spp. completely. *Tylenchorhynchus* spp. are suppressed less effectively or less rapidly, probably because they live ectoparasitically and may withdraw from the roots at intervals. Ectoparasitic vessel feeders with long stylets are apparently not susceptible to the antinemic principles in *Tagetes* at all. Differences in plant characters as well as in nematode behaviour must evidently be taken into account.

CHAPTER 5

AGRICULTURAL SIGNIFICANCE OF *TAGETES*

5.1. Introduction

Tagetes spp. are wellknown ornamental plants. Their value as a source of organic matter, stains, therapeutics or for other purposes is too low to warrant large-scale cultivation, though some cultivation in Mexico is reported (Garret, 1968). Special interest in practical application of *Tagetes* as a crop is raised mainly owing to its drastic suppression of plant nematodes and the marked growth improvement of subsequent crops.

There are many *Tagetes* spp. and cultivars and they differ in their effect and agricultural potencies.

Oostenbrink, Kuiper & s'Jacob (1957) compared the effect of 8 varieties of *T. patula* and *T. erecta* against *P. penetrans* and *P. crenatus*. They concluded that all were effective without much difference, but a scrutiny of their results indicates that cultivars of *T. patula* were more effective than those of *T. erecta* and that the cultivars of the first species varied less than those of the second. These indications are confirmed in our trials, which indicate *T. patula* Harmony and Golden Harmony as most effective. Varieties of *T. patula* are most effective against nematodes. New varieties may be bred for the purpose of nematode control. The data available are recorded under Section 5.2.

The effect of the recommended *T. patula* is studied under Section 5.3. with respect to sowing time and plant density and tested as an autumn crop.

The plant nematodes susceptible to suppression by *Tagetes*, such as *Pratylenchus* and *Meloidogyne* spp., cause widespread damage in agriculture all over the world. Yield increase owing to preceding or sometimes contemporal cultivation of *Tagetes* is marked in many crops on many soils. It requires knowledge about the damage caused by the nematodes at different densities and about the possible significance of other effects introduced by *Tagetes* on the growth of the subsequent crop to decide whether and to what extent yield increase and nematode suppression are causally related. One special case is studied in detail and recorded under section 5.4.

5.2. The choice of a tagetes sp. and cultivar for practical application

There are about 20 species of *Tagetes*, most of them annuals and indigenous to the warmer parts of America (Engler & Prantl, 1897; Munz & Keck, 1959; Chittenden, 1951). *T. minuta* occurs adventively in some temperate regions and as weed in tropical regions in other continents. Four species are cultivated as ornamentals, viz. *T. patula* L., *T. erecta* L., *T. signata* Cav. and *T. lucida* Cav.. The first 2 species, introduced in Europe as early as 1542 and 1573 respectively,

nowadays comprise more than 30 cultivars each (Maatsch, 1962). Complete screening of all *Tagetes* spp. was not pursued, but one or more varieties of the species mentioned above were compared. The factor which is at first instance determinant for the practical value of a *Tagetes* spp. or variety is its effect on nematode populations. Such effects are compared in several tables of results of field trials and laboratory tests throughout this study.

5.2.1. *Nematicidal effect*

The field trial described under Section 3.2.2. and illustrated in Table 6 comprised 8 cultivars of *T. patula*, 6 of *T. erecta*, *T. minuta*, *T. lucida* and *T. signata*. The results are summarized below by recording the percentage survival (post-plant density as a percentage of preplant density) of the *Pratylenchus* population *(crenatus + penetrans)* under each of the plant species:

T. patula	(8 cultivars)	8–21
	Golden Harmony	11
	Harmony	13
T. erecta	(6 cultivars)	20–97
	Aurantiaca	76
T. minuta		150
T. lucida		44
T. signata	Golden Gem	39
Barley		282
Potato		160
Fallow		79

These figures indicate that *T. patula* as a whole was more effective against *Pratylenchus* than the other *Tagetes* spp. There were, however, significant differences between the cultivars of *T. patula* as well as of *T. erecta* and the least effective cultivar of *patula* coincided with the most effective cultivar of *erecta*. It is therefore necessary to indicate the variety used. The cultivars Golden Harmony and Harmony of *T. patula* and Aurantiaca of *T. erecta* are specially recorded because they are often used in our laboratory and field trials.

T. patula Golden Harmony was equally or more effective than any other *Tagetes* spp. against *Pratylenchus*. *Meloidogyne* and *Tylenchorhynchus* spp. in laboratory tests (Tables 1–5, 8–12) *T. patula* Harmony was effective, often very effective in comparison to other crops or fallow, against *Pratylenchus* and *Tylenchorhynchus* populations in many field trials (Table 7, Figs 1, 2, 3).

The results of Chapter 3 evidently show that *Tagetes* spp. are not generally effective in suppressing ectoparasitic plant nematodes, such as *Rotylenchus robustus*, *Hemicycliophora*, *Paratylenchus* and *Trichodorus* spp. In fact Table 13 demonstrates that certain ectoparasites breed freely on *Tagetes* spp. and that this varies with the *Tagetes* spp. and even cultivar used as in other plant-nematode relationships. *Hemicycliophora conida*, for example, reproduced better on *T. patula* Golden Harmony and Harmony than on several other *Tagetes* spp. and varieties. At the other hand Table 7 demonstrates that monoculture of *T. patula* Harmony for several years did not produce high densities of ectoparasites in

any of the 12 field trials concerned, This indicates that ectoparasites for which *T. patula* Harmony is a suitable host plant are not widespread.

5.2.2. *Plant characteristics*

Marigolds, *Tagetes* spp., are intensively improved by selection and breeding as ornamentals, but little is known about their characters for cultivation as a soil hygienic crop.

The following characters hold for most *Tagetes* spp. and varieties when grown in the Netherlands:

Seeds small, oblong achenes, difficult to harvest and to sow mechanically. Germination of seeds and development of seedlings slow, but broadcasted as well as drilled seed or planted seedlings may cover the soil after about 2 months and maintain a dense cover until the next winter. Seedlings as well as fullgrown plants are very susceptible to frost; night frosts in spring as well as in autumn limit the growing season. Slow seedling development and susceptibility to frost exclude development of *Tagetes* spp. and occurrence as weed. Plants sown in April are fullgrown after about 3 months, bloom heavily from mid-summer (mid-June to end of July depending on species) to about October and maintain themselves without leafshedding until winter. The aerial parts consist of many stiffstanding, lignified, branched stems and many flowers in addition to the fine, elastic pinnate leaves; when frost kills the plant the stems remain upright in dried-out condition. Most *Tagetes* spp. seem to thrive best in lighter soils and at relatively high temperatures and light intensities. Root systems are well-developed with white, rather fleshy, strongly branched roots. In sandy soils layers of at least 50 cm depth are intensively rooted by *T. patula* Harmony; rootsystems reach maximum diameters of about 180 cm.

Maatsch (1962) classified cultivars of *T. patula* and *T. erecta* according to characteristics of the inflorescence and gave also particulars with respect to height of the plant, habit, vitality and resistance to the impact of rains. *T. patula* Harmony and Golden Harmony were recorded having a strong vitality and good resistance to rains, which agree with our observations.

The height and size of individual plants, and correspondingly the optimum plant-distance to cover the soil, vary strongly between species and varieties. Table 6 records, that height of the 17 species and varieties tested varied from 20 to 140 cm; dwarf, medium high and high types occur among cultivars of *T. erecta* as well as those of *T. patula*. The variation in height within the genus is known to be much greater; *T. minuta* may reach a height of more than 3 m on the slopes of the Himalaya mountains. Plate II A shows the habit of about two-months-old *T. patula* Golden Harmony, *T. signata* Golden Gem and *T. erecta* Aurantiaca on peaty sand.

T. patula Harmony and Golden Harmony appear in this test as well as in several other field trials as cultivars of medium height which are relatively easy to grow and to handle as a crop.

5.2.3. *Discussion*

T. patula appears to be more effective against *Pratylenchus* and at least as effective against *Meloidogyne* populations than other *Tagetes* spp., and its cultivars Harmony and Golden Harmony are most widely tested. These cultivars seem to be as good or better than other *Tagetes* cultivars to grow and to handle as a crop. On the basis of present knowlegde, therefore, *T. patula* Harmony or Golden Harmony have to be advised for experimental application in practice. The nematicidal effect of these cultivars against plant nematodes is strong enough to be content with. It is clear from the data in Section 5.2.2. that their cultivation as a soil hygienic crop meets several practical difficulties. These may be inherent to all *Tagetes* spp. and cultivars, but a more complete inventory of characters is necessary to analyse the agricultural potencies of the cultivars chosen and those of other *Tagetes* spp. and cultivars. Towner (1961) considers T. patula as a tetraploid offspring from *T. erecta* × *T. tenuifolia*, a concept which is not supported by the extreme nematicidal effect of *T. patula*. At any rate breeding of *Tagetes* spp. appeared to be effective in causing a great variation in the *Tagetes* assortment as ornamentals. This suggests good perspectives for the plant breeder to combine strong nematicidal effect with useful agricultural properties in varieties to be used for nematode control in practice. Seed qualities, rapid development as an autumn crop and winter hardiness could all be improved whereas a search for rentable use of the harvest is desirable. The desired characters will depend on the type of agriculture and the circumstances of application.

5.3. Use of tagetes

Nematode control is costly by all practical means but it is nevertheless performed or desired in many cultures. The usefulness of *Tagetes* in such cultures depends on the profitability of its aplication, in comparison to other methods. This is determined by yield increase, whether related to nematode control or other favourable effects, minus the cost of application. Both yield increase and costs of application vary with the type of agriculture and the method of application of *Tagetes*.

Tagetes may under Dutch conditions be grown as a full crop throughout the growing season, as a second crop in the spring before or in the autumn after a main crop, or simultaneously with the main crop under and around its plants or in rows between.

Tagetes grown as a full crop throughout the season is found effective by several workers. It may cause striking growth increase in nematode susceptible crops as demonstrated in Plate IIB. It is, however, expensive, mainly because no cash crop can be grown during the season. It may find application in areas where valuable main crops are grown and where land rent is low, as in some tropical and subtropical countries. A guide for growing *T. patula* Harmony given by Oostenbrink and coworkers of the Plant Protection Service (cf. also Meyneke & Oostenbrink, 1958) is as follows. Four kg of seed per ha, sown in rows 25 cm

apart in April; little or no fertilizer is necessary. The crop may be ploughed into the soil as green manure or as dry stems and leaves after frost had killed the plants. Sowing by special machines is possible. Early weeds may be suppressed by a pre- or post-emergence treatment of sown *Tagetes* with 4 kg Chloro IPC per ha. The crop does not require special care throughout the rest of the season. The cost of a full-season crop of *Tagetes*, apart from land rent, is estimated at about Df 400 (Df 100 for seed, Df 100 for fertilizers and chemicals and f 200 for labour and use of machines).

Tagetes as a spring crop does not seem to be promising due to its slow seedling growth. There are better possibilities for growing it as an autumn crop after early main plants. Oostenbrink, Kuiper & s'Jacob (1957) recorded postplant *Pratylenchus* densities per 100 ml of soil of 320, 173 and 30 after potato, potato + autumn *Tagetes* and full-season *Tagetes* respectively. Trials to study the relation between sowing time and effect on nematodes and on yield of subsequent crops are recorded under Sections 5.3.1, 5.3.2 and 5.3.3.

Simultaneous cultivation of *Tagetes* with a main plant appeared to be effective around and between trees and woody ornamentals (Oostenbrink, Kuiper & s'Jacob. 1957; Meyneke & Oostenbrink, 1958) and may be promising in more cases. Sowing under cereals and other high crops was not promising in preliminary trials since only few weak *Tagetes* plants survived. It is interesting to note that *Tagetes* is widely grown in rows about 2 meters apart between root-knot susceptible crops on infested land in Uttar Pradesh, India. This *Tagetes* culture is old; the application in regular patterns especially between root-knot susceptible crops is based on experience and may be factual nematode control although growers are not conscious of the investation (Plate II C). Trials to study the effect of row distance on the *Tagetes* effect are recorded under Section 5.3.4.

Our own trials on the practical evaluation of *Tagetes*, therefore, are restricted to growing *Tagetes* as a second crop before or after a main crop.

5.3.1. *Effect of T. patula, T. erecta, T. signata and T. minuta sown at different dates*

On a peaty sand soil with a dense population of *P. penetrans* at Nieuwe Pekela 4 *Tagetes* spp. and red clover were sown compared to fallow. Seeds were sown in a square pattern at 25 cm from each other, in well-separated plots of 3 m × 2.5 m on three different dates, viz. 15 May, 1 July and 15 August 1964. The trial was in a block design with 3 replicates. Initial nematode densities were determined per plot at the moment of sowing, therefore at 15 May, 1 July and 15 August on the plots sown at that particular date; the final density was determined in all plots in the middle of November at which moment all plants were killed by frost except red clover and the last sown *T. minuta*. There were 3 fallow plots in total; the densities in these plots were determined repeatedly, namely at each sowing date and in November. Final densities were also expressed as a percentage of initial densities. Numerical data were not statistically analysed since there was too great a variation in initial densities and percentages survival of nematodes among replicates of some plant treatments. The results summarized

in Table 23, therefore may serve to give a general impression only.

Clover sown on 15 May increased the population density in the soil, but clover sown on 1 July and 15 August suppressed it to about one third. This is probably no real suppression, because it is normal for young host plants to harbour most of the population inside the roots and to require some time before a high density around the roots is restored after exhaustion of the population in the early stages of infection, as was shown earlier. Cf. also Oostenbrink, Kuiper & s'Jacob (1957).

TABLE 23. Postplant density of *P. penetrans* per 300 ml of soil after four different species of *Tagetes* and red clover, sown at three different dates, compared to fallow soil. Between brackets postplant densities as percentage of initial densities. Field trial on peaty sand soil in 1964. Final densities determined medio November.

Sowing dates: Crops	15. May	1. July	15. August
T. patula Golden Harmony	20 (1)	15 (3)	255 (18)
T. erecta Aurantiaca	100 (28)	152 (26)	315 (22)
T. minuta	280 (22)	[1])	395 (39)
T. signata Golden Gem	100 (7)	80 (8)	145 (15)
Red clover	1725 (134)	400 (38)	495 (33)
Fallow	555 (21)[2])	555 (22)[2])	555 (31)[2])

[1]) Not determined
[2]) The same 3 plots were used for consecutive samplings

All *Tagetes* spp. have suppressed the populations in comparison to red clover and fallow at all sowing dates when the actual numbers of nematodes are considered; *T. patula* Golden Harmony again was more effective than the others. There is not much difference between *T. patula* sown on 15 May and 1 July; the plants sown on 15 August were less effective. The same applies for *T. signata* and *T. erecta; T. minuta* sown in August did not cause more reduction of population than when sown in May.

5.3.2. *Effect of T. patula Harmony sown or broken up at different dates.*

Table 24 summarizes the results of a similar field trial as described under Section 5.3.1., now on a *P. crenatus* infested silty sand soil at Ellecom in 1960 with *T. patula* Harmony. Fallow plots were compared to plots sown with *Tagetes* on 1 May, 1 June and 1 July. There were 2 plots of $8\frac{1}{2}$ m × $2\frac{1}{2}$ m per treatment. All plants were dug in on 15 August. *Pratylenchus* densities of all plots were determined on 1 May, before the trial, and in Februari next, therefore after the trial.

The data indicate effective suppression, to below 4 % when *T. patula* Harmony is grown from 1 May or 1 June to 15 August, therefore, for at least $2\frac{1}{2}$ months.

TABLE 24. Final density of *P. crenatus* per 200 ml soil after *T. patula* sown at three different dates and dug in on 14. August compared to fallow. Between brackets final densities (determined February 1961) as percentages of initial densities (determined 1 May 1960). Field trial on silty sand. Figures are means of 2 replicate plots per treatment.

T. patula Harmony, sown 1 May	90 (3.4)
T. patula Harmony, sown 1 June	70 (3.7)
T. patula Harmony, sown 1 July	180 (6.6)
Fallow	725 (41)

Growth from 1 July to 15 August, for 1½ months, suppressed the *Pratylenchus* density to about 7%, and was therefore only a little less effective.

5.3.3. *Effect of T. patula Harmony in a practical design.*

In number VI of the cross-trials described under Section 3.2.2.b *T. patula* Harmony was included as a full-season crop and also in a more practical way as an autumn crop, and the effect was studied. The soil is a sand at Zeijerveld with *P. penetrans* + *crenatus* and *Tyl. dubius* at high and some other plant nematodes at low densities. The trial started in 1961 and is still going on. *Tagetes*, pea + autumn *Tagetes*, pea, and other crops are grown as strips in one direction in 1961, 1963, 1965 and 1967 and at right angles across these strips in 1962, 1964, 1966 and 1968. All crops appear in this scheme as preceding crops and as test crops. Pea + autumn *Tagetes* is therefore grown as monoculture, and there are series of two-year rotations with all other crops.

The preplant density of *Pratylenchus* and *Tylenchorhynchus* in the field before the 1961 season did not differ much. The postplant *Pratylenchus* densities in the monoculture plots of *Tagetes*, pea + *Tagetes* and pea respectively was 30–35–195 after the 1961 crops, 5–60–160 after 1962 and 0–36–500 after 1966. This shows that in the monoculture plots the effect of pea + autumn *Tagetes* was marked, but less than the effect of full-season *Tagetes*.

This correlates with the growth response of the 1962 crops grown across the 1961 strips. Full-season *Tagetes* was generally the best, pea + *Tagetes* as autumn crop was the second best preceding plant for the 1962 crops, except for beet and *Tagetes* itself which probably were the only 2 plants which were not susceptible to the *Pratylenchus*- and *Tylenchorhynchus* population present. Table 25 gives details on the yield of all 9 crops in 1962 following *Tagetes*, pea + *Tagetes* and pea.

In 1963 all crops were grown with rows in the other direction. *Tagetes* 1962 as full-season crop was again the best preceding plant for 1963 crops, except *Tagetes* itself which was slightly better following grass-clover. *Tagetes* 1962 as an autumn crop after pea (= pea + *Tagetes*), however, showed hardly any effect and was not or only slightly better than pea. The nematode figures for 1962/1963 confirm that, like in 1961, the *Tagetes* autumn crop has not been effective in 1962. On the monoculture plot of pea + *Tagetes* the *Pratylenchus* population rose from 35 to 60. The other plots grown with pea + *Tagetes* in 1962 were as

TABLE 25. Yield of 9 different crops following one year of *Tagetes*, pea + *Tagetes* as an autumn crop and pea. The yields following pea are put as 100. Sandy soil at Zeijerveld with high densities of *P. penetrans* + *P. crenatus* and *Tyl. dubius* to start with.

Test plant 1962 \ Preceding plant 1961:	Pea	Pea + *Tagetes*	*Tagetes*
Grass/clover	100	128	149
Potato (tubers)	100	110	130
Beet (roots)	100	101	103
Barley (seed) I	100	149	149
Barley (seed) II	100	109	106
Pea (pods) I (= + *Tagetes*)	100	145	162
Pea (pods) II	100	121	138
English raygrass	100	129	151
Tagetes	100	100	100

heavily infested as the corresponding plots with pea only at the end of the 1962 season. This is undoubtedly due to the fact that all main crops were late in 1962, so that the autumn *Tagetes* could not be sown in this year until 15 August and reached little development. The degree of development of an autumn crop of *Tagetes* is evidently critical for its effect, and this varies strongly from year to year. The data on sowing time and development of *T. patula* Harmony following greenharvested pea of period 1961–1968 are collected in Table 26.

The test crops of 1962 and of 1963 followed one season's application of autumn *Tagetes*. The results in later years indicate that continuous application of autumn *Tagetes* increases its effect and therefore limits the risk of annual variation. This resulted in a markedly improved growth of the 1968 pea crop. It appears that pea + autumn *Tagetes* maintained a good pea crop as two-year

TABLE 26. Sowing dates and development of autumn *T. patula* Harmony following peas (*Pisum sativum* Rovar) harvested green for pods. The sowing dates of *T. patula*, as early as possible, were determined by the dates on which the pea crop could be harvested. Between brackets: second sowing date due to failure of first sowing.

Year	Sowing dates	Amount of seed per ha	Row distance in cm	Development
1961	25 July	5 kg	20 cm	Very good
1962	14 August	5 kg	20 cm	Very poor due to late start
1963	11 July (1 August)	5 kg	25 cm	Very poor due to late start
1964	6 July	5 kg	25 cm	Good
1965	22 July (10 August)	5 kg	25 cm	Very poor due to late start
1966	25 July	5 kg	25 cm	Poor due to rabbit damage
1967	24 July	5 kg	25 cm	Good
1968	22 July	5 kg	25 cm	Very good, heavy crop

rotation with other crops, whereas pea without autumn *Tagetes* did not, unless on the plot where pea + *Tagetes* preceded in the alternate years. This illustrates the yield improving effect of autumn *Tagetes* in two ways.

5.3.4. *Effect of T. patula, T. erecta and T. signata at different plant distances*

Side by side with the trial described in Section 5.3.1. on peaty sand with *P. penetrans*, three *Tagetes* spp., namely *T. patula* Golden Harmony, *T. erecta* Aurantiaca and *T. signata* Golden Gem were each sown in square pattern at 4 row distances, namely 10 cm, 25 cm, 40 cm, and 60 cm. The trial was set up in a block design with 3 replicates per treatment. Initial nematode densities were determined per plot at the date of sowing on 15 May, on 1 July, 17 August and medio November. There were fallow plots for comparison, namely the same fallow plots used in the trial 5.3.1. which were completely comparable.

The results are summarized in Fig. 10. All three *Tagetes* spp. suppressed the *Pratylenchus* population more rapidly and to a lower level than fallow. The population under *Tagetes* dropped steeply at the beginning whereas in fallow soil the population hardly decreased in the first 2 months. The processes, probably suppression and starvation respectively, must therefore be entirely different. *T. patula* acted more rapidly than the 2 other species; the final densities on 15 November were also lowest, apparently followed by *T. signata* and then by *T. erecta*, which is in accordance with former results. Decrease of the population occurs earlier at smaller plant row distances in all 3 species. This is clear on 1 July. However, it disappears again to a large extent in the course of the season and the final densities in November are low at all row distances. This means that the equilibrium density corresponding to each of the *Tagetes* spp. is reached at a row distance of 60 cm as well as at a row distance of 10 cm, although at a later date at the end of the growing season.

5.3.5. *Discussion*

T. patula and other *Tagetes* spp. apparently lost part of their effect against *P. penetrans* when they were sown on 15 August compared to 1 July (Table 23). *T. patula* grown in the spring caused very effective suppression when grown from 1 May or 1 June to 15 August, therefore for more than 2½ months; growth from 1 July to 15 August, therefore 1½ months, was not much less (Table 24). A flourishing crop of *Tagetes*, therefore, requires 1½ to 2½ months to be fully effective; autumn *Tagetes* should be sown between 1 July and 15 August to maintain its full effect. The last point is evidently important for a practical set-up. Autumn *Tagetes* may be very effective in nematode-suppression as well as yield increase in certain years (Table 25); its effect, however, depends on the degree of development, which can be reached after a main crop and this may vary strongly from year to year in practice. The earliest sowing dates after an early pea crop harvested green for pods varied in the period 1961–1967 from 6 July to 14 August and a good development of *Tagetes* was obtained in only 4 out of 8 years. Continuous application of autumn-*Tagetes*, however, may probably be effective despite failures in certain years. Decrease of the *Pratylenchus* population occurs

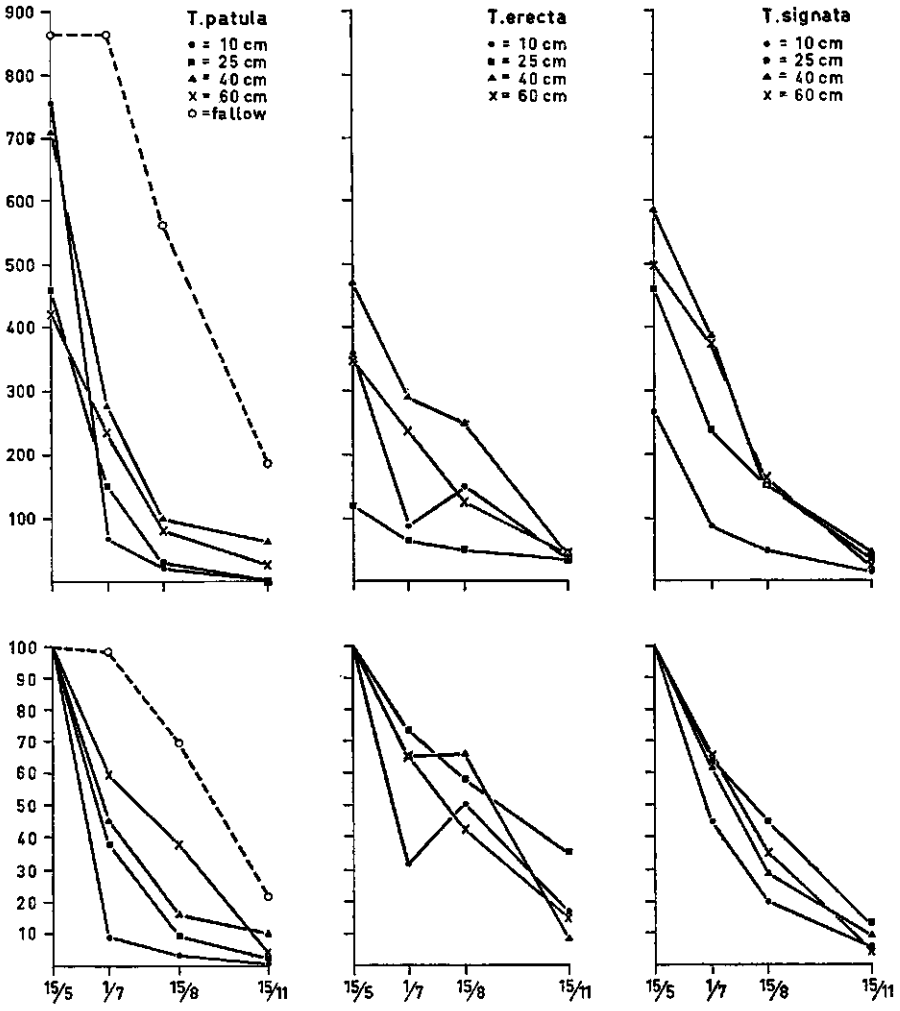


FIG. 10. Effect of *T. patula* Golden Harmony, *T. erecta* Aurantiaca and *T. signata* Golden Gem on a population of *P. penetrans* in peaty sand soil at plant row distances of 10 cm, 25 cm, 40 cm and 60 cm, compared to fallow, as indicated in the graphs.
Abscissa of individual graphs: different sampling dates to determine the population density. Ordinate of individual graphs: number of *Pratylenchus* per 100 ml soil as an average of 3 plots (top row of graphs) or average calculated as a percentage of density at sowing date, 15 May 1964 (bottom row of graphs).

earlier at smaller sowing distances, though the same equilibrium density is reached at 10 or 60 cm row distance under a full-season crop. For the application of autumn *Tagetes* sowing not later than the end of July and application of a sowing distance of 10 rather than 25 cm is advisable.

The fact that a full effect is finally also reached at a row distance of 60 cm, and perhaps more, supports the possibility of full-season *Tagetes* between rows of a main crop. This is evidently practiced on a limited scale, consciously for *P. penetrans* control in some Dutch nurseries and perhaps based on experience without knowledge about the mechanism of the effect in other cases (Plate IIC, D).

Autumn application after a main crop and application between rows of a main crop may both have practical value under certain conditions. The technical possibilities of application increase much when climate allows a better autumn growth, or when agricultural conditions allow a full-season crop.

5.4. Significance of nematicidal and other growth improving effects of Tagetes on apple seedling in soil with P. penetrans

Yield increase of daffodil (Van den Berg-Smit, 1953; Slootweg, 1956) and of many other plants (Oostenbrink, 1960) were the motive for research on *Tagetes* in the Netherlands and elsewhere. Oostenbrink (1960b) recorded specific yield increases of 10–40% in most crops grown after *Tagetes* in light soils. The effect of a full-season crop of *Tagetes* sometimes increased yields of subsequent crops for 3 years; two-year rotation with *T. patula* also gave better yields than with other plants or fallow. *Tagetes* suppressed plant nematodes, especially *Pratylenchus* spp. and the most responsive plants were also generally very susceptible to the nematodes present. The nitrogen level in the soil after *Tagetes* was decreased rather than increased, in comparison to other crops. Oostenbrink supposed that yield responses were mainly due to nematode suppression, though the role of other factors was not excluded. Seinhorst (1963) obtained higher yields of some plants after *Tagetes* than after some other crops in pots without the presence of noxious nematodes.

Yield increase may be due to nematological or to other growth factors or to both at the same time. The nematicidal effect of *Tagetes* has been extensively documented in the previous chapters. However, *Tagetes* may be expected to cause other biological and also chemical and physical changes in the soil. The question is not whether such changes occur, but how important they are as a cause of certain growth responses in plants due to preceding or contemporal growing of *Tagetes*. This problem was studied in the following trials in which apple seedling was grown on nematode infested and uninfested soil after different treatments with growing *Tagetes* plants and with dressings or mulches of *Tagetes* roots and leaves.

5.4.1. *The influence of a T. patula culture*

The soil used was peaty sand partially sterilized by heat. Part of it was left unused for 3 months whereas in the other part a monoculture of *P. penetrans* was reared on red clover. The infested soil was then homogenized by sieving and mixing and the *P. penetrans* density in the soil was checked. Glass tubes were filled with 130 ml of soil, one series with infested and another with uninfested soil. Half the number of tubes of each soil were left fallow; the other half was planted with two-weeks-old seedlings of *T. patula*, which plant was grown for 6 weeks[1]. Then the nematode population in soil and roots if present, was determined in 4 tubes of each of the 4 series, namely *Tagetes* and fallow series of both soils. Eight other tubes of each series were prepared as follows. The soil of fallow tubes was taken from the tube, mixed again and replaced into the tube. The contents of *Tagetes* grown tubes were also taken from the tube, all leaves and stems of the plants were removed, all roots were cut into small pieces and mixed with the soil and returned into the tube. Six weeks later a two-weeks-old seedling of apple was planted and grown as a test plant in each tube. The variety of apple used in this and all following trials was Bittenfelder. Increase of shoot length, dry weight of whole shoots and *Pratylenchus* density in soil and roots were determined 8 weeks later. The results are summarized in Table 27.

The results show that the growth of the test plant apple was promoted by *Tagetes* to 167% of fallow in the infested soil, and was decreased in the uninfested soil. No fertilizer was given in any stage of the trial. The uninfested soil was evidently less fertile than the infested soil, for apple following *Tagetes* showed a smaller increase in length in the uninfested soil. This is probably a nitrogen effect due to the initial growth of red clover for reproduction of *P. penetrans* in the infested soil only.

The fertility of the uninfested soil has further been reduced by *Tagetes* in comparison to fallow, probably again due to loss of nitrogen and other nutrients when the leaves and stems of the *Tagetes* plants were removed.

A lower degree of fertility due to removal of the *Tagetes* tops must also have existed in the infested soil. This means, that the *Tagetes* effect due to suppression of the nematode would have promoted growth of apple seedling to a degree higher than the 167% recorded, if the decrease of soil fertility in the Tagetes grown tubes had been corrected with fertilizer.

Tagetes reduced the nematode density to 403 per tube, or about 20% of fallow. Remarkably the population after *Tagetes* had significantly been further reduced to 71 per tube when the test crop apple was harvested, whereas the population after fallow had increased markedly at that time. This is probably due to a continued suppressive influence of *Tagetes* during the 6 weeks between removal of *Tagetes* and planting of the test crop apple. It must be noted that the *Tagetes* roots were left incorporated in the soil. Probably nematodes surviving the *Tage-*

[1] Apple seedling was also sown as a "preparatory" plant in this trial but it appeared that this plant introduced "specific apple replant disease", as described by Hoestra (1968).
Since this effect had nothing to do with the *Tagetes* effect, nor with normal manure or soil improving influences, it was left out to avoid unnessary complication of the picture.

TABLE 27. Effect of *T. patula* (= T.) grown for six weeks in comparison to fallow on density of *P. penetrans* (= P.) and on growth of a test plant, apple seedling, in infested and in uninfested soil.
Tubes with 130 ml of soil; number of nematodes are means from 4 and plant data from 8 replicates.

Soil treatment	P. per tube after T. and fallow, and before test plant Soil + roots	Test plant apple harvested 8 weeks after planting Increase of shootlength in mm	Dry weight of plant in mg	P. per tube after harvest of test plant Soil + roots
Infested soil (2037 *P. penetrans* per tube reared on red clover):				
T. patula	403	20	310	71
Fallow	1741	12	272	6601
Uninfested soil:				
T. patula	0	13	258	0
Fallow	0	20	354	0

tes treatment were not killed directly but were made less infective or less fertile and that their death was indirectly caused by *Tagetes*. There are, however, no further data to support or reject this possibility. It is at any rate clear that the nematicidal effect of *Tagetes* may continue for some time after the tops have been removed.

5.4.2. *The incorporation of mulches of Tagetes roots and of Tagetes leaves*

To study their direct influence, uninfested *Tagetes* root tissue and uninfested *Tagetes* leaf tissue were incorporated as a mulch into soil with an established population of *P. penetrans* and in uninfested soil. The influence of these mulches on the nematodes and on apple seedling grown as a test plant was studied.

a. *Root mulch into infested and uninfested soil*

Infested and uninfested soil were obtained as described under Section 5.4.1, starting from sterilized peaty sand. *P. penetrans* was again reared to a high density on red clover in the infested soil. Roots were collected from two-months-old seedlings of *T. patula* Harmony and of *Bittenfelder* apple and chopped into pieces of about 1 cm length. Tubes of both soils were mixed with 2 g of *Tagetes* roots, 2 g of apple roots or left untreated. After 6 weeks the nematode densities were determined in 4 tubes per treatment of each soil. Then tubes, 8 per treatment, were planted with 2-weeks-old apple seedling. The tubes were kept moist throughout the trial; no fertilizers were given. Increase of shoot length and nematode densities in root and soil were determined 12 weeks later. The results are given in Table 28.

TABLE 28. Effect of mulch for six weeks with roots of *T. patula* Golden Harmony and apple seedling Bittenfelder in comparison to untreated on density of *P. penetrans* (P.) and on growth of the test plant, apple seedling, in infested and uninfested soil. Tubes with 130 ml soil; 2 g root pieces per tube; number of nematodes are means from 4 and plant data from 8 replicates.

Soil treatments	1 P. per tube after soil treatments and before test plant. Between brackets saprozoic nematodes	2 Test plant apple: increase of shoot length 12 weeks after planting in mm	3 P. per tube after harvest of test plant; soil + roots. Between brackets saprozoic nematodes
Infested soil (1638 *P. penetrans* per tube reared on red clover):			
2 g of *Tagetes* roots	693 (1438)	31	1424 (3737)
2 g of apple roots	1189 (2745)	20	3364 (5183)
Untreated	1445 (811)	20	5744 (2473)
Uninfested soil:			
2 g of *Tagetes* roots	0	85	0
2 g of apple roots	0	92	0
Untreated	0	89	0

The initial infestation of the infested soil was determined as 1638 *P. penetrans* per tube. The population in the fallow tubes dropped slightly, to 88%. In the tubes with mulch of apple roots and *Tagetes* roots the populations dropped to 73% and 42% respectively. The differences in nematode suppression between *Tagetes* on the one side and apple and fallow on the other is highly significant ($p = 0.01$), but the difference between apple and fallow is not significant. The growth of the test plant apple in infested soil is strongly correlated with the nematode density. The test plant grew much better in the uninfested than in the infested soil, the difference in length being significant at the 1% level. Root mulch of apple increased, and root mulch of *Tagetes* suppressed growth of the test plant somewhat, but differences were hardly significant.

The final *Pratylenchus* population on the test plant apple rose in all treatments (Col. 3 in Table 28) compared to the preplant densities (Col. 1), though slightly less in the tubes with *Tagetes* root mulch than in those with apple root mulch and the fallow tubes, namely to 2.1, 2.8 and 4.0 times the preplant densities. This suggests that *Tagetes* root mulch shows a long term nematode-suppressing effect which continues after 6 weeks.

The results indicate that growth promoting *Tagetes* effects in this trial are wholely or largely due to suppression of *P. penetrans.*

b. Increasing dosages of root mulch in infested and uninfested soil

In a trial as described under Section 4.5.2a, 1, 2 and 4 g root pieces of *T. patula*

T. minuta and apple were each incorporated in tubes with soil infested with *P. penetrans*. Tubes with uninfested soil were mixed with 2 and 4 g root pieces of *T. patula* and apple. In both soils untreated control tubes were available. After 6 weeks nematode densities were determined in all tubes with infested soil. The tubes with uninfested soil were then planted with 2-weeks-old apple seedlings and the influence of these mulches was determined 15 weeks later. The results are summarized in Tables 29 and 30.

TABLE 29. Effect of mulch for six weeks with 1, 2, and 4 g root pieces of *T. patula*, *T. minuta* and apple seedling in comparison to untreated on density of *P. penetrans* (P.). Figures are final densities expressed as percentage of pretreatment densities; between brackets the same figures for saprozoic nematodes (S).; all figures are means of 4 replicates.

Soil treatments	Nematodes P. (S.)
T. patula: 1 g – 2 g – 4 g	46 (62) – 34 (87) – 24 (232)
T. minuta: 1 g – 2 g – 4 g	71 (98) – 61 (159) – 45 (379)
Apple: 1 g – 2 g – 4 g	90 (107) – 88 (222) – 72 (423)
Untreated	108 (61)

The *P. penetrans* density in Table 29 increased in the untreated tubes to 108 % after 6 weeks, and the number of saprozoic nematodes dropped to 59 %. This abnormal increase is probably due to the fact that the infested soil was not freed from clover plants in this case until the trial was started, so that part of the *P. penetrans* population was inside undecayed small bits of clover roots left in the soil and which escaped extraction at that moment. The pretreatment densities of *P. penetrans* in the treated tubes must have been underestimated to the same extent and the actual survival percentage should have been correspondingly lower than is indicated in the table.

The results indicate that all root mulches were effective in suppressing *P. penetrans*, that the effect decreased in the order *T. patula* – *T. erecta* – apple, and that 4 g per tube was more effective than the lighter dosage with all plants. The saprozoic nematodes were increased by all mulches, evidently in the order *T. patula* – *T. minuta* – apple, and distinctly more when more roots were added. These data confirm the special effect of roots of *Tagetes*, in the first place of *T. patula* on *P. penetrans*.

Observation of root pieces of *T. patula* buried into infested soil in an additional test revealed, that *P. penetrans* entered freshly excised roots, especially through the cut surfaces. The suppressive effect of root mulch may therefore be due to toxic material emanating from the roots but also to nematode kill inside the roots as in growing *Tagetes* plants.

Tagetes root pieces promoted growth of apple seedling in uninfested soil significantly less than apple root pieces. No specific *Tagetes* effect was therefore noticeable in uninfested soil. Cf. Table 30.

TABLE 30. The effect of mulch for six weeks with 2 and 4 g root pieces of *T. patula*, *T. minuta* and apple seedling in comparison to untreated, on length of subsequent test plant, apple seedling, 15 weeks after planting in tubes with 150 ml uninfested soil. Means of 8 replicates.

Soil treatments		Increase of shoot length in mm
T. patula:	2 g - 4 g	88– 67
T. minuta	2 g - 4 g	62– 65
Apple	2 g - 4 g	88–111
Untreated		50

c. Leaf mulch into infested and uninfested soil

The trial in Section 5.4.2a was repeated, now with 4 g leaf tissue of two-months-old *T. patula* and apple plants per tube. Table 31 gives the results.

Leaf mulch of both plants suppressed *P. penetrans* and increased numbers of saprozoic nematodes. Leaves of *Tagetes*, contrary to roots, do not exert an additional effect in comparison to apple leaves on *P. penetrans*. In fact apple leaves were more effective than *Tagetes* leaves in promoting saprozoic nematodes and in suppressing *P. penetrans*. This is still visible in the postplant nematode densities at the end of the trial.

Shoot growth of the testplant is in the unfertilized, uninfested soil slightly more after *Tagetes* leaf mulch than after apple leaf mulch. Growth increase in

TABLE 31. Effect of mulch for six weeks with leaves of *T. patula* and apple seedling in comparison to untreated on density of *P. penetrans* (P.) and on growth of subsequent apple seedling in infested and uninfested soil.
Tubes with 130 ml of soil; 4 g of leaf pieces per tube; nematode counts are means from 4 and plant data from 8 replicates.

Soil treatments	P. per tube after soil treatments and before test plant. Between brackets saprozoic nematodes	Test plant apple: increase of shoot length 12 weeks after planting, in mm	P. per tube after harvest of test plant; soil + roots. Between brackets saprozoic nematodes
Infested soil (2408 *P. penetrans* per tube reared on red clover):			
4 g of *Tagetes* leaves	1183 (7583)	49	10235 (4953)
4 g of apple leaves	543 (16300)	61	2960 (5695)
Untreated	1660 (865)	26	6681 (5270)
Uninfested soil:			
4 g of *Tagetes* leaves	0	149	0
4 g of apple leaves	0	121	0
Untreated	0	71	0

the infested soil, however, is less after *Tagetes* leaf mulch than after apple leaf mulch and is closely correlated with the *P. penetrans* densities. The influence of the nematode factor evidently dominates over the other soil fertility aspects of leaf mulch.

d. Increasing dosages of leaf mulch in uninfested soil

The effect of *Tagetes* leaf mulch was further studied by mixing increasing dosages of leaf pieces of *T. patula*, *T. minuta* and apple seedling into tubes with soil, compared to untreated, and by growing apple seedling as a test plant in a trial as described in Section 5.4.2b. Table 32 shows the results.

All leaf mulch treatments except with 2 g apple leaf promoted growth of the test plant significantly more than the control in this unfertilized uninfested soil. The differences between 2 g leaves on the one side and 4 and 8 g leaves on the other side were highly significant for each plant species. The differences between the 3 plants were not significant, although leaves of *Tagetes* spp. caused more growth than apple leaves in almost all cases.

TABLE 32. The effect of mulch for six weeks with 2, 4 and 8 g leaf pieces of *T. patula*, *T. minuta* and apple seedling in comparison to untreated, on length of the subsequent test plant, apple seedling, 20 weeks after planting in tubes with 150 ml uninfested soil. Means of 8 replicates.

Soil treatments	Increase of shoot length in mm
T. patula 2 g – 4 g – 8 g	112 – 140 – 161
T. minuta: 2 g – 4 g – 8 g	95 – 183 – 181
Apple: 2 g – 4 g – 8 g	76 – 149 – 152
Untreated	57

5.4.3. *Discussion*

The results indicate that in this particular case the suppression of *P. penetrans* was the dominant growth promoting factor of the *Tagetes* effect and that the green manure or other favourable influence as a whole was of secondary importance, even in the unfertilized soil used.

Tagetes grown for 8 weeks suppressed the nematodes effectively and promoted subsequent growth of apple strongly, despite the removal of aerial parts of the *Tagetes*. This is illustrated in Table 27. The results indicate also that the decline of the nematodes continues markedly after the growth of *Tagetes* has been disrupted by removal of the tops and chopping of the roots: the *Pratylenchus* density was 403 per tube (23 % of fallow) at the disruption of *Tagetes* and 71 per tube (1 % of the former fallow tubes) after growing apple for 8 weeks. The 403 nematodes surviving the growing of *Tagetes* plants may have been lethally weakened, or the remaining roots may have continued the process of suppression. Direct mulch with an amount of *Tagetes* roots comparable to the natural pro-

duction per tube (1–4 g) apparently suppresses *P. penetrans* much better than other mulches or fallow (Tables 28, 29), so that the incorporation of roots is at any rate an important part of the specific aftereffect of a *Tagetes* culture. An earlier trial, described in Section 4.2.2.c also indicated that the aftereffect is not great when roots are removed. The amount of roots produced in the field by a full-season crop of *T. patula* Harmony will be about comparable to 1 g per tube as used here. It is, thus, probable that the aftereffect is also significant under field conditions. Leaf mulches (4 g per tube of 130 ml, which is comparable to 56000 kg per ha) were also effective in suppressing *P. penetrans* (Table 31); *Tagetes* leaves, however, were less effective than apple leaves and no specific nematicidal effect of *Tagetes* leaves could be demonstrated. According to Uhlenbroek & Bijloo (1958) leaves of *Tagetes* contain nematicidal polythienyls, though much less than do roots. This coincides with our results.

The results obtained in uninfested soil show, that cultivation of *Tagetes* and removal of the tops decreases soil fertility, probably in the first place nitrogen content, in unfertilized soil (Table 25). The addition of root mulch and leaf mulch increased soil fertility, and therefore growth of apple seedling in most cases (Tables 28, 29, 30). These green manurial and other soil improving effects could be expected; they are not higher for *Tagetes* than for apple mulches. They would probably have been much smaller if the soils used, uninfested as well as infested, were properly fertilized.

The effect of *P. penetrans* on growth of apple seedling is evidently great and proportional to its population densities. It appears from comparison of artificially infested and uninfested soils, and from the correlation between growth reduction and nematode density in trials on infested soil. These data confirm results by Oostenbrink (1955) and by Hoestra & Oostenbrink (1962), who found growth reduction of apple seedling by inoculation of *P. penetrans* and a good correlation between growth reduction of young apple trees and *P. penetrans* density. One of the trials also confirmed the appearance of specific apple replant disease after six weeks' cultivation of apple seedlings in nematode free soil, in agreement with results of Hoestra's work (1968).

The dominance of nematicidal over non-nematicidal effects of *Tagetes* holds for the growth of apple seedling in soil infested with *P. penetrans*. The relative weight of these factors may of course be different in other plant-nematode relationships.

CHAPTER 6

SUMMARY AND CONCLUSION

Published data revealed that *Tagetes* spp. suppress polyphagous endoparasitic root nematodes, that the effect varies, perhaps between *Tagetes* spp. and cultivars, certainly between nematode genera and perhaps between species and strains. The effect is sometimes striking but the picture in general is far from complete and not clear. This situation determined the three objectives of our investigation: occurrence and significance of *Tagetes* effect, interpretation, and possibilities of application in agriculture.

For most of the trials plants were cultivated under controlled and field conditions, and their growth evaluated; nematode populations were collected, cultivated, maintained and transferred; nematodes in soil and plant tissues were counted and results were analysed statistically. Several special techniques were used occasionally as indicated in the relevant sections.

The occurrence and significance of special *Tagetes* effects on plant nematode population were determined with species of *Pratylenchus, Meloidogyne, Tylenchorhynchus, Rotylenchus* and *Helicotylenchus,* other ectoparasitic nematodes, *Ditylenchus* and *Aphelenchoides.*

Pratylenchus spp. were markedly suppressed by *Tagetes* spp. in tube cultures (Tables 1–5) and in field trials (Tables 6 and 7, Figs 1 and 2). This was true for *P. penetrans, P. crenatus, P. neglectus* and probably *P. thornei.* Soil type may be of influence on the result. There were great differences in effectiveness between *Tagetes* spp. and cultivars. The effectiveness against *Pratylenchus* spp. decreases in the order *T. patula, T. erecta, T. minuta,* with *T. patula* markedly better. *T. patula* Harmony suppresses field populations of *Pratylenchus* spp. in a few months and fallow requires a few years to reach a comparable low final density of these nematodes. Density never fell to zero, probably because of limited reproduction on weeds and limited reproduction on *Tagetes* itself if it is grown for a full season. There was no evidence that resistance of *Tagetes* was broken or that nematode strains resistant to *Tagetes* effects arose even after 7–10 successive crops of *T. patula.* Biennial rotations of *T. patula* and good hosts kept *Pratylenchus* spp. at a low density, except the population of *P. thornei* on heavy soil which fluctuated at a rather high level (Fig. 2).

Tagetes spp. were generally as effective or better than fallow in suppressing *Meloidogyne* spp. in tube cultures (Tables 8–12), although with some notable exceptions. *Meloidogyne* larvae were less persistent than *Pratylenchus* larvae in fallow soil. *T. patula* severely suppressed *M. hapla, M. incognita, M. arenaria* and

M. javanica. *T. erecta* was also suppressive but slightly more syncytia formed in all four *Meloidogyne* spp.; *M. hapla* reproduced and maintained a small population on *T. erecta*. *T. minuta* differed markedly from the earlier species in that it suppressed *M. hapla* and *M. incognita* completely and *M. javanica* almost completely, whereas *M. arenaria* could breed on this plant and reach considerable densities in both roots and soil. Any general effect by *Tagetes* on *Meloidogyne* is therefore complicated by certain exceptions which may account for conflicting published results.

Tube and field trials showed that *Tylenchorhynchus* spp. were suppressed by *T. patula* in different soils (Table 7), that *Tyl. dubius* was suppressed better and more rapidly by *T. patula* than by fallow (Fig. 3A, B; literature), and that *T. erecta* and *T. minuta* were about as effective as *T. patula* against *Tyl. dubius* (Table 6). *Tyl. dubius* was suppressed slightly less effectively and less rapidly than *Pratylenchus* spp. in the same soil by *T. patula*

The data on *R. robustus* supports the view that *Tagetes* spp., at any rate *T. patula* Golden Harmony and Harmony, maintains a rather high density. Unexplained peaks of the density under *Tagetes* and fallow make it difficult to indicate any *Tagetes* effect at all; factors other than the presence of higher plants may govern the population dynamics of this species.

The genera *Hemicycliophora*, *Paratylenchus* and *Trichodorus* are not generally suppressed by *Tagetes* cultivars. Specific host-nematode relationships may vary as widely as is the case with these nematodes on other plants, and no special *Tagetes* effect can be demonstrated against these ectoparasites. Some species breed profusely on certain *Tagetes* spp. but are not affected by other *Tagetes* spp.

The stem nematode *D. dipsaci* may reproduce to a limited extent and cause typical symptoms in *T. patula* and *T. erecta*, but not in *T. minuta*. The same is true for the foliar nematode *A. ritzemabosi* (Plate 1A and B).

The data leave no doubt that certain *Tagetes* spp. suppress certain species of plant nematodes unusually strongly. The *Tagetes* effect manifested itself most clearly with *Pratylenchus* spp., *Meloidogyne* spp. and *Tyl. dubius*, but was evidently not present or not marked against *R. robustus* and several other ectoparasitic genera, nor against *D. dipsaci* and *A. ritzemabosi*. The results alone or combined with published data do not fully explain the mechanism. For a further analysis the exoradicular effects, the effects on the surface of the plant or during penetration, and the endoradicular effects were subsequently studied.

Exoradicular effects may contribute to, but not explain the larger part of the *Tagetes* effect. As with the good host red clover, *P. penetrans* is not particularly attracted nor deterred by growing roots of *T. patula* on agar plates, but there was some aggregation around the roots in soil (Table 14), though it concerned only part of the soil population (Fig. 5). Percolates from pots of *T. patula*, red clover, apple or without a plant did not differ in effect on activity or mortality of *P. penetrans in vitro*. Survival of *P. penetrans* in water cultures of *T. patula* and *T. minuta* was only slightly less than in water culture of apple, control solution

or distilled water, and the effect was at any rate slight and unspecific (Table 15).

Tagetes soil was distinctly nematicidal for some days after the roots have been removed. This effect, however, was not very strong and was not specific for *Tagetes*, because red clover was equally and apple even more effective than *T. patula* and *T. minuta* (Table 16). Damage or kill of nematodes outside the root may therefore play a role, but it is apparently not the essential part of the *Tagetes* effect.

Root systems of three different *Tagetes* spp. were penetrated by *M. hapla* larvae as much as root systems of a suitable host. The same holds for penetration by *P. penetrans*, except perhaps for *T. patula* which fewer nematodes entered than other *Tagetes* spp. or good hosts in most trials (Table 17). As a rule only a few of the larvae around the roots succeed in penetrating.

Endoradicular influences comprise nematode survival and development, nematicidal effects and histological reactions in *Tagetes* tissues. *M. hapla* larvae enter *Tagetes* spp. to the same amount as tomato within a week. Only a few of the larvae around the roots succeed, although potential sites for penetration are present in excess. The unsuccessful nematodes outside the root decrease rapidly in number, more rapidly in the presence of growing plants than in fallow soil, which may be important for the population dynamics of nematodes in general. *M. hapla* survives for at least 4 weeks within roots of *Tagetes* spp., but development beyond the infective second larval stage is hardly noticeable in *T. patula* and *T. minuta*, whereas only a few larvae develop and reach adulthood in *T. erecta* (Table 18).

The picture is similar with *P. penetrans*. The percentage penetration is generally low in all plants, but significantly lower in *T. patula* than in *T. erecta*, *T. minuta* or red clover (Table 19, Fig. 6). *T. patula* may resist penetration by this nematode The main difference between a suitable host such as red clover, and *T. patula* is that nematodes reproduce in the first plant and decline or remain few in *T. patula*. In *T. minuta* the nematodes survive and may even develop and multiply, though at slower rate than in red clover (Figs. 6, 7 and 8). *T. patula*, *T. minuta* and red clover represent degrees of host suitability from almost zero through low to very high (Fig. 7, Table 20). The equilibrium density under *T. patula* is very low but not zero because some reproduction occurs when the plants have grown a long while. The percentage males among the sexually differentiated nematodes is not higher in the very resistant *T. patula* and does not increase with ageing of host plants, as has often been published. *T. patula* allows very little escape or survival of *P. penetrans* once the nematodes have entered the roots, whereas the population in *T. minuta* roots does escape and may be infective as in suitable hosts (Tables 21 and 22).

Root extracts of Tagetes spp., contrary to root exudates, contain a nematicidal principle which manifests itself against *P. penetrans in vitro* from the third day on (Fig. 9A, B, C). Extract of *T. patula* is more effective than extract of *T. minuta* and the latter is more effective than extract of potato or control solutions.

Fractioning of root extracts of *T. patula* over a column of Sephadex G-75 indicate high mortality in a later fraction. No attempt was made to identify the active principle(s) in this fraction. This effect *in vitro* may be related to the *Tagetes* effect on nematodes in soil. It may be caused by the nematicides such as the thiophenes isolated from *Tagetes* roots by Uhlenbroek & Bijloo (1958, 1959). There was some nematode kill in potato root extract too, distinctly less than in *Tagetes* extract but distinctly higher than in the control solutions. This indicates the presence of a weak nematicidal effect in potato root extract and may support the indication recorded earlier that plants establish or induce in general an "antinemic potential" by means of their exudates or other substances in water or soil. The *Tagetes* effects are much stronger and evidently differ from it.

Histological reactions as part of the endoradicular influences of *Tagetes* on penetrated nematodes are not conspicuous. *Tagetes* cultures, unlike most other plants, normally grow well and have well-developed root systems without discoloration in soils with dense populations of *Meloidogyne* and *Pratylenchus* spp. Despite this healthy appearance *Tagetes* roots may show barely visible histological reactions after such nematode infestations.

M. hapla larvae penetrate the root apex of *T. patula* in much the same way as that of tomato. They do not normally develop nor cause marked necrosis or swelling in the roots of *T. patula*. Occasionally, however, small syncytia or sometimes even small galls occur associated with a developing larva. Unlike tomato, *T. patula* develops few and very small syncytia and galls and only slowly, and the nematode often dies and syncytia often abort in *T. patula*.

P. penetrans penetrates young roots of *T. patula*, *T. minuta* and red clover at random sites on their surface except at the apex and causes cortical lesions in all three plants. The lesions in *T. patula* are small, dark and necrotic but do not abort from surrounding cortex tissue; they normally harbour only 1–3 nematodes, often dead, dying or twisted. In *T. minuta* the lesions appear slower, are larger and less dark than in *T. patula;* the number of nematodes per lesion may be up to 38 and often occur outside lesions. Red clover lesions appear still slower, are usually larger and contain a large breeding population. Histological reactions, therefore, largely coincide with nematode development.

The endoradicular effects are apparently instrumental in the nematode suppression by *Tagetes* spp. They are incorporated in or super-imposed upon the common plant-nematode relationships which are different for each association and may therefore influence the result. It is suggested that the special nematicidal principle in *Tagetes* is made up of more components, of which thiophenes recorded up to now from *T. erecta*, and that the components or their relative weights vary between *Tagetes* spp. Some other *Compositae* related closely to *Tagetes* spp. were also found to be effective against *P. penetrans* and also contained the same thiophenes as found in *Tagetes* or hitherto unidentified active principles. *T. patula* is probably superior to other *Tagetes* spp. in its effect against *P. penetrans* because necrosis appears earlier and more acutely. This may be a consequence of more rapid intoxication of the nematodes, as in root extracts.

Ectoparasitic cortex feeders of the genus *Tylenchorynchus* are affected less and root-vessel feeders are evidently not influenced by the nematicidal principles in *Tagetes* roots, probably because they do not undergo the same type or degree of contact with the *Tagetes* tissue. The *Tagetes* effect, therefore, seems to be generally strong for endoparasitic root nematodes or cortical feeders, but varies even within this group with the different plant-nematode associations.

The agricultural value of *Tagetes* as a source of organic matter, stains, therapeutics, or other chemicals, and as ornamentals is limited and has up to now supported only small-scale cultivation. The use of *Tagetes* crops for suppression of plant nematodes and the marked growth improvement obtained in main crops, is handicapped by the lack of value of the crop. Furthermore *Tagetes* spp. and cultivars are limited in their agricultural applicability. Nematode suppression is the primary determinant of their practical value so that *T. patula*, particularly the cultivars Golden Harmony or Harmony are recommended. They are more effective against *Pratylenchus* and at least as effective against *Meloidogyne* populations, and appear to be as good or better than other *Tagetes* cultivars for growing and handling as a crop. Seed characteristics, rapid development as an autumn crop and winter-hardiness could all be improved, whereas a search for profitable use of the crop is desirable. Breeding of *Tagetes* spp. have resulted in a great assortment of ornamentals, and prospects seem good for the plant breeder of combining strong nematicidal effect with useful agricultural properties.

The desired characters will depend on the type of agriculture. *Tagetes* grown as a full-season crop has been found effective by several workers and may cause striking growth and yield increments, it may find use in areas where valuable main crops are grown and where land rent is low, as in some tropical and subtropical countries. *Tagetes* as a spring crop does not seem promising in temperate climates due to its slow seedling growth. Even when sown densely, 10 cm apart on 15 May, minimum densities of *Pratylenchus* could be achieved only after about 2 months; these densities appear later when greater plant distances are taken (Fig. 10). *Tagetes* grown as an autumn crop has better possibilities. The degree of development of autumn *Tagetes* is evidently critical for its effect, and this varies strongly from year to year. Autumn *Tagetes* may be very effective in nematode suppression as well as in yield increase of main plants in certain years (Tables 23, 24 and 25), but the earliest sowing dates after an early pea crop varied in the period 1961–1968 from 6 July to 14 August, and a good development was obtained in only 4 out of 8 years (Table 6). Continuous autumn *Tagetes*, however, may be effective despite failure in certain years. Autumn *Tagetes* should be sown not later than the end of July and 10 rather than 25 cm apart is advisable.

Simultaneous culture of *Tagetes* with a main crop appeared to be effective around and between trees and woody ornamentals and may be promising in more cases, especially since sowing at 60 cm apart completely suppressed nematodes, though slower than when closer sown. Sowing between rows is practized incidentally (Plate IIC and D). Sowing under cereals and other high crops to give the crop a quicker start after harvesting the main crop has not so far been

promising, because only few weak plants survived.

Autumn application after a main crop and application between rows of a main crop may be promising under certain conditions. The technical possibilities increase when climatic conditions allow a better growth in the autumn or when agricultural systems allow a full-season crop.

Yield of main crops after *Tagetes* may be increased by nematological or other growth factors or both. *Tagetes* promoted growth of apple seedlings in soil with *P. penetrans* to 167% of fallow infested soil, though *Tagetes* decreased growth in uninfested soil; allowance for the nitrogen used by *Tagetes* would lead to a higher growth promotion in infested soil (Table 27). The decline in nematode population continued markedly after the growth of *Tagetes* had been disrupted by removal of the tops. Direct mulch with a natural dosage of *Tagetes* roots suppresses *P. penetrans* much better than other mulches or fallow. It is probable that there is a significant aftereffect also under field conditions. Leaf mulches were also effective in suppressing *P. penetrans*, but *Tagetes* leaves were less effective than apple and no specific nematicidal effect of *Tagetes* leaves could be demonstrated. The results in uninfested soil show that cultivation of *Tagetes* and removal of tops decreases soil fertility in unfertilized soil (Table 27). The addition of root or leaf mulch generally increases soil fertility and therefore growth of test plants (Tables 30, 31, 32). These effects, however, are unspecific, and would have been smaller in fertilized soils, uninfested or infested. The dominance of nematicidal over non-nematicidal effects of *Tagetes* is clear for the growth of apple seedlings in soil infested with *P. penetrans*. The relative weight of these factors may of course be different in other plant nematode relationships.

Our results, therefore, confirm or substantiate the marked *Tagetes* effect, but also the variation between plant-nematode relationships. The *Tagetes* effect is exceptional in nature, though not completely restricted to the genus *Tagetes* because it does occur in some genera of the *Heleniae*. The effect appears to be centred inside the roots and is evidently correlated with the presence in these plants of strongly nematicidal thiophenes, which are rare elsewhere in nature. The fact that it is conspicuous against endoparasitic polyphagous root nematodes and not against epidermal and vessel feeders is understandable from the difference in parasitic way of life.

Histopathological reactions to nematode invasion vary between *Tagetes* spp. This may explain the stronger effect of *T. patula* on *P. penetrans* than of other *Tagetes* spp. The concept of nematode intoxication by nematicidal thiophenes and histopathological resistance phenomena may be interrelated and are not necessarily contradictory.

Use of *Tagetes* spp. often markedly increases yield of main crops, but is limited by type of agriculture. In temperate regions *Tagetes* grown simultaneously with the main crop or grown in the autumn after the main crop is considered promising. Slow seedling growth, high light requirement and frost susceptibility of available cultivars are disadvantages. *Tagetes* has better prospects in tropical and subtropical agriculture.

SAMENVATTING EN CONCLUSIES

Uit de literatuur bleek, dat *Tagetes* spp. de populatie van polyphage endoparasitaire wortelnematoden drukken en dat dit effect waarschijnlijk varieert tussen de verschillende *Tagetes* spp. en cultivars, in ieder geval ten aanzien van verschillende nematoden geslachten en mogelijk ook tussen soorten en rassen. In bepaalde gevallen is dit effect opvallend, maar in het algemeen is het beeld onvolledig en onduidelijk. Deze omstandigheid bepaalde de drie doelstellingen van dit onderzoek: het nagaan van vóórkomen en betekenis van *Tagetes* effecten, de interpretatie van deze effecten, en de landbouwkundige toepassingsmogelijkheden.

De in dit onderzoek regelmatig gebruikte methoden werden beschreven; deze omvatten methoden voor het kweken van gewassen onder gecontroleerde en onder veldomstandigheden, groei- en opbrengstbepalingen van gewassen, het verzamelen, kweken en inoculeren van nematoden, het bepalen van de populatiedichtheid van nematoden in de grond en in plantenweefsels en de wiskundige verwerking van proefresultaten. Bij kasproeven werden als regel kweekbuizen gebruikt, waarin planten in al dan niet besmette grond werden gekweekt. In sommige gevallen werden speciale methoden gebezigd; deze zijn in de desbetreffende hoofdstukken beschreven.

Het vóórkomen en de betekenis van *Tagetes* effecten werd bepaald ten aanzien van soorten van *Pratylenchus*, *Meloidogyne*, *Tylenchorhynchus*, *Rotylenchus* en *Helicotylenchus*, andere ectoparasieten, *Ditylenchus* en *Aphelenchoides*.

Zowel in buiscultures (Tabellen 1–5) als in het veld (Tabel 6 en 7; Fig. 1 en 2) drukten *Tagetes* spp. de populatie van verschillende *Pratylenchus* spp. in de grond, nl. *P. penetrans*, *P crenatus* en *P. neglectus* opmerkelijk sterk. Het is mogelijk dat de grondsoort invloed heeft op dit effect. Er kwamen grote verschillen voor in effectiviteit tussen *Tagetes* spp en varieteiten daarvan. De effectiviteit vermindert in de volgorde *T. patula*, *T. erecta*, *T. minuta*, waarbij *T. patula* opvallend effectiever is dan beide andere soorten. Onder veldomstandigheden werden *Pratylenchus* spp. door *T. patula* Harmony binnen enkele maanden tot zeer lage dichtheden gedrukt, terwijl onder braak vergelijkbare dichtheden pas na enkele jaren werden bereikt. Een dichtheid nul wordt nooit bereikt, vermoedelijk doordat enige vermeerdering zowel op onkruiden als op *Tagetes* zelf mogelijk is wanneer deze laatste gedurende een heel groeiseizoen wordt geteeld. Er zijn geen aanwijzingen dat de resistentie van *Tagetes* doorbroken wordt of dat nieuwe rassen ontstaan die resistent zijn tegen het *Tagetes* effect, zelfs niet na 7–10 opeenvolgende jaren teelt van *Tagetes*. Bij tweejarige rotatie van *T. patula* met goede waardplanten bleef de populatie dichtheid van *Pratylenchus* spp. op

een laag niveau gehandhaafd, behalve die van *P. thornei* op zware gronden, die op tamelijk hoog niveau fluctueerde (Fig. 2).

Op enkele uitzonderingen na werden populaties van *Meloidogyne* spp. door *Tagetes* spp. even effectief of zelfs beter gedrukt dan door braak (Tabellen 8–12). In braak grond zijn *Meloidogyne* larven minder persistent dan *Pratylenchus* larven. *T. patula* bleek zeer effectief te zijn tegen *M. hapla, M. javanica, M. incognita* en *M. arenaria. T. erecta* was eveneens effectief, maar alle genoemde *Meloidogyne* spp. induceerden deze *Tagetes* spp. tot een grotere mate van vorming van voor de voeding van de nematoden noodzakelijke syncytia. *M. hapla* bleek in staat te zijn zich op *T. erecta* op beperkte schaal te vermeerderen en een bescheiden populatie op te bouwen. *T. minuta* verschilt sterk van beide andere *Tagetes* spp. doordat het een tamelijk goede waardplant is van *M. arenaria.* Vermeerdering van *M. javanica* op *T. minuta* kwam incidenteel voor, terwijl *M. hapla* en *M. incognita* door dit gewas volkomen werden onderdrukt. De mogelijkheid dat het *Tagetes* effect algemeen geldt ten aanzien van *Meloidogyne* spp. wordt hierdoor in twijfel getrokken.

Buis- en veldproeven toonden aan dat *Tylenchorhynchus* spp. in verschillende gronden door *T. patula* worden gedrukt (Tabel 7), dat *Tyl. dubius* beter en sneller wordt gedrukt door *T. patula* dan door braak (Fig. 3A en B; overzicht van gegevens uit de literatuur), en dat *T. erecta* en *T. minuta* ten aanzien van *Tyl. dubius* in effectiviteit niet verschilde van *T. patula* (Tabel 6). *Tyl. dubius* werd in dezelfde grond minder effectief en minder snel gedrukt dan *Pratylenchus* spp.

De gegevens in 3.5 bevestigen de bevinding, dat bij de teelt van *Tagetes* spp., in ieder geval van *T. patula* Harmony en Golden Harmony, *R. robustus* zich op tamelijk hoge populatie dichtheden kan handhaven. De dichtheidscurven kunnen bij de teelt van *Tagetes* evenals bij braak pieken vertonen, waardoor het niet mogelijk is aan te tonen dat in dit geval sprake is van enig *Tagetes* effect; het is mogelijk dat andere factoren dan de aanwezigheid van hogere planten een overheersende rol spelen in de populatie dynamiek van deze methode.

Blijkens de gegevens in 3.6 worden *Hemicycliophora, Paratylenchus* en *Trichodorus* in het algemeen niet gedrukt door *Tagetes* spp. De relatie plant-parasiet kan in het geval met *Tagetes* even grote variaties vertonen als tussen deze nematoden geslachten en andere planten; er is ten aanzien van deze ectoparasieten geen sprake van een *Tagetes* effect. Sommige soorten vermenigvuldigen zich intensief op bepaalde *Tagetes* cultivars maar worden door andere cultivars niet beinvloed.

Het stengelaaltje *D. dipsaci* vermenigvuldigt zich in *T. patula* en *T. erecta* in beperkte mate en veroorzaakt typische aantastingsbeelden; dit is niet het geval bij *T. minuta.* Hetzelfde geldt voor het bladaaltje *A. ritzemabosi* (Fig. 1A en B).

Uit het voorgaande blijkt dat *Tagetes* spp. bepaalde soorten plantennematoden opvallend sterk drukken. Het *Tagetes* effect komt het sterkst tot uiting ten aanzien van *Pratylenchus* spp., *Meloidogyne* spp. en *Tyl dubius,* maar manifesteert zich niet of op onduidelijke wijze ten aanzien van *R. robustus* en verscheidene andere ectoparasitaire geslachten, *D. dipsaci* en *A. ritzemabosi.* Om een inzicht te krijgen in het mechanisme van het *Tagetes* effect wordt de invloed van de

plant in de drie phasen van het aantastingsproces nagegaan, nl. het exoradiculaire effect, het effect op het oppervlak van de plant of tijdens het penetratie proces, en de endoradiculaire effecten.

Exoradiculaire effecten kunnen enige bijdrage leveren tot het totale *Tagetes*-effect, maar kunnen geen verklaring geven voor het grootste deel hiervan. Op agar platen bleek, dat *P. penetrans* niet wordt aangetrokken noch afgestoten door groeiende wortels van *T. patula* en de geschikte waardplant, rode klaver; in de grond is echter sprake van verzameling rondom de wortels (Tabel 14), hoewel dit een klein gedeelte van de populatie betreft (Fig. 5). Percolaten verkregen uit potten waarin *T. patula*, rode klaver appel en geen plant groeiden vertoonden in vitro geen verschillen in effect op de activiteit en sterfte van *P. penetrans*. In watercultures van *T. patula* en *T. minuta* bleek de overleving van *P. penetrans* slechts iets geringer te zijn dan die in watercultures van appel, controle oplossingen of gedestilleerd water; het effect was in ieder geval zeer gering en niet specifiek (Tabel 15). In grond waarin *Tagetes* werd geteeld, werd binnen een periode van enige dagen nadat de wortels uit de grond werden verwijderd, een duidelijk nematicide effect geconstateerd. Dit effect is echter zwak en aangezien rode klaver een even groot en appel een groter effect vertoonde, is dit niet specifiek voor *Tagetes* (Tabel 16). Een buiten het worteloppervlak werkende ongunstige of dodende invloed op de nematoden kan daarom wel een rol spelen, maar is blijkbaar geen essentieel onderdeel van het *Tagetes*-effect.

M. hapla bleek de wortelstelsels van drie verschillende *Tagetes* spp. in dezelfde mate binnen te dringen als die van de goede waardplant, tomaat. Slechts een klein gedeelte van het aantal larven rondom de wortels is in staat, meestal binnen een week, de wortels binnen te dringen hoewel worteltoppen, dus potentiële penetratiepunten, in ruime mate aanwezig zijn. Het overige gedeelte wordt snel kleiner; bij aanwezigheid van groeiende planten is deze afbraak sneller dan bij braak. Dit kan van belang zijn in de algemene populatie dynamiek van plantennematoden. Binnengedrongen larven van *M. hapla* handhaven zich in de wortels van *Tagetes* spp. gedurende minstens 4 weken; een ontwikkeling verder dan het slanke tweede larvale stadium vindt in *T. patula* en *T. minuta* echter praktisch niet plaats, terwijl het bij *T. erecta* slechts enkele individuen gelukt zich verder te ontwikkelen tot volwassen individuen (Tabel 18).

P. penetrans vertoont hetzelfde algemene beeld; het percentage dat binnendringt is in alle toetsgewassen in het algemeen laag. In *T. patula* dringen echter belangrijk minder nematoden binnen dan in *T. erecta*, *T. minuta* of in rode klaver (Tabel 19, Fig. 6). *T. patula* vertoont dus resistentie tegen het binnendringen van *P. penetrans*, maar niet tegen dat van *Meloidogyne* spp.

Bij de beoordeling van endoradiculaire effecten werden de overlevingskansen, de ontwikkeling en vermeerdering van binnengedrongen nematoden nagegaan, tevens het vóórkomen van nematicide effecten en histopathologische reacties van *Tagetes* weefsels. Het voornaamste verschil tussen een goede waardplant

als rode klaver en *T. patula* ligt hierin, dat binnengedrongen nematoden zich in het eerste gewas snel vermeerderen, terwijl zij in *T. patula* in aantal afnemen of zich op een zeer lage evenwichtsdichtheid blijven handhaven. In *T. minuta* handhaaft het merendeel der gepenetreerde nematoden zich en kan zich verder ontwikkelen en vermeerderen, zij het langzamer en in geringere mate dan in rode klaver (Fig. 6, 7 en 8). De waardplantgeschiktheid van *T. patula*, *T. minuta* en rode klaver vormt dus een reeks van gradaties lopend van bijna nihil, langs gering naar zeer groot (Fig. 7, Tabel 20). De evenwichtsdichtheid is bij *T. patula* uiterst laag; bij een voldoende lange teelt van dit gewas is enige vermeerdering mogelijk. De ontsnappings- en overlevingskansen van zich in de wortels bevindende *P. penetrans* zijn bij *T. patula* zeer gering; evenals bij goede waardplanten kunnen grotere gedeelten van populaties uit het weefsel van *T. minuta* in de grond rondom de wortels ontsnappen en herinfectief zijn.

In tegenstelling tot wortelexudaten vertoonden perssapppen, bereid uit wortels van *Tagetes* spp. *in vitro* een duidelijk nematicide werking tegen *P. penetrans* (Fig. 9A, B en C). Wortelperssappen van *T. patula* bleken effectiever te zijn dan die van *T. minuta* en de laatsten zijn effectiever dan die van de geschikte waardplant, aardappel. Fractionering van wortelperssappen van *T. patula* toonde aan dat de hoogste mortaliteit werd verkregen in een volgens volgorde van eluatie hooggenummerde fractie en dat deze niet correleert met het eiwit gehalte. Er werden geen analyses verricht om de identiteit van de actieve bestanddelen vast te stellen. Dit *in vitro* effect hangt waarschijnlijk samen met het *Tagetes* effect in gronden en wordt waarschijnlijk veroorzaakt door de door Uhlenbroek & Bijloo (1958, 1959) uit *Tagetes* wortels geïsoleerde nematicide thiophenen. In aardappelwortelperssap trad ook enige doding van nematoden op en wel lager dan in *Tagetes*wortelperssap maar hoger dan in de controles. Dit zou een aanwijzing kunnen zijn voor de aanwezigheid van een zwak nematidice agens in aardappelwortelperssap; het steunt de eerder genoemde veronderstelling dat planten door middel van exudaten of andere bestanddelen een algemeen "antinemisch potentiaal" in het groeimedium vestigen of induceren. *Tagetes*effecten zijn duidelijk verschillend en sterker dan deze algemeen antinemische effecten.

In met *Meloidogyne* en *Pratylenchus* zwaar besmette gronden vertoont *Tagetes* in tegenstelling tot de meeste andere planten een goede groei en geeft een goed ontwikkeld wortelstelsel met blanke gezonde wortels. Desondanks kunnen aan het wortelstelsel onopvallende kleine lesies voorkomen als gevolg van nematodenaantastingen. De larven van *M. hapla* dringen de worteltoppen van *T. patula* op dezelfde wijze binnen als die van tomaat. Meestal ontwikkelen zij zich in *T. patula* niet verder en veroorzaken ook geen necroses of verdikkingen van de worteltoppen. Incidenteel worden echter ook wel kleine syncytia, soms omgeven door enig galweefsel, gevormd, welke samengaan met het voorkomen van een verder ontwikkelde larve. De vorming van syncytia en galweefsel is bij *T. patula* vergeleken met tomaat sterk gereduceerd en vertraagd; de larve sterft vaak terwijl de syncytia steeds in gedegenereerde conditie worden aangetroffen, met een roodbruin gekleurde celinhoud. *P. penetrans* dringt de jonge wortels van *T. patula*, *T. minuta* en rode klaver op ieder willekeurig punt van het oppervlak

binnen, behalve in de worteltoppen, waarbij lesievorming optreedt. Deze lesies zijn bij *T. patula* donkergekleurd, necrotisch en klein en bevatten 1 tot 3 nematoden, die vaak reeds dood zijn en in een gekronkelde houding liggen. Bij *T. minuta* zijn de lesies groter van omvang en lichter gekleurd dan bij *T. patula*, hoewel necrotische lesies hier ook wel voorkomen; de lesies worden bij *T. minuta* trager gevormd dan bij *T. patula* en bevatten meer, namelijk tot 38 nematoden. Bij rode klaver worden lesies nog later gevormd; zij zijn groot en bevatten een grote, zich sterk vermeerderende *P. penetrans* populatie. De histologische reactie van de plant hangt blijkbaar voor een belangrijk deel samen met de ontwikkeling van de nematoden. Endoradiculaire effecten spelen blijkbaar een belangrijke rol in de nematoden dodende invloed van *Tagetes* spp. In de verhouding plant-nematode in het algemeen, die voor iedere associate verschillend kan zijn, vormen deze effecten een deel of komen er extra bij en bepalen mede het uiteindelijke resultaat. Verondersteld wordt dat de specifiek nematicide factor in *Tagetes* uit verschillende componenten bestaat, waaronder de tot nu toe uit *T. erecta* bekende nematicide thiophenen, en voorts dat deze componenten of hun relatieve gewichten verschillend zijn bij de diverse *Tagetes* spp. Sommige plantensoorten die binnen de *Compositae* nauw verwant zijn aan *Tagetes* zijn eveneens effectief tegen *P. penetrans* en blijken dezelfde thiophenen te bevatten als *Tagetes* of tot nu toe niet geïdentificeerde actieve principes. De grotere effectiviteit van *T. patula* ten opzichte van andere getoetste *Tagetes* spp. is waarschijnlijk het gevolg van het sneller en heftiger optreden van necroses. Dit zou een gevolg kunnen zijn van een snellere doding van de nematoden in het plantenweefsel, zoals geconstateerd is in wortelperssappen. Ectoparasitaire zich aan de schors voedende nematoden van het geslacht *Tylenchorhynchus* worden minder, en ectoparasitaire vaatweefselvoeders worden niet beïnvloed door de nematicide principes in *Tagetes* wortels, waarschijnlijk omdat zij niet onderhevig zijn aan dezelfde soort of mate van contact met het *Tagetes* weefsel. Het *Tagetes*-effect schijnt daarom in het algemeen sterk te zijn ten aanzien van endoparasitaire wortelnematoden of ectoparasitaire schorsvoeders, maar zelfs binnen deze groep nematoden is er, afhankelijk van de betreffende plant-nematode combinatie, een variatie in effect mogelijk.

Als bron van organisch materiaal, voor het winnen van kleurstoffen, medicinale of andere stoffen en als siergewas heeft *Tagetes* een beperkte landbouwkundige betekenis; er is tot op dit ogenblik slechts een teelt op kleine schaal. De toepassing van *Tagetes* als onderdrukker van plantennematoden en ter verbetering van de groei van hoofdgewassen wordt voornamelijk bemoeilijkt door gebrek aan marktwaarde van het gewas. Het bestaande sortiment van *Tagetes* spp. en hun varieteiten heeft ook beperkte landbouwkundige teeltmogelijkheden. Uitgaande van de gedachte dat de waarde als nematodenbestrijder voorop staat zijn varieteiten van *T. patula*, in het bijzonder Golden Harmony en Harmony, aan te bevelen. Zij zijn in vergelijking met andere *Tagetes* spp. en cultivars effectiever tegen *Pratylenchus* en minstens even effectief tegen *Meloidogyne;* als ge-

was zijn zij ook beter of minstens even goed te telen en te bewerken. Zaadeigenschappen, snelheid van jeugdgroei en kouderesistentie dienen te worden verbeterd, terwijl het wenselijk is de mogelijkheden voor het verhandelen van het gewas te vergroten. Door veredeling is een groot sortiment van *Tagetes* als siergewas ontstaan; dit suggereert dat er voor de plantenverdelaar goede perspectieven zijn om sterke nematicide effecten met gunstige landbouwkundige eigenschappen te verenigen.

Het kiezen van de gewenste eigenschappen zal men afhankelijk stellen van het landbouwkundig systeem en de toepassingsomstandigheden. Verschillende onderzoekers hebben reeds gevonden dat het effectief is wanneer *Tagetes* het hele groeiseizoen geteeld wordt; dit kan opvallende groeiverbeteringen veroorzaken. Het kan in gebieden toegepast worden waar kostbare gewassen geteeld worden en waar de pacht laag is, zoals in sommige tropische en subtropische gebieden. Aangezien de jeugdgroei langzaam is biedt *Tagetes* in gematigde luchtstreken weinig perspectieven voor de toepassing in het voorjaar. Zelfs wanneer het gewas dicht gezaaid wordt, nl. op 10 cm onderlinge afstand en de zaaidatum 15 mei is, worden minimum dichtheden van de nematoden pas na ongeveer 2 maanden bereikt; bij grotere plantafstanden worden deze dichtheden ook later verkregen (Fig. 10). Als najaarsgewas biedt *Tagetes* grotere mogelijkheden. De groeikracht van najaars-*Tagetes*, welke uiteraard van belang is voor het nematoden bestrijdende effect, kan van jaar tot jaar sterk verschillen. In sommige jaren kan een najaars-*Tagetes* teelt de nematodenpopulatie sterk drukken en de groei van hoofdgewassen verbeteren (Tabellen 23, 24 en 25); de vroegste zaaidata na een vroeg hoofdgewas lagen in de periode 1961–1968 echter tussen 6 juli en 14 augustus, terwijl een goede ontwikkeling van het *Tagetes* gewas werd verkregen in slechts 4 van de 8 jaren (Tabel 26). Voortdurende toepassing van najaarsteelt van *Tagetes*, kan echter effectief zijn, ondanks het vóórkomen van mislukkingen in bepaalde jaren. Bij toepassing als najaarsgewas, is het aan te bevelen niet later dan eind juli te zaaien en dichte plantafstanden te nemen, b.v. 10 cm in plaats van 25 cm.

Gelijktijdige teelt van *Tagetes* en een hoofdgewas, kan in het geval van teelt rondom en tussen boompjes en houtige siergewassen effectief zijn; het kan in andere gevallen ook perspectieven bieden, vooral omdat bij de teelt gedurende een heel groeiseizoen bij 60 cm plantafstand maximale daling van nematodenpopulaties verkregen wordt, zij het na langere tijd dan bij kleinere zaaidichtheden. In de praktijk komt inzaai tussen de rijen van een hoofdgewas incidenteel voor (Fig. IIC en D). Als ondergewas bij de teelt van granen en andere hoogopgaande gewassen met de bedoeling een volgend gewas een snellere start te geven is *Tagetes* tot op dit ogenblik niet hoopgevend gebleken, daar slechts enkele zwak groeiende *Tagetes* planten overbleven.

De toepassing als nagewas in het najaar en tussen de rijen van een hoofdgewas biedt onder bepaalde omstandigheden gunstige perspectieven. De technische mogelijkheden worden belangrijk vergroot wanneer klimatologische omstandigheden een betere groei in het najaar mogelijk maken, of wanneer de teelt van *Tagetes* gedurende een heel groeiseizoen mogelijk is.

Opbrengstverhogingen van hoofdgewassen na de teelt van *Tagetes* kunnen door nematologische en andere factoren of door beide veroorzaakt worden. In een door *P. penetrans* besmette grond werd na een voorgewas *Tagetes* een groeiverbetering van 167% in vergelijking met braak verkregen, terwijl bij dezelfde behandeling in onbesmette grond een groeivermindering optrad. Wanneer gecorrigeerd wordt op het verschil in stikstof niveau tengevolge van de *Tagetes*-behandeling, dan zou een grotere groeiverbetering worden verkregen (Tabel 27). De daling van de populatiedichtheid zet zich voort nadat de bovengrondse delen van *Tagetes* zijn verwijderd. Door vermenging van de grond met een natuurlijke hoeveelheid *Tagetes* wortels werd een grotere daling van de *P. penetrans* populatie verkregen dan door vermenging met appelwortels of door braak. Het is mogelijk dat ook onder veldomstandigheden een na-effect optreedt. Vermenging met bladmateriaal gaf ook een drukkende werking op *P. penetrans; Tagetes* blad was echter minder effectief dan appelblad en vertoonde hier geen specifiek nematicide effect. De resultaten van proeven met onbesmette grond tonen aan dat de teelt van *Tagetes* en het verwijderen van de bovengrondse delen de vruchtbaarheid van onbemeste grond deed verminderen (Tabel 27). In het algemeen verhoogde de toevoeging van wortel- of bladmateriaal de vruchtbaarheid van de grond en veroorzaakte het een verbetering van de groei van toetsgewassen (Tabellen 30, 31 en 32). Deze effecten zijn echter niet specifiek en zouden in bemeste gronden kleiner zijn, zowel in het geval dat deze gronden onbesmet als besmet zijn. Het is duidelijk dat in door *P. penetrans* besmette en met *Tagetes* als voorgewas behandelde grond de factor "nematoden" bij de groei van appelzaailingen overheersend is ten opzichte van de factor "niet-nematoden". Het relatieve gewicht van deze factoren kan bij andere plant-nematode verhoudingen uiteraard verschillend zijn.

De resultaten van dit onderzoek bevestigen het voorkomen van opmerkelijke "*Tagetes*-effecten" en wijzen ook op de varierende invloed van de individuele plant-nematode relaties. Hoewel dit effect niet tot het geslacht *Tagetes* is beperkt maar ook door sommige geslachten der *Heleniae* wordt vertoond, is het in de natuur uitzonderlijk. Er bestaan sterke aanwijzingen dat de voornaamste oorzaken van dit effect zijn gelegen binnen de wortels en dat zij samenhangen met de aanwezigheid van sterke nematicide thiopheenverbindingen, die overigens zeldzaam zijn in de natuur. Het feit dat het effect zich het duidelijkst manifesteert tegen polyphage endoparasitaire wortelnematoden en niet tegen epidermis- en vaatweefselvoeders is uit het oogpunt van verschil in parasitaire levenswijze begrijpelijk. Het verschijnsel van doding van nematoden door bepaalde nematicide thiophenen enerzijds en dat van histopathologische resistentie reacties van de plant anderzijds, kunnen met elkaar in verband staan en hoeven niet tegenstrijdig te zijn.

De toepassing van *Tagetes* veroorzaakt vaak een verhoogde opbrengst van hoofdgewassen, maar er zijn beperkingen van praktische aard die afhankelijk zijn van het landbouwkundig systeem. In de gematigde luchtstreken kan gelijk-

tijdige teelt van *Tagetes* met een hoofdgewas of een najaarsteelt na een vroeg hoofdgewas veelbelovend zijn. Een langzame jeugdgroei, behoefte aan hoge lichtintensiteiten en vorstgevoeligheid vormen bezwaarlijke eigenschappen van de bestaande cultivars. In de tropen en subtropen kunnen betere perspectieven voor de landbouwkundige toepassing van *Tagetes* aanwezig zijn.

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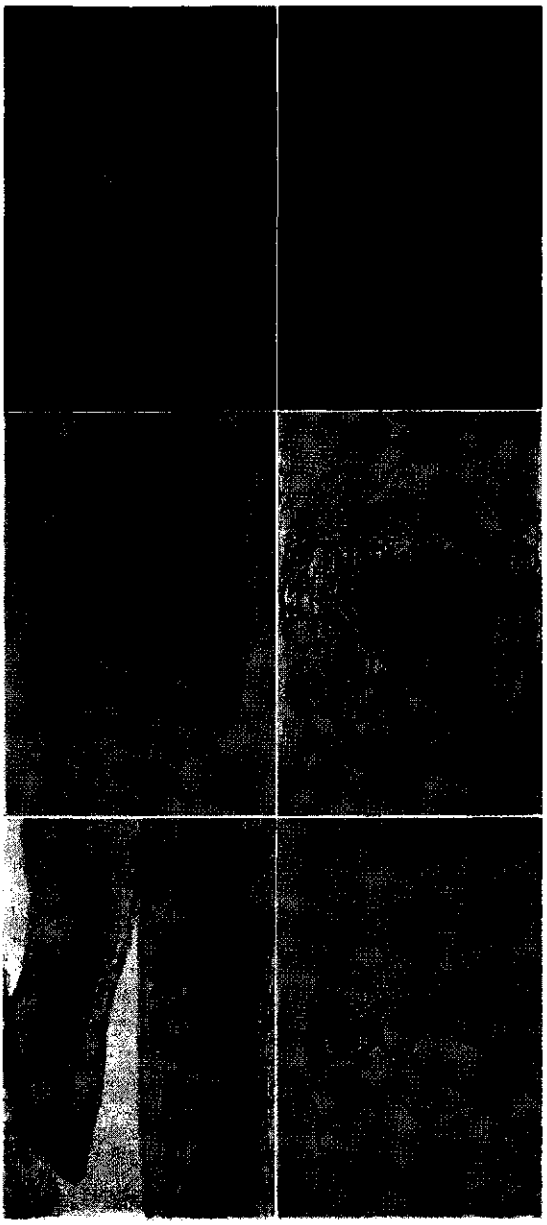

PLATE I. A Symptoms in *T. erecta* caused by *Ditylenchus dipsaci*.
B Leaf of *T. erecta* (left) and of *T. patula* (right) infested with *Aphelenchoides ritzemabosi*.
C Longitudinal section of a root apex of *T. patula* showing syncytium as incidentally caused by *Meloidogyne hapla*; giant cells with brownish cytoplasm, nuclei and nucleoli; giant cells may be aborted at a later stage.
D Longitudinal section of root apex of *T. patula* showing a dark brown cluster of giant cells, initiated by *M. hapla*.
E Unsectioned roots of *T. patula* showing *Pratylenchus penetrans* in necrotic lesions; root on the left showing one nematode with the body largely outside the root and the head end in a necrotic lesion.
F Cross section of a root of *T. patula* showing a lesion as recorded in E; necrotic epidermal and cortical cell with dark brown content, each enclosing an orange staining nematode body section.

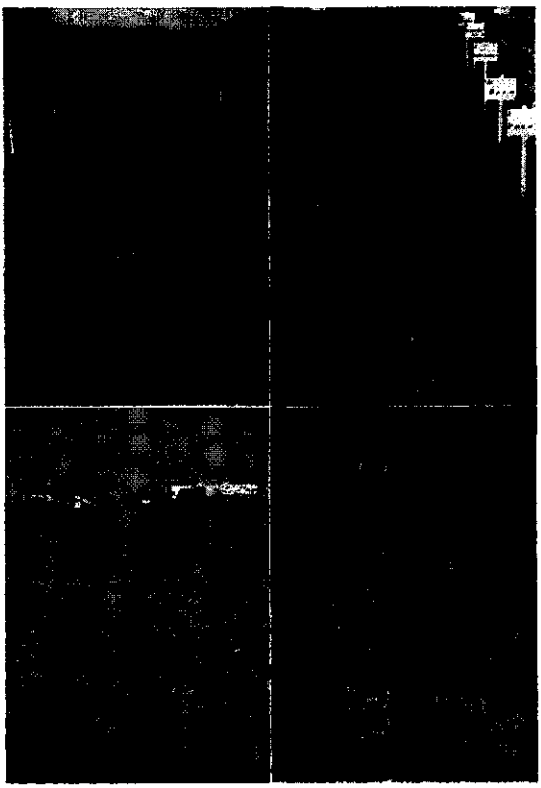

PLATE II A From front to rear: two-months-old plants of *T. patula* cv. Golden Harmony, *T. signata* cv. Golden Gem and *T. erecta* cv. Aurantiaca on peaty sand

B Effect of *T. patula* on the growth of ornamental *Geum* in a sandy soil at Driebergen infested with *P. penetrans*. Preceding plants from front to rear: *Nepeta faaseni*, *T. patula*, *Phlox paniculata*, *Geum* hybrid, *Trollius* hybrid, *Delphinium* hybrid and *Helenium* hybrid. Good growth of *Geum* only following *Tagetes* and *Helenium*

C *Tagetes* grown in rows between egg-plants on *Meloidogyne* infested soil at Aligarh, Uttar Pradesh, India

D *Tagetes* grown between rows of conifer seedlings in a nursery at Gaasterland