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NEMATICIDAL PRINCIPLES IN *COMPOSITAE*

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1. REVIEW OF LITERATURE

1.1. INTRODUCTION

Nematicidal and nematostatic principles are known from plants. Different groups of chemicals have such effects and plants containing or releasing such compounds are found in widely different groups of the plant kingdom. Nematicidal compounds originating from certain bacteria, fungi and actinomycetes are also known.

Most plant species with nematicidal or nematostatic properties were discovered because of their marked suppression of nematode populations in the field. If densities of plant parasitic nematodes decrease as the result of cultivation of certain plant species there is a chance that nematicidal or nematostatic substances are present in the roots or released by the roots although other phenomena may be involved. A review of these substances and the plants which produce them, arranged according to the chemical groups concerned, is given below.

1.2. ISOTHIOCYANATES

MORGAN (1925) and TRIFFIT (1929; 1930) found an inhibiting effect of *Sinapis alba* L. (*Cruciferae*) on the emergence of larvae of *Heterodera rostochiensis* WOLLENWEBER. *Brassica nigra* (L.) KOCH showed the same effect (ELLENBY, 1945a; 1945b). This effect is considered to be due to the presence of isothiocyanates in root diffusates of these plant species (TRIFFIT, 1929; ELLENBY, 1945a; 1945b).

1.3. THIOPHENICS

TYLER (1938) and STEINER (1941) recorded resistance of *Tagetes* species (*Compositae*) against *Meloidogyne*. In 1956 *Tagetes* reappeared in the literature because of its suppressing effect on populations of *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN (SLOOTWEG, 1956). This author gave a theoretical base to the experiences of VAN DEN BERG-SMIT (1953) a grower who successfully used *Tagetes* as a preceding crop for narcissus bulbs in root rot infested soil. Root rot of bulbs was considered to be related to *P. penetrans*. OOSTENBRINK et al. (1957) stated that the cultivation of one crop of *Tagetes* approximated to the effect of soil treatment with a nematicide. WINOTO SUATMADJI (1969) found *Tagetes patula* L. var. 'Golden Harmony' to be the *Tagetes* species with the best suppressing properties on *P. penetrans*.

UHLENBROEK and BIJLOO (1958; 1959) isolated from a variety of *Tagetes erecta* L. two thiophenic compounds, α-terthienyl and 5-(3-buten-1-ynyl)-2,2'-bithienyl, with in vitro strong nematicidal activity. α-Terthienyl had been iso-

lated before from the flowering parts of *Tagetes* (ZECHMEISTER and SEASE, 1947). Toxicity of root extracts of *Tagetes* for *Panagrellus redivivus* (L.) GOODEY (STESSEL and SAKKINEN, 1961), larvae of *Meloidogyne* (SWARUP and SHARMA, 1967) and *P. penetrans* (WINOTO SUATMADJI, 1969) has also been recorded. A number of synthetically prepared thiophenic compounds showed in vitro nematicidal activity (UHLENBROEK and BIJLOO, 1960; HANDELÉ, 1971).

1.4. GLYCOSIDES

'Mary Washington', a variety of *Asparagus officinalis* L. (*Liliaceae*) effectively reduced populations of *Trichodorus christiei* (now *Paratrichodurus minor* (ALLEN) SIDDIQI). From roots of this variety an unidentified nematicidal glycoside has been isolated (ROHDE and JENKINS, 1958a). Drenching the root zone of growing tomatoes, a good host plant for, *T. christiei*, with an extract of *Asparagus* roots reduced the population of this nematode. Spraying leaves of tomato with a 1000 ppm solution of the glycoside also prevented the development of *T. christiei* (ROHDE and JENKINS 1958a; 1958b). So a systemic action of this nematicidal principle may be present. The glycoside showed an anticholinesterase effect on the nerve system of the amphidial pouch of *T. christiei* (ROHDE, 1960).

Additional evidence for the presence of nematostatic or nematicidal compounds within the genus *Asparagus* is provided by the work of SWARUP and SHARMA (1967) who found that root extracts of *Asparagus racemosus* WILLD. inhibited the hatching of eggs of *Meloidogyne javanica* (TREUB) CHITWOOD and *Meloidogyne arenaria* (NEAL) CHITWOOD.

1.5. ALKALOIDS

Physostigmine (eserine), the cholinesterase inhibiting alkaloid of the Calabar bean, *Physostigma venenosum* BALFOUR (*Papilionaceae*) exerts a systemic nematicidal activity as demonstrated in the '*Pisum* test' (BIJLOO, 1966). Certain analogues of this alkaloid also have nematicidal properties (WELLE, 1964).

In *Maclea chordata* (WILLD.) R. BR. (*Papaveraceae*) three nematicidal alkaloids occur: sanguinarine, cheletrine and bocconine (ONDA et al., 1965). Several *Crotalaria* species (*Leguminosae*) suppress populations of *Meloidogyne* species (OCHSE and BREWTON, 1954; RHOADES, 1965). Chopped roots of *Crotalaria striata* DC. killed *Trichodorus christiei* (ROHDE, 1965). In *Crotalaria spectabilis* ROTH. unidentified substances with nematicidal properties are present (BIJLOO, 1968). FASSULIOTIS and SKUCAS (1969) isolated from *C. spectabilis* monocrotaline, a pyrrolizidine ester toxic to vertebrates. Monocrotaline inhibited in vitro the mobility of larvae of *Meloidogyne incognita* (KOFOID and WHITE) CHITWOOD. According to the investigators, the presence of this alkaloid is not directly related with resistance of *Crotalaria* to *Meloidogyne* since certain

Cytisus and *Echium* species also containing the alkaloid are susceptible to this nematode.

α-Chaconine, a steroidglycoalkaloid from *Solanum tuberosum* L. (*Solanaceae*) is toxic to the free-living nematode *Panagrellus redivivus*. From studies with buffered solutions it became clear that the free base is the nematicidal form of this compound (ALLEN and FELDMESSER, 1971). Similar observations were made with α-tomatine (ALLEN and FELDMESSER, 1970).

1.6. PHENOLICS

TAYLOR and MURANT (1966) found in vitro a poisoning effect of aqueous extracts of raspberry roots and canes, *Rubus idaeus* L. (*Rosaceae*), on *Longidorus elongatus* (DE MAN) THORNE and SWANGER. The chemicals involved were not identified but the authors found strong indications that tannins and polyphenols were responsible for this effect. The incorporation into the soil of hydroquinone, catechol and resorcinol reduced the numbers of *L. elongatus* and those of some other nematodes. SCHEFFER et al. (1962) found catechol as a nematicidal compound in root diffusate of *Eragrostis curvula* (SCHRAD.) NEES, a plant species with suppressing properties on *Meloidogyne*.

Phenolics may also play a role, via IAA-oxydase activity, in resistance of potato varieties against *Heterodera rostochiensis* (GIEBEL, 1970; GIEBEL et al., 1971).

1.7. FATTY ACIDS

Nematicidal principles are present in decomposing plant material. LINFORD et al. (1938) were among the first who observed the suppressing effect of decomposing organic matter on nematode populations. Organic soil amendments, such as castor bean pomace (LEAR, 1959), finely divided oil cakes (SINGH and SITARAMAIAH, 1967), corn meal (HOLLIS and RODRIGUEZ-KABANA, 1966), chitine (MANKAU and DAS, 1969) and fish oil (RENNINGER et al., 1958) were able, if very large quantities were used, to suppress certain nematode populations. A review on this subject was recently given by SAYRE (1971). This effect is partially related to an increase of predacious fungi and animals. VAN DER LAAN (1956) in his studies with *Heterodera rostochiensis*, suggested an increase of the resistance of the potato plant to result from physiological changes induced by organic manuring. The production of low fatty acids, in addition to increased concentrations of H_2S (RODRIGUEZ-KABANA et al., 1965) and of NH_3 (MANKAU and MINTEER, 1962; WALKER, 1969; 1971; VASSALLO, 1967), is probably one of the other causes for nematode suppression.

Extracts of decomposing plant residues demonstrate a selective nematicidal action against *Melodoigyne incognita* and *Pratylenchus penetrans* but not against saprozoic forms (SAYRE et al., 1964; PATRICK et al., 1965). One of the active principles was isolated from decomposing rye (*Secale cereale* L.) and

timothy (*Phleum pratense* L.) and identified as butyric acid. The nematicidal activity of this acid is restricted within the pH range 4.0–5.3 (SAYRE et al., 1965). Similar results have been found with formiate, calciumformiate acting as a nematicide only in soils with a pH/KCl below 5.5 (VAN BERKUM, 1961). According to JOHNSTON (1959) mixtures of formic, acetic, propionic and butyric acids produced by the bacterium *Clostridium butyricum* PRAZMOWSKI were toxic to *Tylenchorhynchus martini* FIELDING. BANAGE and VISSER (1965), HOLLIS and RODRIGUEZ-KABANA (1966) and ELMILIGY and NORTON (1973) also recorded nematicidal activity of lower fatty acids.

TARJAN and CHEO (1956a; 1956b) found nematicidal activity in acids with longer chains. The optimum activity was found with chain lengths of C_9–C_{11}.

1.8. NEMATICIDAL ACTIVITY OF PLANT MATERIAL CONTAINING UNIDENTIFIED PRINCIPLES

Juices of a number of plants, parts of it and root leachings have been tested for nematicidal activity. These observations and some other cases in which unidentified nematicidal or nematostatic principles are involved or seem to be involved are listed below. In many cases specific active principles are present, but no direct nematicidal relation has been proved (for instance saponosides containing plants).

Polygonum hydropiper L. (water pepper) when cultured in combination with wheat (*Triticum aestivum* L.) decreased the number of attacks of the gall nematode *Anguina tritici* (STEINBUCH) FILIPJEV. This effect is possibly due to released compounds from the roots of water pepper (SUKUL, 1970). Nematoxicity for *Panagrellus redivivus* was also found in root juice of *Polygonum cuspidatum* SIEB. et ZUCC. (STESSEL and SAKKINEN, 1961). Root exudates of *Sesamum orientale* L. have nematicidal properties against *Meloidogyne incognita* (ATWAL and MANGAR, 1969). Roots of citrus contain a toxic compound to *Tylenchulus semipenetrans* COBB (VAN GUNDY and KIRKPATRICK, 1964). Root juice of *Cornus florida* L., a plant resistant to *Meloidogyne*, decreased the infectivity of larvae of *Meloidogyne incognita acrita* on tomato (DROPKIN and BOONE, 1966). An extract of poison ivy was toxic to *Meloidogyne* when sprayed on tomato leaves (TARJAN and CHEO, 1956a), suggesting a systemic activity of this extract. According to AYALA et al. (1967) the roots of *Panicum glabrum* GAUD. (pangola grass) contain toxic principles to *Meloidogyne*. Root juices of the *Helenium* hybrid 'Moerheim Beauty' and the *Gaillardia* hybrid 'Burgunder', like juice of roots of *Tagetes*, exerted a stronger nematicidal activity on females of *Pratylenchus penetrans* then root juice of potato (WINOTO SUATMADJI, 1969). SWARUP and SHARMA (1967) found nematostatic effects on *Meloidogyne* of root juice of *Tagetes* and of *Polygonum cuspidatum*. LUC (1961) and LUC et al. (1969) demonstrated an unknown factor from millet roots (*Pennisetum typhoideum* RICH.) inhibiting the movements of *Hemicycliophora paradoxa* LUC. Aqueous extracts of the aerial parts of *Erigeron filifolius* NUTT. exert a reversible nema-

tostatic influence on larvae of *Anguina tritici* (NENE and KUMAR, 1967). Aqueous extracts of leaves of *Anagallis arvensis* L. are toxic to larvae of *Anguina tritici* (NENE and THAPLIYAL, 1966). These extracts also possess antifungal properties (NENE and THAPLIYAL, 1965). Fruit juice of *Duranta plumieri* JACQ. and *Solanum xanthocarpum* SCHRAD. et WENDL. and seed extracts of *Argemone mexicana* L., *Cucurbita pepo* L. and *Cucurbita maxima* DUCHESNE exert nematicidal influences on *Hoplolaimus*, *Tylenchorhynchus*, *Ditylenchus*, larvae of *Meloidogyne* and certain saprozoic nematodes (HUSAIN and SAXENA, 1969). STESSEL and SAKKINEN (1961) recorded nematicidal properties of juices of several plant species. Nematoxicity of juices from the following plants is listed in decreasing order of potency: *Tagetes lucida* (roots), *Phytolacca decandra* L. (roots), *Arctium minus* (HILL) BERNH. (roots), *Calendula officinalis* L. (roots), *Tropaeolum majus* L. (aerial), *Erucastrum gallicum* (WILLD.) SCHULZ (whole plant), *Ambrosia trifida* L. (roots), *Polygonum cuspidatum* (roots), *Solanum dulcamara* L. (berries), *Rhus typhina* L. (aerial), *Scirpus eriophorum* MICHX (aerial), *Saponaria officinalis* L. (aerial), *Ambrosia artemisiifolia* L. (aerial), *Verbena hastata* L. (aerial), *Solanum dulcamara* (aerial), *Lycopodium complanatum* L. (whole plant), *Fagopyrum esculentum* MOENCH. (roots) and *Portulaca oleracea* L. (roots).

Culture filtrates of *Aspergillus niger* VAN TIEGHEM have been demonstrated to be toxic to the mycophagous nematode *Aphelenchus avenae* BASTIAN (MANKAU, 1969), possibly because of the presence of oxalic acid. *Pratylenchus penetrans* was found to be sensitive to toxic metabolites of several actinomycetes and bacteria (WALKER et al. 1965, 1966). Aqueous extracts of *Amanita muscaria* (L.) FR., a basidiomycete extremely toxic to man, killed the nematode *Aphelenchoides ritzemabosi* (SCHWARTZ) STEINER (MILLER and STODDARD, 1965). Extracts of mycelia of *Nematoctonus haptocladus* DRESCH. and *N. concurrens* DRESCH. and media in which they were grown, had nematicidal properties (GIUMA and COOKE, 1971).

1.9. CONCLUDING REMARKS

It is clear that a number of plant species contain or secrete compounds with nematicidal or nematostatic properties, and that certain micro organisms also produce nematicidal metabolites. It is possible that many of these plant juices are toxic because decomposition products or exudates of micro organisms thriving in them comprise one or more common nematicidal principles.

The elucidation of the structure of the nematicidal principles from *Tagetes* roots initiated the synthesis of a number of analogues (UHLENBROEK and BIJLOO, 1960; HANDELÉ, 1971). A number of these thiophenics exerted excellent nematicidal activity in vitro, but never found practical use because they showed no or only weak nematicidal activity when mixed into the soil (HANDELÉ, 1971). Nevertheless the isolation and elucidation of the structure of natural nematicidal principles can give information on the nature of the suppressing properties of plant species and opens the possibility of discovery of new groups of nematicides.

2. SCOPE OF THE INVESTIGATIONS

2.1. Screening of plant species

Screening of plant species with suppressing properties on nematode populations, especially *Pratylenchus penetrans*, was, after preliminary research, mainly restricted to members of the *Compositae*. It is clear from the studies of Hijink and Winoto Suatmadji (1967) that the *Compositae* having this effect are chemotaxonomically related. They found these properties in *Tagetes* and also in species of *Helenium*, *Gaillardia*, *Rudbeckia*, *Echinops*, *Solidago*, *Eriophyllum* and *Erigeron*.

In the present study plant species were selected mainly on a chemotaxonomical base. For instance the nematicidal principle 5-(3-buten-1-ynyl)-2,2'-bithienyl from *Tagetes* (Uhlenbroek and Bijloo, 1959) seems to be common within the tribe *Helenieae* (Bohlmann and Herbst, 1962).

Bohlmann (1967), Bohlmann and Sucrow (1963), Sörensen (1962) and Bohlmann and Mannhardt (1957) reviewed the acetylenic (including thiophenic) compounds present within the *Compositae*. Romo and Romo de Vivar (1967) summarized the literature on the pseudoguaianolides and Hegnauer (1964) compiled the general chemotaxonomical relationships between *Compositae*. These studies, supplemented with recent literature, guided the choice of the *Compositae* used in the screening programme.

2.2. Inoculation trials

Density-dependent relations between nematodes and plants exist (Oostenbrink, 1966; Seinhorst, 1966a). Selected plant species from the screening programme were therefore studied in more detail with various initial nematode densities and nematode species.

2.3. Isolation of nematicidal principles

Some plant species from the screening programme were selected for the isolation and eventual structural identification of their nematicidal principles.

2.4. Activity of nematicidal principles

The nematicidal principles from *Tagetes* killed, in vitro, several nematode species in concentrations of 1–5 ppm (Uhlenbroek and Bijloo 1958; 1959). Mixing of α-terthienyl into the soil up to a concentration of 200 ppm did not

control *Meloidogyne javanica* (DAULTON and CURTIS, 1963). Some nematode species, for instance *Hemicycliophora similis* THORNE (SEINHORST and KLINKENBERG, 1963) *Trichodorus teres* (now *Paratrichodorus teres* (HOOPER) SIDDIQI) (KUIPER, 1963) and *Longidorus maximus* (now *Paralongidorus maximus* (BÜTSCHLI) SIDDIQI) (STURHAN, 1963) build up high populations on *Tagetes*. Saprozoic forms are also not affected by cultivation of *Tagetes*. These observations suggest differences between the in vitro and in vivo action of these natural nematicides, if indeed they do have in vivo activity. Experiments were done to obtain more insight in this subject.

3. MATERIALS AND METHODS

The special techniques for the extraction and purification of nematicidal principles and other chemical and physical methods are described in chapter 5.

3.1. PLANT MATERIAL AND ITS CULTURE

Seeds of most plant species used were obtained from a large number of Botanical Gardens in Europe and America.[1]

The seeds were germinated in a glasshouse on a glass covered bench in steam sterilized standard potting soil. Germination took from three days to about four weeks, depending on plant species. For glasshouse trials seedlings were transplanted into wooden boxes containing sterilized soil three to four days after germination. The plants were used in the experiments one to three weeks after transplanting, depending on the rate of growth of the different species. Perennial hybrids, such as the *Helenium* hybrid 'Moerheim Beauty', were increased by allowing young shoots to make roots in steam sterilized soil. During the experiments the temperature was maintained at 20–25 °C. From October until the beginning of May extra light was supplied with 80 W daylight incandescent lamps so that the plants received a total of 16 hours of light. Different types of pots were used, and are therefore described for the different experiments.

For field experiments seedlings were transplanted to wooden boxes of steam sterilized soil. Hardening of the young plants occurred after about three weeks. Plants were therefore transferred under unheated flat glass. Usually plants were planted out in the field during May.

3.2. NEMATODES AND SOILS

Monospecific cultures of *Pratylenchus penetrans* and *Tylenchorhynchus dubius* (BÜTSCHLI) FILIPJEV were used in certain glasshouse trials and laboratory experiments. These populations were established in a sandy soil from a newly reclaimed Flevopolder, which did not contain plant parasitic nematodes. *P. penetrans* was cultured on the good host plants *Trifolium pratense* L., *Avena sativa* L. and *Digitalis purpurea* L. *Tylenchorhynchus dubius* was reared on oats (*Avena sativa*).

For glasshouse trials with *P. penetrans* soils with well established monocultures were used. One to two months before starting an experiment the roots

[1] Through Botanical Garden, University of Groningen and Laboratory of Plant Taxonomy and Plant Geography, Agricultural University, Wageningen.

of the host plant were cut into pieces and mixed again with the soil. Most of the root pieces disintegrated during this time and nematodes moved into the soil. Before starting the experiments root debris was removed by carefully sieving the soil.

In laboratory experiments *Pratylenchus penetrans*, *Ditylenchus dipsaci* (KÜHN) FILIPJEV and larvae of *Heterodera rostochiensis* were used. *P. penetrans* was extracted from roots of the stock cultures, and *D. dipsaci* from infected onions or potato tubers by standard procedures described in section 3.3. Larvae of *H. rostochiensis* were hatched from dry cysts as described by JANZEN (1968). Dry cysts were presoaked in a solution of flavianic acid (100 mg/l). Five days later these cysts were transferred to a solution of potato root diffusate[2] (10 mg/l). Incubation for three days in this medium gave many hatched larvae. The potato root diffusate was a dried product obtained by lyophilization of demineralized crude potato root diffusate.

Field experiments were carried out in the 'Kruidentuin' at Buitenpost and in a nursery in Gaasterland, in the northern part of the Netherlands. The soil at Buitenpost is a sandy clay and trials were usually on plots previously cropped with *Digitalis purpurea*. The soil in Gaasterland is a poor sandy soil, and at this site trials were situated on areas with an even distribution of plant parasitic nematodes according to soil sample results. Plant species were planted out at a distance of about 25 cm on plots of about 3 m^2 in three replicates. The plots were arranged at random over the trial site. In a number of cases a block design was chosen. In these experiments *Digitalis purpurea*, a good host plant for *P. penetrans* and also very susceptible to attack by this nematode (OOSTENBRINK, 1966) was used as a control. *Tagetes patula*, the *Helenium* hybrid 'Moerheim Beauty' the *Gaillardia* hybrid 'Burgunder', poor host plants for this nematode, (SLOOTWEG, 1956; OOSTENBRINK, et al., 1957; HIJINK and WINOTO SUATMADJI, 1967) and fallow were also used as controls.

3.3. ESTIMATION OF NEMATODE DENSITIES

Soil samples from the field plots were taken with an auger of 1 cm width to a depth of about 25 cm. From each plot a bulk comprising 30–40 cores was stored in waxed paper bags in a cold room at 4 °C until extraction. Active nematodes were extracted from 100 ml of mixed and sieved soil according to the elutriator method of OOSTENBRINK (1960) and counted under a dissecting microscope. For glasshouse trials usually the whole pot contents were processed in the elutriator.

In glasshouse experiments with low quantities of soil (10–20 ml) a modification of the sugar flotation method was used (CAVENESS and JENSEN, 1955). The soil was carefully mixed, with the aid of a vibrator, with a 50% sugar solution. After 2 minutes centrifugation at 1800 r.p.m. the supernatant, with the nema-

[2] Kindly supplied by Dr. G. J. Janzen.

todes, was poured onto 22 μm aperture sieves and the catch immediately counted under a dissecting microscope.

Densities of active endoparasitic nematodes in the roots were assessed according to the blender-cottonwool filter method (STEMERDING, 1963) or the funnel spray method (OOSTENBRINK, 1960).

In some cases the endoparasitic nematodes inside the roots were counted directly under the dissecting microscope after staining with cotton blue in lactophenol (GOODEY, 1963).

4. FIELD AND GLASSHOUSE EXPERIMENTS

Screening of a number of *Compositae* and some other plant species with expected suppressing properties on nematode populations was done in the field and in glasshouses. Plant species with suppressing properties on *Pratylenchus penetrans* in the field were nearly all tested on the two different soils in different years. Numbers of the endoparasitic *P. penetrans* in the roots and in the soil were counted. Data were also collected on the behaviour of populations of certain ectoparasitic nematodes on cultivation of these *Compositae*. Most *Compositae* with suppressing properties on *P. penetrans* were also tested in the pots in the controlled environment of a glasshouse.

Selected *Compositae* were studied more in detail in glasshouse experiments. Rate of penetration of *P. penetrans* in roots and the eventual density-dependent relationships between these *Compositae* and *P. penetrans*, *Meloidogyne hapla* and *Tylenchorhynchus dubius* were assessed.

4.1. FIELD EXPERIMENTS[3]

4.1.1. *Field experiment 1967*

In a preliminary experiment on the Buitenpost site 32 plant species were tested in two replicates for their ability to suppress populations of *Pratylenchus penetrans*. The initial and final densities and sampling dates are given in table 1.

Only *Tagetes patula* and the *Helenium* hybrid 'Moerbeim Beauty' (controls) suppressed *Pratylenchus* to low densities. Of the other *Compositae* tested *Gaillardia aristata* PURSH and possibly *Calendula officinalis* reduced densities of *Pratylenchus* slightly, and the same was true for *Asparagus officinalis*. *Borago officinalis* L. (*Boraginaceae*), *Delphinium elatum* L., *Delphinium grandiflorum* L. (*Ranunculaceae*), *Primula veris* L. and *Primula elatior* (L.) HILL (*Primulaceae*) slightly decreased or did not raise the population of *Pratylenchus* when compared with fallow. The other plant species tested turned out to be good host plants for this endoparasitic nematode. Although *Chenopodium ambrosioides* L. and *Chenopodium ambrosioides* (L.) var. *anthelminticum* GRAY did not support *Meloidogyne* (MILLER, 1946) these two species appeared to be good hosts for *Pratylenchus*. *Sinapis alba*, *Brassica nigra* and *Armoracia rusticana* G.M. et SCH. supported high populations of *Pratylenchus*, even though these plant species contain isothiocyanates which are reported to have, in vitro, nematicidal or nematostatic properties (ELLENBY, 1945a; 1945b).

4.1.2. *Field experiments 1968*

The indications obtained from the experiment in 1967 were checked in 1968

[3] Published in part (Gommers, 1971a; 1972a).

TABLE 1. Effect of 32 plant species and fallow in a field trial at Buitenpost on a mixed population of soil-inhabiting nematodes. Two replicates per treatment; planting time spring 1967; evaluation august 1967.
Average nematode figures per 100 ml of soil and per 10 g of roots.
P. = *Pratylenchus crenatus* LOOF and *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN,
T. = *Tylenchorhynchus dubius* (BÜTSCHLI) FILIPJEV,
R. = *Rotylenchus robustus* (DE MAN) FILIPJEV,
Pa. = *Paratylenchus* sp.
O + S = Other stylet-bearing and saprozoic nematodes.

	Numbers of nematodes					
	in soil					in roots
Initial density	P. 105	T. 45	R. 65	Pa. 10	O + S 870	P.
Final densities						
COMPOSITAE						
Tagetes patula L.	8	12	5	0	770	14
Gaillardia aristata PURSH	44	5	18	7	1520	8
Helenium hybrid 'Moerheim Beauty'	0	15	2	8	1240	5
Calendula officinalis L.	80	20	15	10	980	140
Senecio aquaticus HILL	186	15	20	5	1730	1200
Senecio viscosus L.	355	30	50	10	1480	2830
Eupatorium rugosum HOUTT.	215	15	30	10	1110	6500
Achillea ptarmica L.	220	50	70	5	730	3610
BORAGINACEAE						
Borago officinalis L.	40	20	22	150	1940	940
Heliotropium arborescens L.	300	55	35	40	970	730
CARYOPHYLLACEAE						
Herniaria glabra L.	190	5	20	15	1150	2360
Herniaria hirsuta L.	210	20	60	30	1710	2780
Gypsophila muralis L.	365	5	50	10	970	1450
PAPAVERACEAE						
Chelidonium majus L.	180	15	20	8	800	1310
Papaver somniferum L.	210	20	7	20	1640	7380
RANUNCULACEAE						
Delphinium elatum L.	60	7	12	5	940	1120
Delphinium grandiflorum L.	40	10	5	20	965	870
Delphinium ajacis L.	180	5	5	10	860	670
UMBELLIFERAE						
Conium maculatum L.	350	12	30	7	1025	7480
CHENOPODIACEAE						
Chenopodium ambrosioides (L.) var. *anthelminticum* GRAY	350	5	30	8	1410	11300
Chenopodium ambrosioides L.	370	0	40	5	1140	9400
PRIMULACEAE						
Primula veris L.	90	0	15	5	955	360
Primula elatior (L.) HILL	50	0	25	0	950	280
Anagallis arvensis L.	130	0	20	5	1005	310

TABLE 1. (continued)

	Numbers of nematodes					
	in soil					in roots
Initial density	P. 105	T. 45	R. 65	Pa. 10	O + S 870	P.
CRUCIFERAE						
Armoracia rusticana G., M. et SCH.	300	10	40	35	955	1180
Brassica nigra (L.) KOCH	560	35	5	160	1250	6400
Sinapis alba L.	430	60	15	120	1370	8900
SCROPHULARIACEAE						
Digitalis purpurea L.	405	125	35	0	1195	9800
ROSACEAE						
Geum rivale L.	280	60	10	15	1480	6300
Geum urbanum L.	370	20	20	35	910	6800
LABIATAE						
Galeopsis segetum NECK.	410	8	30	20	1780	4700
LILIACEAE						
Asparagus officinalis L.	30	5	10	10	715	20
Fallow	85	30	40	10	1280	

in field experiments in Buitenpost and in Gaasterland. The initial and final nematode densities from the experiments at Buitenpost and Gaasterland are given in table 2 and table 3 respectively.

It can be seen from table 2 that none of the plant species tested suppressed *Pratylenchus penetrans* to the same degree as *Tagetes patula* and the *Gaillardia* hybrid 'Burgunder'. *Borago officinalis* decreased the density of *P. penetrans* to some extent when compared with fallow. The roots of this plant species, however, harboured fairly high numbers of this nematode. The *Compositae Saussurea albescens* HOOK., *Bidens dahlioides* WATS. and *Helenium hoopesi* GRAY, the *Primulaceae Anagallis arvensis* and *Lysimachia nummularia* L. and *Delphinium elatum* (*Ranunculaceae*) did not suppress *P. penetrans*. These plant species also had high numbers of *P. penetrans* within the roots. Cultivation of *Digitalis purpurea* resulted in high densities of *P. penetrans* in the soil and the roots.

The influence of these plants species on *Tylenchorhynchus dubius* was less pronounced. *Bidens dahlioides*, the *Gaillardia* hybrid 'Burgunder', *Delphinium elatum* and *Borago officinalis* significantly reduced the density of *T. dubius* compared with fallow and the other plant species tested.

The population densities of *Rotylenchus robustus* (DE MAN) FILIPJEV showed no significant differences as the result of cultivation of the different plant species.

TABLE 2. Effect of 10 plant species and fallow in a field trial at Buitenpost on a mixed population of soil-inhabiting nematodes. Three replicates per treatment; planting time May 1968; evaluation September 1968.
Average nematode figures per 100 ml of soil and per 10 g of roots.
P. = *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN,
T. = *Tylenchorhynchus dubius* (BÜTSCHLI) FILIPJEV,
R. = *Rotylenchus robustus* (DE MAN) FILIPJEV,
O + S = Other stylet-bearing and saprozoic nematodes.

	Numbers of nematodes				
	In soil				In roots
Initial densities	P. 330	T. 177	R. 23	O + S 680	P.
Final densities					
Digitalis purpurea L.	433 a[1]	138 a[1]	88 a[1]	1373 a[1]	10353 a[1]
Saussurea albescens Hook.	285 a	53 a	63 a	900 a	6233 a
Bidens dahlioides Wats.	193 b	38 b	17 a	1532 a	2753 b
Delphinium elatum L.	135 b	25 b	38 a	1013 a	880 c
Lysimachia nummularia L.	157 b	52 a	92 a	893 a	750 c
Helenium hoopesi GRAY	177 b	48 a	80 a	758 a	1673 c
Anagallis arvensis L.	188 b	98 a	167 a	1277 a	927 c
Borago officinalis L.	102 c	25 b	195 a	980 a	540 d
Gaillardia hybrid 'Burgunder'	40 d	30 b	23 a	843 a	40 e
Tagetes patula L.	15 d	48 a	28 a	1690 a	33 e
Fallow	220 b	108 a	32 a	627 a	–

[1] Multiple range test; treatments sharing a common letter do not differ significantly at 95% probability level.

In Gaasterland (table 3) the initial density of *Pratylenchus penetrans* was 35 specimens per 100 ml soil. The *Helenium* hybrid 'Moerheim Beauty', *Helenium autumnale* L., *Gaillardia pulchella* FOUG., *Eriophyllum caespitosum* DOUGL., *Tagetes patula*, *Asparagus officinalis* and *Borago officinalis* lowered the population density of *P. penetrans* in the soil when compared with fallow. In contrast with the aforementioned *Compositae* and *Asparagus officinalis*, *Borago officinalis* had high numbers of *P. penetrans* inside its roots. *Echinops sphaerocephalus* L., *Echinops ritro* L. and the *Gaillardia* hybrid 'Burgunder' dit not significantly decrease the population of *P. penetrans* in the soil compared with fallow. The *Gaillardia* hybrid 'Burgunder', *Echinops ritro* and also *Eriophyllum caespitosum* were reported to be poor hosts for this nematode (HIJINK and WINOTO SUATMADJI, 1967). A good indication that this is true is found in the low numbers of *P. penetrans* inside the roots. In this regard these three plants resemble the aforementioned *Compositae*. *Digitalis purpurea*, *Primula veris*, *P. elatior*, *Anagallis arvensis*, *Lysimachia nummularia* and *Delphinium elatum* did not significantly change the densities of *P. penetrans* in the soil when compared with fallow. In the roots of these plant species high to very high densities of *P. penetrans* were found.

TABLE 3. Effect of 16 plant species and fallow in a field trial at Gaasterland on a mixed population of soil-inhabiting nematodes. Three replicates per treatment; planting time spring 1968; evaluation September 1968. Average nematode figures per 100 ml of soil and per 10 g of roots.
P. = *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN,
T. = *Tylenchorhynchus dubius* (BÜTSCHLI) FILIPJEV,
R. = *Rotylenchus robustus* (DE MAN) FILIPJEV,
O + S = Other stylet-bearing and saprozoic nematodes.

	Numbers of nematodes				
	In soil				In roots
Initial densities	P. 35	T. 93	R. 162	O + S 983	P.
Final densities					
Digitalis purpurea L.	120 a[1]	138 a[1]	387 ab[1]	640 c[1]	7750 a[1]
Primula veris L.	38 b	127 a	182 abc	820 b	273 bc
Anagallis arvensis L.	15 b	60 a	852 a	1948 a	172 bc
Echinops sphaerocephalus L.	17 b	38 a	267 abc	1418 b	13 d
Lysimachia nummularia L.	22 b	88 a	212 abc	702 b	97 c
Delphinium elatum L.	15 b	17 b	68 bc	998 b	223 bc
Echinops ritro L.	17 b	82 a	110 bc	533 c	20 d
Gaillardia hybrid 'Burgunder'	15 b	30 a	17 c	2043 a	17 d
Borago officinalis L.	7 c	7 b	1052 a	735 b	1023 ab
Primula elatior (L.) HILL	30 b	127 a	253 ab	932 b	363 bc
Asparagus officinalis L.	10 c	28 a	218 ab	897 b	27 d
Tagetes patula L.	7 c	15 b	42 c	1382 b	33 d
Eriophyllum caespitosum DOUGL.	10 c	107 a	55 bc	1422 b	13 d
Gaillardia pulchella FOUG.	5 c	22 a	23 c	1515 b	0 e
Helenium autumnale L.	2 c	35 a	68 bc	1015 b	7 d
Helenium hybrid 'Moerheim Beauty'	7 c	90 a	150 bc	672 b	17 d
Fallow	23 b	90 a	140 bc	755 b	–

[1] Multiple range test; treatments sharing a common letter do not differ significantly at 95% probability level.

The population densities of *Tylenchorhynchus dubius* were hardly affected by the plant species tested. Only *Delphinium elatum*, *Borago officinalis* and *Tagetes patula* significantly lowered the densities of this nematode.

Cultivation of *Anagallis arvensis* and *Borago officinalis* significantly increased the populations of *Rotylenchus robustus*. The lowest densities were found after cultivation of the *Gaillardia* hybrid 'Burgunder', *G. pulchella* and *Tagetes patula*. These densities, however, did not differ significantly from those in the fallow plots.

4.1.3. *Field experiments 1969*

The effect of the cultivation of different plant species on the population

TABLE 4. Effect of 13 *Compositae*, *Digitalis purpurea* L. and fallow in a field trial at Buitenpost on a mixed population of soil-inhabiting nematodes. Three replicates per treatment, planting time May 1969; evaluation September 1969. Average nematode figures per 100 ml of soil and per 10 g of roots.
P. = *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN,
T. = *Tylenchorhynchus dubius* (BÜTSCHLI) FILIPJEV,
R. = *Rotylenchus robustus* (DE MAN) FILIPJEV,
O + S = Other stylet-bearing and saprozoic nematodes.

	Numbers of nematodes				
	In soil				In roots
Initial densities	P. 330	T. 70	R. 80	O + S 1690	P.
Final densities					
Anthemis ruthenica BIEB.	843 a[1]	45 c[1]	132 a[1]	1338 a[1]	10437 a[1]
Anthemis tinctoria L.	288 b	35 c	75 ab	950 a	1143 ab
Tanacetum balsamita L.	372 b	93 b	95 ab	1990 a	2630 a
Chrysanthemum parthenium (L.) BERNH.	321 b	48 c	72 ab	943 a	2743 a
Digitalis purpurea L.	110 bc	278 a	47 ab	1028 a	3270 a
Flaveria repanda LAG.	65 c	12 d	50 ab	770 a	4530 a
Xanthium strumarium L.	103 c	8 d	20 b	813 a	7673 a
Anthemis nobilis L.	22 d	22 cd	75 ab	902 a	363 b
Gaillardia hybrid 'Burgunder'	18 d	5 e	10 c	792 a	30 c
Echinops sphaerocephalus L.	10 d	7 d	18 b	827 a	10 c
Iva xanthiifolia NUTT.	3 e	8 d	103 ab	918 a	17 c
Tagetes patula L.	5 e	18 cd	3 c	933 a	10 c
Ambrosia trifida L.	3 e	3 e	3 c	677 a	17 c
Helenium hybrid 'Moerheim Beauty'	7 e	18 cd	8 c	937 a	33 c
Fallow	120 bc	27 cd	50 ab	840 a	–

[1] Multiple range test; treatments sharing a common letter do not differ significantly at 95% probability level.

densities of the soil-inhabiting nematodes in the experiment 'Buitenpost 1969' are summarized in table 4.

A number of *Compositae* reduced the population densities of *Pratylenchus penetrans* when compared with the initial density and with the population in the fallow plots. Population densities were, as expected, significantly lower in the plots with *Tagetes patula*, the *Helenium* hybrid 'Moerheim Beauty', the *Gaillardia* hybrid 'Burgunder' and *Echinops sphaerocephalus*. In addition densities of *P. penetrans* were significantly lower after cultivation of *Iva xanthiifolia* NUTT., *Ambrosia trifida* and *Anthemis nobilis* L. Although few *P. penetrans* were found in the soil after cultivation of *Anthemis nobilis* a significantly higher number of *P. penetrans* were present inside the roots when compared with the other aforementioned *Compositae*. *Anthemis ruthenica* BIEB., *Anthemis tinc-*

toria L., *Tanacetum balsamita* L., *Chrysanthemum parthenium* (L.) BERNH., *Digitalis purpurea*, *Flaveria repanda* LAG. and *Xanthium strumarium* L. proved to be very good hosts for *P. penetrans*. In the soil and in the roots high numbers of this nematode were present.

Ambrosia trifida and the *Gaillardia* hybrid 'Burgunder' reduced the density of *Tylenchorhynchus dubius* significantly when compared with fallow. After cultivation of *Digitalis purpurea* and *Tanacetum balsamita* numbers of *T. dubius* were significantly higher than in the fallow plots. None of the other plant species tested significantly changed the densities of this nematode.

The *Gaillardia* hybrid 'Burgunder', *Tagetes patula*, *Ambrosia trifida* and the *Helenium* hybrid 'Moerheim Beauty' significantly reduced the densities of *Rotylenchus robustus*. *Iva xanthiifolia* seemed to be a reasonably good host for *R. robustus*. Only cultivation of *Anthemis ruthenica* significantly increased the population density of this nematode.

Most of the plant species tested in the experiment at Buitenpost were also tested in a field experiment in Gaasterland where the initial density of *P. penetrans* was only 15 specimens per 100 ml of soil. The densities of *Tylenchorhynchus dubius* and *Rotylenchus robustus* were also low. The initial densities and final densities of nematode species as the result of cultivation of different crops and of fallow are given in table 5.

After growing the *Helenium* hybrid 'Moerheim Beauty', *Iva xanthiifolia*, *Tagetes patula* and *Ambrosia artemisiifolia* no *Pratylenchus penetrans* were recovered from the soil. In the roots none or only a few nematodes were detectable. Cultivation of *Flaveria repanda* and the *Gaillardia* hybrid 'Burgunder' did not change the population density of *P. penetrans* when compared with fallow. However, the *Gaillardia* hybrid and *Flaveria repanda* differed significantly from each other regarding the numbers of *P. penetrans* inside the roots. In this respect the *Gaillardia* hybrid resembled *Ambrosia artemisiifolia*, *Tagetes patula* and *Iva xanthiifolia*, whereas *Flaveria repanda* harboured high numbers of *P. penetrans* and must be considered as a good host plant. Growing *Anthemis tinctoria*, *A. ruthenica*, *A. nobilis*, *Tanacetum balsamita*, *Chrysanthemum parthenium* and *Digitalis purpurea* increased the densities of *P. penetrans* when compared with fallow. High numbers of this nematode were also found inside the roots.

In this experiment none of the plant species tested significantly influenced the densities of *Tylenchorchynchus dubius* which were low to start with.

Ambrosia artemisiifolia and the *Helenium* hybrid 'Moerheim Beauty' significantly decreased the population density of *Rotylenchus robustus* whereas *Anthemis tinctoria*, *Tanacetum balsamita* and *Iva xanthiifolia* built up significantly higher populations compared with the fallow. The final densities of *R. robustus* did not differ significantly from fallow after cultivation of the other plant species tested.

TABLE 5. Effect of 11 *Compositae*, *Digitalis purpurea* L. and fallow in a field trial at Gaasterland on a mixed population of soil-inhabiting nematodes. Three replicates per treatment; planting time April-May 1969; evaluation September 1969. Average nematode figures per 100 ml of soil and per 10 g of roots.
P. = *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN,
T. = *Tylenchorhynchus dubius* (BÜTSCHLI) FILIPJEV,
R. = *Rotylenchus robustus* (DE MAN) FILIPJEV,
O + S = Other stylet-bearing and saprozoic nematodes.

	Numbers of nematodes				
	In soil				In roots
Initial densities	P. 15	T. 10	R. 33	O + S 625	P.
Final densities					
Anthemis tinctoria L.	133 a[1]	5 a[1]	147 a[1]	1138 b[1]	2213 a[1]
Tanacetum balsamita L.	98 a	15 a	105 a	1286 b	1286 a
Anthemis ruthenica BIEB.	68 ab	8 a	73 ab	975 b	3353 a
Chrysanthemum parthenium (L.) BERNH.	78 ab	23 a	55 ab	1288 b	3093 a
Digitalis purpurea L.	33 b	3 a	32 b	858 b	2340 a
Anthemis nobilis L.	33 b	2 a	45 ab	858 b	290 b
Flaveria repanda LAG.	5 cd	5 a	37 ab	522 c	1947 a
Gaillardia hybrid 'Burgunder'	7 cd	2 a	13 b	2808 a	77 c
Ambrosia artemisiifolia L.	0 d	3 a	7 c	798 b	20 d
Tagetes patula L.	0 d	10 a	52 ab	1507 b	20 d
Iva xanthiifolia NUTT.	0 d	5 a	93 a	1307 b	33 cd
Helenium hybrid 'Moerheim Beauty'	0 d	7 a	8 c	598 c	0 e
Fallow	11 c	5 a	28 b	960 b	–

[1] Multiple range test; treatments sharing a common letter do not differ significantly at 95% probability level.

4.1.4. *Field experiments 1970*

In 1970 another series of plants was selected. This series included a number of previously untested *Compositae* as well as species which had already proved to be poor hosts for *Pratylenchus penetrans*. The results of the experiment 'Buitenpost 1970' are given in table 6. At the beginning of the experiment the soil harboured 160 *Pratylenchus penetrans*, 360 *Tylenchorhynchus dubius* and 50 specimens of an unidentified species of *Trichodorus*.

Ambrosia trifida, *Iva xanthiifolia*, both tested in 1969, *Milleria quinqueflora* L., *Eriophyllum lanatum* (PURSH) FORBES, *Schkuhria senecioides* NEES et DC. and *Ambrosia maritima* L. decreased the population density of *Pratylenchus penetrans* in the soil to the same extent as *Tagetes patula*. The roots of these plant species contained low numbers of this nematode. Cultivation of *Baeria chrysostoma* F. et M., *Baeria minor* (DC.) FERRIS ssp. *maritima* (GRAY) FERRIS and *Baeria californica* (HOOK.) CHAMB. did not significantly change the densities of *P. penetrans* compared with fallow, but in respect of the numbers

TABLE 6. Effect of 14 *Compositae*, *Digitalis purpurea* L. and fallow in a field trial at Buitenpost on a mixed population of soil-inhabiting nematodes. Three replicates per treatment; planting time May 1970; evaluation September 1970. Average nematode figures per 100 ml of soil and per 10 g of roots.
P. = *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN,
T. = *Tylenchorhynchus dubius* (BÜTSCHLI) FILIPJEV,
Tr. = *Trichodorus* sp.,
O + S = Other stylet-bearing and saprozoic nematodes.

	Numbers of nematodes				
	In soil				In roots
Initial densities	P. 160	T. 360	Tr. 50	O + S 990	P.
Final densities					
Layia patyglossa (F. et M.) GRAY ssp. *campestris* NECK.	343 a[1]	90 bc[1]	27 ab[1]	1540 a[1]	6333 a[1]
Madia sativa MOL.	177 ab	110 ab	10 ab	1463 a	6000 a
Eriophyllum nevinii GRAY	173 ab	377 a	20 ab	1860 a	613 b
Digitalis purpurea L.	150 ab	133 ab	27 ab	1763 a	6000 a
Baeria californica (HOOK.) CHAMB.	80 ab	190 ab	43 ab	2090 a	1 c
Baeria minor (DC.) FERRIS ssp. *maritima* (GRAY) FERRIS	90 ab	447 a	27 ab	2693 a	7 c
Gaillardia aristata PURSH	40 b	127 ab	23 ab	1583 a	227 b
Baeria chrysostoma F. et M.	27 bc	133 ab	20 ab	1653 a	3 c
Schkuhria senecioides NEES et DC.	7 c	10 d	0 c	1647 a	30 c
Eriophyllum lanatum (PURSH) FORBES	10 c	53 bc	7 b	1693 a	21 c
Tagetes patula L.	7 c	440 a	23 ab	1697 a	24 c
Milleria quinqueflora L.	7 c	143 ab	137 a	1203 a	9 c
Ambrosia maritima L.	3 c	3 d	0 c	1570 a	26 c
Iva xanthiifolia NUTT.	3 c	37 c	10 b	1933 a	7 c
Ambrosia trifida L.	0 d	13 c	3 b	1193 a	7 c
Fallow	120 ab	97 bc	43 ab	1680 a	–

[1] Multiple range test; treatments sharing a common letter do not differ significantly at 95% probability level.

of this nematode inside their roots these three *Baeria* species clearly belong to the aforementioned group of *Compositae*. Only *Layia patyglossa* (F. et M.) GRAY ssp. *campestris* NECK. significantly raised the population density of *P. penetrans*. *Madia sativa* MOL., *Eriophyllum nevinii* GRAY, *Digitalis purpurea* and *Gaillardia aristata* did not significantly influence the densities of *P. penetrans* in the soil. *Madia sativa* and *Digitalis purpurea* were extremely good host plants for this nematode as shown by the high numbers of *P. penetrans* found inside the roots. *Eriophyllum nevinii* and *Gaillardia aristata* clearly behaved differently with significantly lower number of *P. penetrans* in the roots.

Schkuhria senecioides and *Ambrosia maritima* significantly suppressed the densities of *Tylenchorhynchus dubius*. *Tagetes patula*, *Baeria minor* ssp. *maritima* and *Eriophyllum nevinii* acted as hosts for this nematode. The other plant species tested did not significantly change the densities of *T. dubius* when compared with fallow.

Because of the presence of an unidentified *Trichodorus* species in the soil data could be gathered on the host suitability of the plant species for this nematode. The plots cropped with *Schkuhria senecioides* and *Ambrosia maritima* did not contain detectable numbers of *Trichodorus*. *Milleria quinqueflora* which greatly suppressed densities of *Pratylenchus penetrans*, acted as a very good host for *Trichodorus*. The other plant species included in this experiment did not significantly change the population densities of *Trichodorus* when compared with fallow.

A number of *Compositae* from the experiment in Buitenpost and some other species were also planted in Gaasterland. The initial and the final nematode densities in this experiment are given in table 7.

Eriophyllum lanatum, *Ambrosia trifida*, *Ambrosia maritima*, *Baeria californica* and *Tagetes patula* significantly reduced the population densities of *Pratylenchus penetrans*. The effect of cultivation of *Schkuhria pinnata* (LAM.) KUNTZE, *Schkuhria senecioides*, *Baeria chrysostoma* and *Baeria minor* ssp. *maritima* on the population densities of *P. penetrans* in the soil did not differ significantly from that of fallow plots. The numbers of this nematode inside the roots were low and in this regard these plant species belong to the same group as the aforementioned *Compositae*. *Gaillardia lutea* GREENE, with 197 *P. penetrans* per 10 g of roots, had significantly less nematodes than the apparently good host plants *Lindheimera texana* GRAY, *Melampodium perfoliatum* L., *Layia patyglossa* ssp. *campestris*, *Madia sativa*, *Madia elegans* DON. and *Digitalis purpurea*.

The densities of *Tylenchorhynchus dubius* were not significantly changed by any of the plant species tested.

None of the plant species used suppressed populations of *Rotylenchus robustus*. *Melampodium perfoliatum* acted as an extremely good host for this nematode.

4.2. GLASSHOUSE EXPERIMENTS

4.2.1. *Screening of some selected Compositae in pot tests*

Certain *Compositae* used in the field experiments and a number of related species were tested in a glasshouse for their ability to reduce populations of *Pratylenchus penetrans*. The experiment was set up to compare the efficiency of the suppressing properties of different *Compositae*, and to explore the discrepancy, sometimes found in the field experiments, between the low numbers of *P. penetrans* in the roots and the relatively high numbers in the soil. The soil used originated from the 'Kruidentuin' at Buitenpost. The plant species

TABLE 7. Effect of 15 *Compositae*, *Digitalis purpurea* L. and fallow in a field trial at Gaasterland on a mixed population of soil-inhabiting nematodes. Three replicates per treatment; planting time May 1970; evaluation September 1970. Average nematode figures per 100 ml of soil and per 10 g of roots.
P. = *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN,
T. = *Tylenchorhynchus dubius* (BÜTSCHLI) FILIPJEV,
R. = *Rotylenchus robustus* (DE MAN) FILIPJEV,
O + S = Other stylet-bearing and saprozoic nematodes.

	Numbers of nematodes				
	In soil				In roots
Initial densities	P. 78	T. 132	R. 232	O + S 1387	P.
Final densities					
Madia elegans DON.	137 a[1]	90 a[1]	440 b[1]	445 b[1]	4333 b[1]
Madia sativa MOL.	108 a	48 a	492 b	490 b	21800 a
Layia patyglossa (F. et M.) GRAY ssp. *campestris* NECK.	100 a	28 a	400 b	608 b	27933 a
Digitalis purpurea L.	221 a	98 a	300 b	647 b	15967 a
Lindheimera texana GRAY	67 ab	20 a	277 b	402 b	4783 b
Gaillardia lutea GREENE	37 ab	27 a	242 c	568 b	197 c
Baeria minor (DC.) FERRIS ssp. *maritima* (GRAY) FERRIS	47 ab	85 a	223 c	1050 a	2 d
Melampodium perfoliatum H.B.K.	67 ab	72 a	842 a	693 a	1033 b
Schkuhria senecioides NEES et DC.	15 bc	32 a	143 c	627 b	2 d
Baeria chrysostoma F. et M.	20 bc	47 a	187 c	735 a	3 d
Schkuhria pinnata (LAM.) KUNTZE	27 bc	43 a	188 c	993 a	1 d
Baeria californica (HOOK.) CHAMB.	10 cd	42 a	105 c	751 a	2 d
Ambrosia maritima L.	8 cd	20 a	275 b	797 a	1 d
Ambrosia trifida L.	10 cd	38 a	290 b	1113 a	0 d
Tagetes patula L.	3 d	28 a	180 c	1393 a	2 d
Eriophyllum lanatum (PURSH) FORBES	0 e	65 a	113 c	1026 a	3 d
Fallow	92 ab	197 a	227 c	857 a	–

[1] Multiple range test; treatments sharing a common letter do not differ significantly at 95% probability level.

and hybrids were planted, in five replicates, in plastic pots filled with carefully sieved and mixed soil. No fertilizers were supplied and watering occurred two to three times a week. Each week the pots were randomly rearranged on the bench in order to avoid external differences in the glasshouse as much as possible.

TABLE 8. Effect of 41 *Compositae* and fallow in a glasshouse trial on a mixed population of soil-inhabiting nematodes. Five replicates per treatment; planting time spring 1970; evaluation August-September 1970. Average nematode figures per 100 ml of soil and per 10 g of roots. In brackets the averages of the naperian logarithm of the nematode counts + 1. P = *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN, Pa = *Paratylenchus* sp., T = *Tylenchorhynchus dubius* (BÜTSCHLI) FILIPJEV, R = *Rotylenchus robustus* (DE MAN) FILIPJEV, O + S = Other stylet-bearing and saprozoic nematodes.

	Numbers of nematodes					
	In soil					In roots
Initial densities	P 44	Pa 4	T 83	R 93	O + S 1850	P
Final densities						
HELIANTHEAE						
Melampodiinae						
Melampodium perfoliatum H.B.K.	8 (1.57)	2 (0.48)	42 (3.70)	26 (2.70)	918 (6.70)	187 (5.12)
Melampodium divaricatum DC.[1]	4 (0.61)	0 (0.00)	20 (2.70)	40 (3.65)	938 (6.72)	0 (0.00)
Melampodium divaricatum DC.[1]	0 (0.00)	2 (0.48)	26 (2.87)	16 (2.31)	872 (6.61)	0 (0.00)
Silphium perfoliatum L.	23 (2.68)	6 (1.73)	44 (3.72)	57 (4.01)	1446 (7.26)	548 (6.22)
Silphium asterides L.	4 (0.96)	30 (2.28)	40 (3.68)	126 (4.47)	1542 (6.56)	60 (3.20)
Lindheimera texana GRAY	174 (5.02)	12 (1.78)	42 (3.62)	86 (4.20)	1858 (7.40)	12335 (9.14)[2]
Ambrosiinae						
Iva xanthiifolia NUTT.	0 (0.00)	8 (1.22)	10 (1.70)	52 (3.84)	1342 (7.19)	5 (1.38)
Ambrosia trifida L.	4 (0.96)	0 (0.00)	22 (2.67)	4 (0.96)	608 (6.23)	1 (0.28)
Ambrosia artemisiifolia L.[1]	4 (0.61)	2 (0.48)	12 (2.53)	2 (0.48)	396 (5.76)	0 (0.00)
Ambrosia artemisiifolia L.[1]	2 (0.48)	20 (2.43)	2 (0.48)	6 (1.09)	1502 (7.20)	1 (0.28)
Ambrosia maritima L.	2 (0.55)	6 (0.69)	16 (1.87)	16 (1.91)	1034 (6.44)	0 (0.00)
Franseria artemisioides CAV.	2 (0.48)	2 (0.48)	4 (0.61)	8 (1.57)	822 (6.95)	8 (1.21)
Milleriinae						
Milleria quinqueflora L.	0 (0.00)	0 (0.00)	6 (1.09)	14 (2.25)	1650 (7.38)	0 (0.00)
Madiinae						
Madia gracilis (SM.) NECK.	688 (6.22)	166 (4.86)	88 (4.24)	148 (4.73)	1800 (7.40)	75702 (10.76)[2]
Madia anomala GREENE	330 (5.69)	74 (4.11)	54 (3.84)	90 (4.17)	1494 (7.26)	31135 (9.86)[2]
Madia sativa MOL.	108 (4.50)	150 (4.86)	100 (4.58)	116 (4.49)	1270 (7.04)	12105 (9.27)[2]
Madia elegans DON.	48 (2.49)	322 (5.21)	68 (3.53)	88 (4.28)	870 (6.59)	3238 (7.77)
Hemizonia corymbosa ssp. (DC.) TORR. et GRAY						
macrocephala NUTT.	234 (5.16)	14 (2.31)	48 (3.66)	110 (4.28)	1758 (7.40)	9199 (8.96)
Hemizonia corymbosa (DC.) TORR. et GRAY	138 (4.50)	0 (0.00)	28 (3.11)	148 (4.85)	1303 (7.04)	4974 (8.37)
Layia patyglossa (F. et M.) GRAY ssp.						
campestris NECK.	456 (5.89)	689 (5.27)	76 (4.29)	282 (5.26)	2158 (7.61)	12065 (9.38)[2]
Layia elegans TORR. et GRAY	166 (4.81)	116 (3.84)	39 (3.50)	40 (2.95)	1440 (7.09)	11665 (9.21)[2]
HELENIEAE						
Heleniinae						
Baeria californica (HOOK.) CHAMB.[1]	8 (1.22)	82 (3.58)	18 (2.26)	36 (2.85)	895 (6.37)	0 (0.00)

TABLE 8. (continued)

Initial densities	Number of nematodes					
	In soil					In roots
	P 44	Pa 4	T 83	R 93	O + S 1850	P
Baeria californica (HOOK.) CHAMB.[1]	6 (0.69)	103 (4.33)	36 (3.42)	24 (2.55)	1276 (7.12)	2 (0.48)
Baeria minor (DC.) FERRIS ssp. *maritima* (GRAY) FERRIS[1]	2 (0.48)	58 (3.13)	8 (1.92)	12 (2.53)	655 (6.13)	0 (0.00)
Baeria minor (DC.) FERRIS ssp. *maritima* (GRAY) FERRIS[1]	0 (0.00)	48 (2.64)	24 (3.05)	48 (3.23)	2878 (7.54)	0 (0.00)
Baeria chrysostoma F. et M.	2 (0.48)	8 (1.17)	18 (2.86)	4 (0.96)	1530 (7.24)	0 (0.00)
Flaveria repanda LAG.	126 (4.47)	14 (2.31)	88 (4.24)	110 (4.28)	1850 (7.40)	31243 (9.85)[2]
Schkuhria senecioides NEES et DC.[1]	2 (0.48)	0 (0.00)	32 (3.34)	24 (2.55)	1224 (7.37)	0 (0.00)
Schkuhria senecioides Nees et DC.[1]	0 (0.00)	4 (0.96)	6 (1.44)	0 (0.00)	1746 (7.41)	0 (0.00)
Schkuhria pinnata (LAM.) KUNTZE	0 (0.00)	0 (0.00)	15 (2.33)	16 (1.96)	965 (6.81)	0 (0.00)
Eriophyllum nevinii GRAY	212 (5.14)	4 (0.61)	64 (4.17)	108 (4.54)	1642 (7.34)	4743 (8.29)
Eriophyllum lanatum (PURSH) FORBES	6 (1.09)	4 (0.61)	8 (0.74)	26 (3.17)	2002 (7.58)	0 (0.00)
Eriophyllum confertiflorum GRAY	6 (1.09)	90 (3.09)	16 (2.31)	10 (1.22)	2004 (6.72)	0 (0.00)
Eriophyllum caespitosum DOUGL.	2 (0.48)	712 (3.87)	8 (1.92)	34 (2.92)	1138 (6.83)	0 (0.00)
Helenium hybrid 'Moerheim Beauty'	6 (1.09)	8 (1.22)	8 (1.92)	8 (1.22)	1434 (7.66)	1 (0.46)
Helenium nudiflorum NUTT.	2 (0.48)	8 (1.17)	18 (2.46)	60 (4.02)	1696 (7.33)	1 (0.36)
Helenium bolanderi GRAY	0 (0.00)	0 (0.00)	20 (2.92)	76 (4.31)	1112 (6.98)	12 (1.73)
Helenium flexuosum RAFIN.	0 (0.00)	0 (0.00)	20 (2.48)	32 (2.79)	1632 (7.35)	6 (1.06)
Gaillardia pulchella FOUG.	4 (0.96)	2 (0.48)	22 (2.49)	10 (1.70)	2164 (7.56)	1 (0.42)
Gaillardia amblyodon GRAY	2 (0.48)	0 (0.00)	20 (2.94)	20 (2.94)	1458 (7.15)	1 (0.41)
Gaillardia lanceolata MICHX	2 (0.48)	6 (1.09)	12 (1.70)	2 (0.48)	2682 (7.78)	0 (0.00)
Gaillardia arizonica GRAY	2 (0.48)	0 (0.00)	10 (1.70)	6 (1.44)	2404 (7.77)	2 (0.58)
Gaillardia aristata PURSH	1 (0.48)	0 (0.00)	16 (2.38)	6 (1.44)	1766 (7.44)	1 (0.28)
Gaillardia lutea GREENE	0 (0.00)	6 (1.09)	14 (2.18)	40 (2.50)	1684 (7.36)	2 (0.81)
Tagetinae						
Tagetes patula L.	8 (1.57)	0 (0.00)	28 (3.30)	8 (1.22)	432 (5.89)	3 (1.03)
Tagetes tenuifolia CAV.	0 (0.00)	0 (0.00)	4 (0.96)	10 (1.65)	872 (6.66)	1 (0.42)
Fallow	34 (2.92)	8 (1.22)	25 (2.68)	61 (3.20)	2012 (6.72)	
Significant differences						
at p. 0.05	(2.73)	(3.47)	(2.91)	(3.29)	(1.53)	(1.71)
at p. 0.01	(3.03)	(3.85)	(3.23)	(3.60)	(1.70)	(1.90)

[1] Originating from different sources

[2] Nematode numbers calculated from less than 10 g of roots.

The initial densities and the final densities of the nematode populations resulting from cultivation of plant species and fallow are given in table 8. In some cases, if root weights were lower than 10 g, the numbers of *P. penetrans* inside the roots were calculated per 10 g of fresh roots. The significance of differences between population densities of nematodes after growing the plants, was calculated according to the method of TUKEY (SNEDECOR, 1962). The poor hosts *Tagetes patula* and the *Helenium* hybrid 'Moerheim Beauty' and fallow served as controls.

Within the subtribus *Melampodiinae* only *Lindheimera texana* clearly acted as a good host for *Pratylenchus penetrans*. The soil and the roots harboured large numbers of this nematode. *Melampodium divaricatum* DC., originating from two localities, effectively suppressed the densities of *P. penetrans*. *Melampodium perfoliatum*, *Silphium perfoliatum* and to a lesser extent *Silphium asterides* L. behaved in a somewhat different way. Regarding the numbers of *P. penetrans* in the soil, these plant species seemed to suppress the densities of this nematode, the numbers found not significantly differing (at 95% level) from those found in pots after *Tagetes patula* or the *Helenium* hybrid. The roots, however, contained fair numbers of *P. penetrans*. Within the subtribus *Ambrosiinae* none of the species tested supported the original density of *P. penetrans*. *Iva xanthiifolia*, *Ambrosia trifida*, *A. artemisiifolia* and *A. maritima* showed the same suppressing effect as already recorded in field experiments. *Ambrosia elatior* and *Franseria artemisioides* CAV. also proved to be poor host plants for this nematode. Of the *Ambrosiinae* tested, pots cropped with *Iva xanthiifolia* contained the highest numbers of *Rotylenchus robustus*.

Milleria quinqueflora (*Milleriinae*) also did not support *P. penetrans*, none being detected in either soil or roots.

None of the *Madiinae* tested suppressed *Pratylenchus penetrans*. *Madia sativa*, *M. elegans*, *M. gracilis* (Sm.) KECK., M. *anomala* GREENE, *Hemizonia corymbosa* (DC.) TORR. et GRAY, *Hemizonia corymbosa* (DC.) TORR. et GRAY, ssp. *macrocephala* NUTT., *Layia elegans* TORR. et GRAY and *L. patyglossa* ssp. *campestris* were good hosts for *P. penetrans*. The numbers of *P. penetrans*, *Tylenchorhynchus dubius* and *Rotylenchus robustus* were, in general, significantly higher after cultivation of these *Madiinae* than in the fallow pots.

With the exception of *Flaveria repanda* and *Eriophyllum nevinii* the *Heleniinae* tested suppressed the population densities of *P. penetrans*. In the soil and in the roots none or only a few *P. penetrans* could be found. The numbers of this endoparasitic nematode in the soil were always significantly lower when compared with the numbers in the fallow pots. *Tagetes patula* and also *T. tenuifolia* CAV. behaved as expected and decreased densities of *P. penetrans*.

Cultivation of *Baeria californica* and *B. minor* ssp. *maritima* significantly raised the densities of the *Paratylenchus*. *B. chrysostoma* and the *Schkuhria* species tested did not affect the population densities of this nematode. From the *Eriophyllum* species tested *E. nevinii* also significantly raised the densities of *Rotylenchus robustus*. *E. caespitosum*, in contrast with the other *Eriophyllum* species tested, acted as an extremely good host for *Paratylenchus*.

4.2.2. *Inoculation trial with Pratylenchus penetrans and some Compositae*

The suppressing effect of *Milleria quinqueflora, Ambrosia trifida, Schkuhria pinnata, Baeria californica* and the *Helenium* hybrid 'Moerheim Beauty' on *Pratylenchus penetrans* were studied in detail in a glasshouse trial.

The plant species were grown in soil with different densities of this nematode. *Tagetes patula, Avena sativa,* a good host plant, and fallow served as controls. As discussed by OOSTENBRINK (1966) and SEINHORST (1966a) the maximum density of a particular nematode species on a particular plant is the equilibrium density. If this is the case with these *Compositae* one can expect density-dependent relations between nematode and plant. *P. penetrans,* originating from a monoculture was used. This population was propagated on red clover. After removal of the root debris in the usual way the soil contained 723 *P. penetrans* per 300 ml of soil. Thoroughly mixing with different quantities of nematode-free soil on which red clover had also been grown, resulted in 4 additional densities with respectively 430, 190, 80 and 33 *P. penetrans* per 300 ml of soil. Plastic bags in PVC tubes were filled with 300 ml of the appropriate soil and planted, in four replicates, with 2 to 4 week old seedlings of the aforementioned plants. Four tubes of each density were left unplanted as fallow controls. Three months after planting the roots and the soil were assessed for infestation by *P. penetrans.* In table 9 the numbers of *P. penetrans* in the roots and soils are given.

After cultivation of *Tagetes patula, Milleria quinqueflora, Ambrosia trifida* and *Schkuhria pinnata* none or only a few *P. penetrans* were found in the roots of these plants or in the soil. In the tubes cropped with *Baeria chrysostoma* and the *Helenium* hybrid 'Moerheim Beauty' small numbers of *P. penetrans* were still present in the soil. From the roots no nematodes could be recovered.

P. penetrans multiplied vigorously on oats. Most of the nematodes were present in the roots. If the final densities are plotted against the initial ones there is a density-dependent relation, in contrast with the *Compositae* tested.

In the fallow tubes the densities of *P. penetrans* dropped by 15 to about 40% when compared with the initial levels of infestation. These final densities were always higher than in tubes cropped with any of the *Compositae.*

4.2.3. *Penetration of the roots of some Compositae by Pratylenchus penetrans*

The inability of endoparasitic plant nematodes to invade the roots of a plant could be a reason for the suppressing effect of this plant on a nematode population. WINOTO SUATMADJI (1969) could not find statistically significant differences between the rate of penetration of *Pratylenchus penetrans* in the roots of *Tagetes* species and the good host red clover. In a few cases the degree of penetration in *Tagetes patula* seemed to be lower than in *T. erecta, T. minuta* and red clover. As discussed by this investigator several external factors, apart from specific features of nematode and plant, have to be taken into account in measuring the rate of penetration. The developmental stage of the inoculum, the amount of active inoculum i.e. the population density, the amount of roots or potential penetration sites, the damage caused by nema-

TABLE 9. The effect of cultivation of six *Compositae*, *Avena sativa* L. and fallow on five densities of *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN. Glasshouse trial with tubes of 300 ml of soil; planting date 7-9-1970. Nematode figures are means of 4 replicates from 300 ml of soil and in whole rootsystems three months after planting.

Initial densities	Final densities fallow	Numbers of nematodes													
		Tagetes patula L.		*Milleria quinqueflora* L.		*Ambrosia trifida* L.		*Schkuhria pinnata* (LAM.) KUNTZE		*Baeria chrysostoma* F. et M.		*Helenium* hybrid 'Moerheim Beauty'		*Avena sativa* L.	
		soil	roots	soil	roots	soil	roots	soil	roots	soil	roots	soil	roots	soil	roots
723	450	0	0	0	0	0.3	0	0.3	0	23.3	0	28.5	0	650	1872
430	383	0	0	0	0	0	0	6.6	0	40	0	5.7	0	466	2653
190	130	0	0	0	0	0	0	0	0	8	0	22.5	0	775	2547
80	60	0	0	0.3	0	0.3	0	0	0	0.3	0	0	0	300	1117
33	23	0	0	0	0	0	0	0	0	0	0	0	0	200	580
Average fresh weights of roots in g	-	1.75		10.39		1.73		0.66		0.70		4.52		1.75	

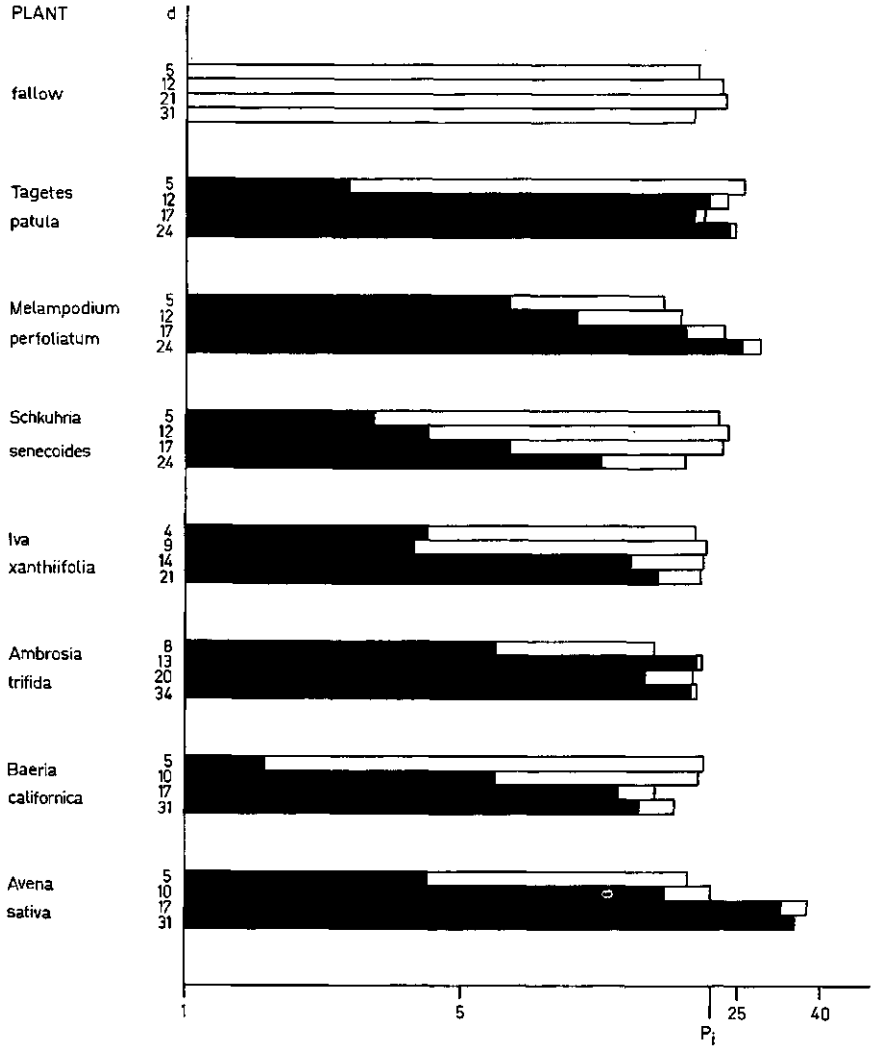


FIG. 1. Penetration of *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN, at different times, in the roots of 6 *Compositae*. *Avena sativa* L. and fallow served as controls. Figures (black = numbers in the roots; white = numbers in the soil) are means of 4 replicates. Abscissa: numbers of nematodes per tube with one seedling and 12 ml of soil (P_i = initial density).
Ordinate: harvest times of plant species (in days).

todes to growing roots, rhizosphere influences on nematodes and environmental factors such as soil type, moisture content and temperature may all affect penetration.

The rate of penetration of *P. penetrans* into the roots of growing *Schkuhria senecioides*, *Baeria californica*, *Ambrosia trifida*, *Iva xanthiifolia* and *Melam-*

podium perfoliatum was studied. *Tagetes patula* var. 'Golden Harmony' and the good host *Avena sativa* served as controls. The numbers of *P. penetrans* in tubes without plants (fallow) were also determined at every harvest time. Young seedlings with only two cotyledons were planted in glass tubes, 8 cm high with a diameter of about 1,5 cm. Tubes were filled with 12 ml of soil originating from the Flevopolder in which a monoculture of *P. penetrans* had been propagated on red clover. The soil was cleaned from root debris in the usual way. At the start of the experiment each tube was estimated to contain about 20 *P. penetrans*. At different times after planting the seedlings the numbers of *P. penetrans* in the roots and in the soil were counted. Nematodes were recovered from the soil with the aid of a modified sugar flotation method. *P. penetrans* in the roots were stained with a solution of cotton blue in lactophenol and counted under a dissecting microscope.

Data, as means of four replicates, are shown graphically in fig. 1. 20 days after the beginning of the experiment nearly all *P. penetrans* entered the root systems of *Tagetes patula* var. 'Golden Harmony', *Melampodium perfoliatum* and *Avena sativa*. In the tubes with *A. sativa* some multiplication took place, since after 17 days the total numbers of *Pratylenchus penetrans* were higher than in tubes without plants. In the tubes with *Iva xanthiifolia, Schkuhria senecioides* and *Baeria californica* 20 to 30% of the nematodes still remained in the soil at the end of the trial. Although this could be the result of inability of the nematodes to enter the roots of these plants, more probably the cause is incomplete rooting through the soil.

4.2.4. *Inoculation trial with Tylenchorhynchus dubius and some Compositae*

A number of *Compositae* with suppressing properties on *Pratylenchus penetrans* were also tested on their ability to suppress populations of the ectoparasitic nematode *Tylenchorhynchus dubius*. The *Compositae* were therefore grown in a soil with five different densities of this nematode. The experiment was carried out with *Milleria quinqueflora, Schkuhria pinnata, Baeria californica*, the *Helenium* hybrid 'Moerheim Beauty' and *Tagetes patula*. Oats (*Avena sativa*), a good host for *T. dubius* and fallow served as controls. *T. dubius* was propagated on oats in a sandy peat soil originating from the Flevopolder. This soil contained 1200 nematodes per 300 ml. Mixing with nematode free soil, in which oats were also grown, resulted in four additional densities with about 500, 275, 115 and 50 nematodes per 300 ml of soil. The experiment was set up in the same way as described in 4.2.2.

The results are shown graphically in figure 2. Regression lines were calculated assuming a straight line relationship between initial and final densities. *Milleria quinqueflora, Schkuhria pinnata* and the *Helenium* hybrid 'Moerheim Beauty' suppressed densities below the maintenance line as did fallow. All four lines are parallel with the maintenance line, and do not differ significantly from each other (at 95% level). Using the definition of Seinhorst (1967), these three plant species behave as non-hosts for *T. dubius* for the initial densities used.

Another relation was found for *Tagetes patula, Baeria californica* and oats.

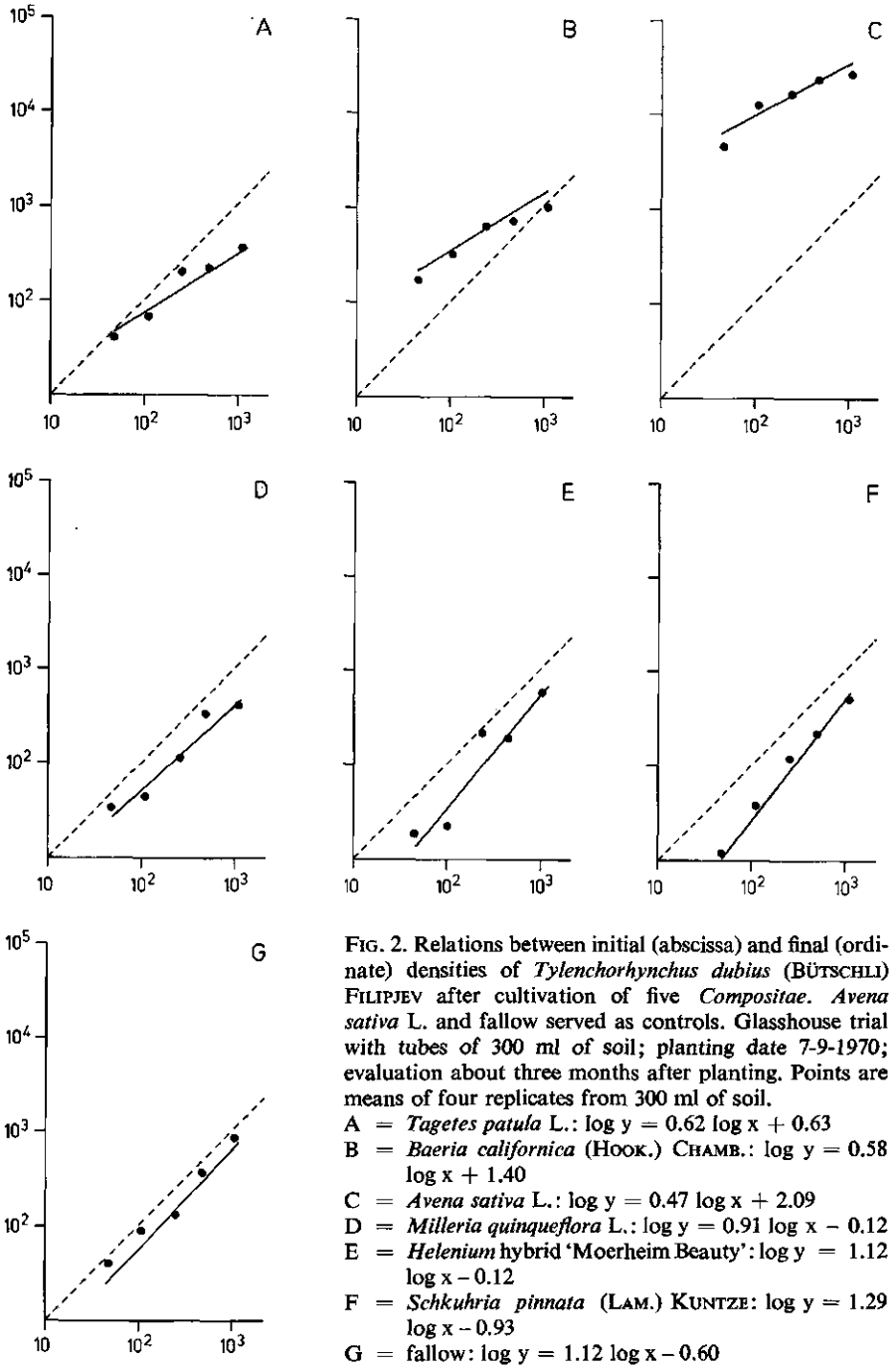


FIG. 2. Relations between initial (abscissa) and final (ordinate) densities of *Tylenchorhynchus dubius* (BÜTSCHLI) FILIPJEV after cultivation of five *Compositae*. *Avena sativa* L. and fallow served as controls. Glasshouse trial with tubes of 300 ml of soil; planting date 7-9-1970; evaluation about three months after planting. Points are means of four replicates from 300 ml of soil.

A = *Tagetes patula* L.: $\log y = 0.62 \log x + 0.63$
B = *Baeria californica* (HOOK.) CHAMB.: $\log y = 0.58 \log x + 1.40$
C = *Avena sativa* L.: $\log y = 0.47 \log x + 2.09$
D = *Milleria quinqueflora* L.: $\log y = 0.91 \log x - 0.12$
E = *Helenium* hybrid 'Moerheim Beauty': $\log y = 1.12 \log x - 0.12$
F = *Schkuhria pinnata* (LAM.) KUNTZE: $\log y = 1.29 \log x - 0.93$
G = fallow: $\log y = 1.12 \log x - 0.60$

The regression lines are mutually parallel, but they are at different levels and are not parallel with the maintenance line (95% probability). It is clear that in these cases a density-dependent relation exists and that the host suitability decreases in the order oats, *B. californica*, *T. patula*. In fact *Tagetes patula* suppressed the populations for all densities tested, but especially for the higher densities of *T. dubius*.

4.2.5. *Host suitability of certain Compositae for Meloidogyne hapla*

Some *Compositae* which effectively reduced populations of *Pratylenchus penetrans* were tested on their ability to support populations of *Meloidogyne hapla* CHITWOOD. *Schkuhria pinnata*, *S. senecioides*, *Milleria quinqueflora*, the *Helenium* hybrid 'Moerheim Beauty', *Ambrosia trifida*, *Iva xanthiifolia*, *Baeria chrysostoma* and *B. californica* were therefore grown in soil infested with *Meloidogyne hapla*. *Tagetes patula* var. 'Golden Harmony', a poor host for *M. hapla* and the good host tomato ('Moneymaker') served as controls. In order to detect possible density dependent relations 4 densities of *M. hapla* were used.

Plants were cultivated in 300 ml of soil in plastic bags supported by PVC tubes as described before. Tubes without plants were also included in the experiment. The tubes were filled with 300 ml of nematode free soil from the Flevopolder and inoculated with 1, 2, 4 and 8 egg masses respectively. Each plant species was planted in four replicates on each density of *M. hapla*. Egg masses were obtained from roots of tomato, var. 'Moneymaker', propagated in the glasshouse on a soil heavily infested with *M. hapla*. The egg masses were picked from the roots under a dissecting microscope with forceps. The average content of an egg mass was estimated to be 300 eggs. The variation in egg content of egg masses was rather great, from about 100 to more than 400 eggs. Directly before planting the three to four weeks old seedling tubes were inoculated with the appropriate number of egg masses. Three months later the numbers of nematodes in the soil and in the roots were counted. The soil was carefully removed from the roots by gently rinsing the roots with tapwater. The free moving larvae and males of *M. hapla* were recovered from the soil by the elutriator method. The number of root knots on a root system often gives an indication on the rate of infestation by root knot nematodes, so the knots on the roots were counted or estimated. These numbers were arranged in an index (gall index) according to the following system:

0: no root knots
1: 1–5 root knots
2: 5–10 root knots
3: 30–50% of the root system covered with root knots
4: 50–80% of the root system covered with root knots
5: more then 80% of the root system covered with root knots

Afterwards nematodes in the roots were stained with cotton blue and counted or estimated under a dissecting microscope.

Microtome sections of the roots of plants from this experiment were examined. Root knots were fixed in FAA. After removal of the air from the plant

TABLE 10. The effect of 9 *Compositae*, *Solanum lycopersicum* L. and fallow on 4 densities of *Meloidogyne hapla* CHITWOOD. Inoculum: 1, 2, 4 and 8 egg masses per tube of 300 ml of soil. Gall indices, final densities of larvae (l_2), males, females and fresh root weights (g) were determined three months after planting date. Figures are averages of 4 replicates.

Plant species	Inoculum (No. of egg masses)	Gall-index	*Meloidogyne hapla*		Root weights
			Roots	Soil	
Tagetes patula L.	1	0	0	28	10.8
	2	0	0	4	13.9
	4	¼	0; 1 ♀	2; 1 ♂	8.9
Helenium hybrid 'Moerheim Beauty'	8	¼	0; 1 ♀	12	7.7
	1	0	0	8	8.3
	2	0	0	47	8.1
	4	0	0	15	8.2
	8	0	0	40	8.3
Ambrosia trifida L.	1	0	0	13	2.6
	2	¼	0; 1 ♀	45	4.1
	4	¼	0	43	3.4
Schkuhria pinnata (LAM.) KUNTZE	8	0	0	112	5.6
	1	0	1	5	1.4
	2	¼	0; 1 ♂	37	1.3
	4	¼	1	5	1.9
Schkuhria senecioides NEES et DC.	8	¼	0; 1 ♂	35	1.4
	1	0	0	0	0.7
	2	0	0	10; 1 ♂	2.1
	4	0	0	8	0.6
	8	¼	1; 1 ♀	98; 3 ♂	1.5
Milleria quinqueflora L.	1	0	0	0	4.8
	2	1	1; 1 ♀	0	7.8
	4	½	0	30	7.5
Baeria californica (HOOK.) CHAMB.	8	1	0; 1 ♀	50	7.6
	1	1	0	5	1.2
	2	½	0; 1 ♂	0	0.3
	4	½	3; 5 ♀	10	1.3
	8	2½	25; 1 ♂; 11 ♀	50; 20 ♂	1.1
Baeria chrysostoma F. et M.	1	1	6; 2 ♂; 10 ♀	0	1.8
	2	1	10; 1 ♂; 5 ♀	5	1.3
	4	1½	28; 3 ♂; 5 ♀	10	2.1
	8	2¼	20; 2 ♂; 10 ♀	175	0.7
Iva xanthiifolia NUTT.	1	1½	26; 1 ♂; 2 ♀	47	2.8
	2	2¼	28; 4 ♂; 10 ♀	117	6.2
	4	2	63; 3 ♂; 50 ♀	37	6.3
Solanum lycopersicum L. var. 'Moneymaker'	8	4½	74; 30 ♀	122	6.8
	1	2½	24; 7 ♂; 2 ♀	7	6.5
	2	2¾	400; 9 ♂; 80 ♀	61; 2 ♂	5.4
	4	4¾	77; 4 ♂; 110 ♀	19; 1 ♂	5.3
	8	5	121; 9 ♂; 420 ♀	149; 19 ♂	3.5
Fallow	1	–	–	40	–
	2	–	–	45	–
	4	–	–	90	–
	8	–	–	200	–

material under reduced pressure the FAA was gradually replaced by tertiary butylalcohol (TBA). The TBA in turn was substituted by paraplast. Transverse, tangential and longitudinal sections of 15 or 20 microns were cut on a rotary microtome. The sections were stained with safranine, counterstained with fast green and mounted in Canada balsam. The whole procedure is fully described by GERLACH (1969).

The giant cell formation in the roots of tomato was considered to be the normal picture of an infestation with *M. hapla*. A complete description was given by WINOTO SUATMADJI (1969) and is briefly reported here. 4 to 6 giant cells surround the head region of the nematode. The giant cells possess thick walls and dense cytoplasm with a number of nuclei in which nucleoli can be observed. The adjacent gall tissue consists of tracheidal and vessel elements in the centre and in the radial direction of parenchymatous cells of pericyclic and hypertrophied cortical origin.

The results are summarized in table 10. The *Helenium* hybrid 'Moerheim Beauty'was the only plant tested which did not show any evidence of an attack by *M. hapla*. No root knots were formed, no nematodes could be traced inside the roots and only larvae could be detected in the soil. The number of nematodes in the soil was less than for fallow. *M. hapla* behaved in the same way on *Schkuhria pinnata, S. senecioides, Ambrosia trifida* and *Milleria quinqueflora* as on *Tagetes patula* var. 'Golden Harmony'. The total number of nematodes in the roots and in the soil was always lower than in the comparable fallow tubes. None or only a single larvae or male could be detected in the roots of these plants. Inside the few root knots formed mostly normal giant cells and females were present as will be reported later. Some larvae were found in the soil but males only rarely occurred.

In spite of the fact that *Baeria californica, B. chrysostoma* and *Iva xanthiifolia* hardly supported populations of *Pratylenchus penetrans* these plants were fairly good hosts for *M. hapla*. It can be seen from the data in table 10 that *B. californica* roots contained fewer root knots and males than *B. chrysostoma*. This suggests a difference in host suitability between these two *Baeria* species. The root knots on *Iva xanthiifolia* were abundant, big and often arranged like a bead necklace. The roots of tomato, a control, were also heavily infested with *M. hapla*.

The arrangement of the knot tissues was not principally different from tomato in the few root knots on *Tagetes patula*. The root knots, however, were tiny when compared with those formed on tomato roots. Usually only 2–4 syncytial giant cells surrounded the head region of the nematodes. The tissue involved in the root knot formation was mainly of xylemic origin. The pericycle tissue seemed not to be involved in root knot formation. The few, and usually tiny, root knots formed on *Schkuhria senecioides, Milleria quinqueflora* and *Ambrosia trifida* appeared to be of the same constitution as the root knots on *Tagetes patula*. The number of giant cells varied from 1–4 per female, and again, tissue mainly of xylemic origin contributed to the formation of the galls.

The pericycle was usually intact and often flattened against the slightly hypertrophied cortex.

On roots of *Schkuhria pinnata* a few root knots were formed but in serial sections of these galls no giant cells and no nematodes could be detected. Nevertheless some males were found in the soil (table 10) which implies the completion of a lifecycle in the roots of these plants.

4.3. Discussion

4.3.1. *Influence on Pratylenchus penetrans*

The effects of the *Compositae* tested in these field and glasshouse experiments on populations of *P. penetrans* supplemented with the results of Oostenbrink et al. (1957) and Hijink and Winoto Suatmadji (1967) are summarized in table 11. *Compositae* with suppressing properties on *P. penetrans* are mainly restricted to the genera *Melampodium*, *Iva*, *Ambrosia*, *Franseria*, *Milleria* (*Heliantheae*), *Baeria*, *Schkuhria*, *Eriophyllum*, *Helenium*, *Gaillardia*, *Tagetes* (*Helenieae*) and *Echinops* (*Cardueae*). The same properties were also found in some species of the genera *Coreopsis* and *Rudbeckia* (*Heliantheae*), in a *Solidago* hybrid, in *Erigeron speciosus* (*Astereae*) and in a hybrid of *Arctotis* (*Arctoteae*). These few positive records justify a closer investigation of the suppressing properties of these last five genera on *P. penetrans*.

Eriophyllum nevinii (table 6, 13) and to a lesser extent *Helenium hoopesi* (table 2) acted as host plants for *P. penetrans* in contrast to the other tested species of these genera. *Eriophyllum nevinii* also has a somewhat separate position within the genus both from a phyletic point of view and from its geographical distribution (Constance, 1937).

The inability of *Ambrosia artemisiifolia* (Townshend and Davidson, 1960) and of *Milleria quinqueflora* (Winoto Suatmadji, personal communication) to support *P. penetrans* has already been noticed. Koen (1967) reported *Schkuhria pinnata* as a host plant for *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans Stekhoven.

In all cases where *Compositae* decreased densities of *P. penetrans* in the soil no or only a few nematodes could be traced inside the roots at the end of an experiment. In fact low numbers of *P. penetrans* in the roots always indicated plant species that suppressed densities of this nematode.

Exceptionally relatively high numbers of *P. penetrans* were found in the soil after cultivation of some of these suppressing plant species in the field. Cf. table 3 where *Echinops sphaerocephalus*, *E. ritro* and the *Gaillardia* hybrid 'Burgunder' and table 6 where some *Baeria* and *Schkuhria* species exerted no negative influence on densities of *P. penetrans* in the soil. In other field experiments (table 4, 5, 7) these *Compositae* nevertheless reduced *P. penetrans* to low densities. The results of the experiments were confirmed in a glasshouse trial (table 9). The explanation for the discrepancy is simple. In the pot experiments there was always good root development throughout the soil. In the field,

TABLE 11. Host suitability of *Compositae* for *Pratylenchus penetrans*. Summary of our field experiments and glasshouse trials (1), the results of HIJINK and WINOTO SUATMADJI (1967) (2) and of OOSTENBRINK et al. (1957) (3). Plant species are arranged according to the system of ENGLER (1964).
In bold print: plants that suppressed *P. penetrans*.
In normal print: host plants for *P. penetrans*.

EUPATORIEAE	*Eupatoriinae*	*Ageratum mexicanum* (2); *Eupatorium rugosum* (1).
	Kuhniinae	*Liatris spicaea* (2).
ASTEREAE		***Solidago* hybrid** (2).
	Bellidinae	*Bellis perennis* (2); *Brachicome iberidifolia* (2).
	Asterinae	*Callistephus chinensis* (2); *Erigeron speciosus* (2).
INULAE	*Gnaphaliinae*	*Leontopodium alpinum* (2); *Helichrysum bracteatum* (2).
HELIANTHEAE	*Coreopsidinae*	*Guizotia abyssinica** (1); ***Coreopsis lanceolata*** (2); ***Coreopsis grandiflora*** (2); *Coreopsis verticillata* (2); *Coreopsis basalis* (2); *Coreopsis tinctoria* (2); *Dahlia* hybrid (2); *Bidens dahlioides* (1); *Cosmos sulphureus* (2).
	Helianthinae	***Rudbeckia laciniata*** (2); ***Rudbeckia serotonina*** (2); ***Rudbeckia bicolor*** (2); *Rudbeckia speciosa* (2); *Rudbeckia purpurea* (2); *Wyethia elata** (1); *Helianthus annuus* (2); *Encelia virginensis** (1); *Encelia californica** (1).
	Melampodiinae	*Melampodium perfoliatum* (1); ***Melampodium divaricatum*** (1); *Silphium perfoliatum* (1); ***Silphium asterides*** (1); *Lindheimera texana* (1); ***Parthenium argentatum**** (1); *Parthenium hysterophorus** (1).
	Ambrosiinae	***Iva xanthiifolia*** (1); ***Ambrosia trifida*** (1); ***Ambrosia artemisiifolia*** (1); ***Ambrosia maritima*** (1); ***Ambrosia chamissonis**** (1); ***Franseria artemisioides*** (1); ***Franseria chenopodifolia**** (1); *Xanthium strumarium* (1).
	Milleriinae	***Milleria quinqueflora*** (1).
	Zinniinae	*Zinnia elegans* (2); *Sanvitalia procumbens** (1); *Heliopsis glabra* (2).

TABLE 11. (continued)

	Madiinae	*Madia gracilis* (1); *Madia anomala* (1); *Madia sativa* (1); *Madia elegans* (1); *Hemizonia corymbosa* (1); *Layia patyglossa* (1); *Layia elegans* (1).
HELENIEAE	*Heleniinae*	***Baeria californica*** (1); ***Baeria minor*** (1); ***Baeria chrysostoma*** (1); *Flaveria repanda* (1); ***Schkuhria pinnata*** (1); ***Schkuhria senecioides*** (1); ***Eriophyllum lanatum*** (1); ***Eriophyllum confertiflorum*** (1); ***Eriophyllum caespitosum*** (1,2); *Eriophyllum nevinii* (1); ***Helenium autumnale*** (1); ***Helenium nudiflorum*** (1); ***Helenium bolanderi*** (1); ***Helenium flexuosum*** (1); *Helenium hoopesi* (1,2); ***Helenium*** **hybrid 'Moerheim Beauty'** (1.2); ***Helenium*** **hybrid 'Riverton Gem'** (2); ***Gaillardia pulchella*** (1); ***Gaillardia amblyodon*** (1); ***Gaillardia lanceolata*** (1); ***Gaillardia arizonica*** (1); ***Gaillardia aristata*** (1); ***Gaillardia lutea*** (1); ***Gaillardia*** **hybrid 'Burgunder'** (1,2).
	Tagetinae	***Tagetes patula*** (1,2,3); ***Tagetes erecta*** (2,); ***Tagetes tenuifolia*** (1).
ANTHEMIDEAE	'*Anthemidinae*'	*Anthemis tinctoria* (1); *Anthemis ruthenica* (1); *Anthemis nobilis* (1); *Achillea ptarmica* (1,2); *Achillea filipendula* (3).
	'*Chrysantheminae*'	*Matricaria* hybrid (2); *Chrysanthemum maximum* (2); *Chrysanthemum carinatum* (2); *Chrysanthemum parthenium* (1); *Chrysanthemum* hybrid (2); *Tanacetum balsamita* (1); *Artemisia absinthium* (2); *Artemisia dracunculus* (3).
SENECIONEAE	*Senecioninae*	*Doronicum caucasicum* (2); *Doronicum orientale* (3); *Cineraria candicans* (2); *Senecio vulgaris* (2); *Senecio aquaticus* (1); *Senecio viscosus* (1).
CALENDULEAE		*Calendula officinalis* (1,2).
ARCTOTEAE	*Arctotinae*	***Arctotis*** **hybrid** (2).

(continued on next page)

TABLE 11. (continued)

CARDUEAE	*Carduinae*	*Saussurea albescens* (1).
	Centaureinae	*Centaurea cyanus* (2).
	Echinopinae	***Echinops sphaerocephalus*** (1); ***Echinops ritro*** (1,2).
CICHORIEAE	*Cichoriinae*	*Cichorium endiva* (2,3); *Cichorium intybus* (2,3).
	Scorzonerinae	*Scorzonera hispanica* (3).
	Crepidinae	*Lactuca sativa* (2,3); *Taraxacum officinale* (3).

* Incidental observations

however, plants were not always rooted through the whole plot because of poor growth, so that soil samples, which were taken at random in the plots, contain soil that had not been penetrated by the root systems and therefore may be considered as fallow soil. Poor growth of the tiny *Baeria* and *Schkuhria* species were mostly due to attacks by *Botrytis*, *Baeria* species being especially sensitive to attack by this fungus.

The possibility that tough cellwalls of the roots of the *Compositae* prevent penetration of *P. penetrans* and therefore suppress populations of this nematode can be excluded since the results, summarized in fig. 1, show that the rate of penetration in *Tagetes patula*, *Schkuhria senecioides*, *Melampodium perfoliatum*, *Iva xanthiifolia* and *Baeria californica* did not differ significantly from that in oats, a good host plant for this nematode. Although the rate of lesion formation as a result of penetration by *P. penetrans* differed from plant species to plant species no excessive necrotic tissue could be observed in the early stage of infection. Generally lesion formation was most pronounced on the roots of *Tagetes patula*. In the long term experiments, for example the field experiments, no lesions could be detected on the roots of those *Compositae* with suppressing properties. Root systems seemed always to be unaffected, in contrast with the *Compositae* which were good hosts for *P. penetrans*, where root systems were always abundantly covered with dark coloured lesions.

The suppressing effect of a number of *Compositae* on *P. penetrans*, shown in section 4.1 and 4.2, could be governed by phenomena related to equilibrium densities as discussed by SEINHORST (1966a) and OOSTENBRINK (1966) and not by specific suppressing substances. Therefore a glasshouse trial was set up as described in par. 4.2.2. Cultivation of the plant species used in this experiment i.e. *Milleria quinqueflora*, *Schkuhria pinnata*, *Baeria chrysostoma*, the *Helenium* hybrid 'Moerheim Beauty', *Ambrosia trifida* and *Tagetes patula* resulted in the disappearance of nearly all *P. penetrans*. The few nematodes still present in the tubes with the *Helenium* hybrid and *Baeria chrysostoma* could be explained by incomplete rooting of these plant species through the tubes.

If a certain low equilibrium density exists one would expect, at least in the

tubes with the higher initial nematode densities, a certain part of the nematode population to survive. Nevertheless all *Compositae* used in this experiment suppressed all initial densities of *P. penetrans* to zero or nearly to zero. It is clear that these plant species with respect to *P. penetrans*, not properly fit the definitions of a bad host or a non-host as given by SEINHORST (1967). In inoculation trials with *P. penetrans* and *Tagetes erecta*, SEINHORST (1966a) observed in some cases multiplication of *P. penetrans* on this plant species but a density-dependent relationship always existed. In his studies with *P. penetrans* and different *Tagetes* species WINOTO SUATMADJI (1969) found differences in suppressing properties.

4.3.2. *Influence on Meloidogyne hapla*

Certain *Compositae* with suppressing properties on populations of *Pratylenchus penetrans* showed different effects on *Meloidogyne hapla* (see par. 4.2.5. and table 12). *Tagetes patula*, the *Helenium* hybrid 'Moerheim Beauty', *Ambrosia trifida*, *Schkuhria senecioides*, *S. pinnata* and *Milleria quinqueflora* acted as non-hosts, whilst, in contrast, *Baeria californica*, *B. chrysostoma* and *Iva xanthiifolia* were hosts for *M. hapla*.

Roots of the *Helenium* hybrid did not show any sign of attack by this nematode.

Serial sections of the few root knots formed on *Tagetes patula*, *Ambrosia trifida*, *Milleria quinqueflora* and *Schkuhria senecioides* showed tiny giant cells. A few mature females and males were found in roots and soil. Giant cells were absent in the root galls of *Schkuhria pinnata*. Nevertheless in two cases a male was found in the soil. This indicates that larvae were able to penetrate the roots and in some cases complete their development. The absence of giant cells in the case of *Schkuhria pinnata* is rather curious. The only records in literature where full-grown *Meloidogyne* were found without giant cell formation are those of CHRISTIE (1949) in *Pelargonium graveolens* L'HERIT and of ORION (1971) who often found malformed *Meloidogyne* females in tissue cultures of carrot.

No studies were made on the early stages of infection of *M. hapla* in the roots of the *Compositae* tested. WINOTO SUATMADJI (1969) could not find significant differences between the rate of penetration of *Meloidogyne* larvae in roots of *Tagetes patula* and the host plant tomato. In contrast, DAULTON and CURTIS (1963) noticed a failure of *Meloidogyne* to invade in appreciable numbers.

It remains possible that the inability of *M. hapla* to develop in great numbers on those *Compositae* that hardly support them, could be due to delayed development of the nematodes or anatomic abnormalities of the plants. For instance FASSULIOTIS (1970) found in the case of the *Meloidogyne* resistant *Cucumis metuliferus* and *C. ficifolius* a hindrance of larval development beyond the second stage and a stimulation to maleness.

There are a few records on these *Compositae* and related species which did not support *Meloidogyne*. *Ambrosia artemisiifolia* was shown not to support *Heterodera marioni* (= *Meloidogyne* s.l.) (MILLER, 1946), *M. arenaria*, *M. incognita*, *M. javanica* and *M. hapla* (SASSER, 1954). LUC (1959) recorded

Ambrosia artemisiifolia as a non-host for *M. javanica*. *Ambrosia psilostachya* DC. did not support *Meloidogyne arenaria* (GASKIN, 1958) nor *M. incognita* (GASKIN, 1958; DAVIDSON and TOWNSHEND, 1967) and *M. hapla* (TOWNSHEND and DAVIDSON, 1962). *Milleria quinqueflora* decreased populations of *M. incognita* and *M. iavanica* (WINOTO SUATMADJI, personal communication).

4.3.3. *Influence on ectoparasitic nematodes*

The population dynamics of ectoparasitic *Tylenchorhynchus dubius* on a number of *Compositae* differed markedly from that of the endoparasitic *Pratylenchus penetrans* (see par. 4.2.4.). Oats acted as a good host plant, the host suitability of *Baeria californica* was less and *Tagetes patula* suppressed populations of *T. dubius*, at least at the densities used in experiment. In all cases density-dependant relations were found.

Schkuhria pinnata, the *Helenium* hybrid 'Moerheim Beauty' and *Milleria quinqueflora* acted as non-hosts.

These results and the incidental observations on host suitability of *Compositae* for other ectoparasitic nematodes in the field, supplemented with the host suitability of some selected *Compositae* for *Meloidogyne hapla*, are summarized in table 12.

Iva xanthiifolia slightly suppressed *T. dubius* but acted as a host plant for *Rotylenchus robustus*. *Ambrosia trifida* negatively influenced densities of *T.*

TABLE 12. Host suitability of certain *Compositae* for *Pratylenchus penetrans*, *Paratylenchus* sp., *Tylenchorhynchus dubius*, *Rotylenchus robustus*, *Trichodorus* sp. and *Meloidogyne hapla*. Figures are selected from the preceding field experiments and glasshouse trials.
+ = a good host plant; − = a plant species with suppressing properties, taking into account the initial levels of infestation in the field and the initial levels of infestation used in the glasshouse trials; ○ = no significant differences from fallow; • = no observations made.

	Pratylenchus penetrans	*Paratylenchus* species	*Tylenchorhynchus dubius*	*Rotylenchus robustus*	*Trichodorus* species	*Meloidogyne hapla*
Iva xanthiifolia	−	○	−	+	○	+
Ambrosia trifida	−	○	−	○	−	−
Milleria quinqueflora	−	○	−	○	+	−
Baeria californica	−	+	+	○	○	+
Baeria chrysostoma	−	○	○	○	○	+
Schkuhria senecioides	−	○	−	○	−	−
Schkuhria pinnata	−	○	−	○	•	−
Eriophyllum lanatum	−	○	○	○	−	•
Eriophyllum caespitosum	−	+	○	○	•	•
Helenium hybrid 'Moerheim Beauty'	−	○	−	○	•	−
Gaillardia hybrid 'Burgunder'	−	○	−	−	•	•
Tagetes patula	−	○	−	○	○	−

dubius and of an unidentified *Trichodorus* species. *Milleria quinqueflora* acted as a host plant for *Trichodorus*. The *Baeria* species tested were suitable hosts for *T. dubius* and an unidentified *Paratylenchus* species. *Eriophyllum caespitosum*, in contrast with *E. lanatum* and *E. confertiflorum*, was an extremely good host for this *Paratylenchus*. The *Helenium* hybrid 'Moerheim Beauty', the *Gaillardia* hybrid 'Burgunder' and both *Schkuhria* species hardly suppressed any of the ectoparasites tested. The same was true for *Tagetes patula* which is in agreement with the results of WINOTO SUATMADJI (1969).

From the foregoing it is clear that *Compositae* which effectively reduced densities of *Pratylenchus penetrans*, either hardly affected population densities of other plant parasitic nematodes, or suppressed them or even acted as good host plants. This also holds for *Tagetes* species. Since the discovery of the suppressing properties of *Tagetes* on certain nematode species (STEINER, 1941; SLOOTWEG, 1956; OOSTENBRINK et al., 1957; MILLER and AHRENS, 1969) many investigations have been carried out with this plant genus and the records on reproduction are summarized.

Reproduction on *Tagetes* species was found with *Criconemoides mutabile* (now *Nothocriconema mutabile* (TAYLOR) DE GRISSE and LOOF) (STEINER, 1941), *Trichodorus teres* (KUIPER, 1963), *Hemicycliophora similis* (SEINHORST and KLINKENBERG, 1963), *Hemicycliophora conida* THORNE (WINOTO SUATMADJI, 1969), *Longidorus maximus* (STURHAN, 1963), *Aphelenchoides besseyi* CHRISTIE (SHER, 1954) and *Aphelenchoides ritzemabosi* (WINOTO SUATMADJI, 1969).

5. NATURAL NEMATICIDAL PRINCIPLES AND THEIR ACTIVITIES

5.1. INTRODUCTION

The screening experiments described in the preceding chapter show that *Compositae* with suppressing properties on *Pratylenchus penetrans* are restricted to certain genera (table 11). A number of *Coreopsidinae*, *Helianthinae*, *Melampodiinae*, *Ambrosiinae*, *Milleriinae*, most of the *Heleniinae*, *Tagetinae*, two *Echinopinae* and a few other plants tested show this effect. These plant species are chemotaxonomically related (BOHLMANN and MANNHARDT, 1957; BOHLMANN and SUCROW, 1963; BOHLMANN, 1967; HEGNAUER, 1964; ROMO and ROMO DE VIVAR, 1967; SÖRENSEN, 1962). UHLENBROEK and BIJLOO (1958; 1959) isolated two nematicidal principles from roots of a variety of *Tagetes erecta*: α-terthienyl and 5-(3-buten-1-ynyl)-2,2'-bithienyl. ZECHMEISTER and SEASE (1947) were the first to isolate α-terthienyl from the aerial parts of *Tagetes*.

Both aforementioned nematicidal thienyl-derivatives were amongst other thiophenic derivatives discovered in roots of *Tagetes patula* (BOHLMANN and HERBST, 1962; HORN and LAMBERTON, 1963), *T. minuta* (ATKINSON et al., 1964), *Echinops sphaerocephalus*, *E. commutatus* JUR., *E. ritro*, *E. strigosus* L., *E. dahurieus* FISCH., *E. persicus* STEV. et FISCH., *E. horridus* DESF., *E. niveus* WALL., *E. cornigerus* DC., *E. bannaticus* ROCHEL et SCHRAD. (BOHLMANN et al., 1965), *Eclipta alba* (L.) HASSK., *E. erecta* L., *E. prostrata* L. (BOHLMANN et al., 1964; BOHLMANN and ZDERO, 1970), *Berkheya adlami* HOOK. and *B. radula* BURTT-DAVY (BOHLMANN et al., 1967). α-Terthienyl has also been isolated from *Flaveria repanda* (BOHLMANN and KLEINE, 1963). SÖRENSEN (1961) found from spectrophotometric data strong evidence for the presence of 5-(3-buten-1-ynyl)-2,2'-bithienyl in the roots of *Berkheya macrocephala* WOOD. and *Echinops sphaerocephalus*. BOHLMANN and HERBST (1962) stated that this compound is common within the *Helenieae*.

It is shown below that roots of *Iva xanthiifolia*, *Ambrosia trifida*, *A. artemisiifolia*, *Milleria quinqueflora*, *Schkuhria pinnata*, *Eriophyllum caespitosum*, the *Helenium* hybrid 'Moerheim Beauty', the *Gaillardia* hybrid 'Burgunder' and *Echinops sphaerocephalus* also contain nematicidal substances. Diluted aqueous solutions of root extracts made up with a mixture of light petroleum and diethylether killed, in vitro, plant parasitic nematodes such as *Pratylenchus penetrans*, *Ditylenchus dipsaci* and larvae of *Heterodera rostochiensis*. Chromatography of concentrated root extracts of all these plants on columns of silicagel yielded two or more fractions with nematicidal activity in vitro. Some of these fractions were sufficiently pure to allow identification.

5.2. Isolation of nematicidal compounds from roots of two *compositae*

5.2.1. *The Helenium hybrid 'Moerheim Beauty'*

Nematicidal principles were purified from concentrated root extracts made with an excess of a mixture of light petroleum (b.p. 40–60 °C) and diethylether. The extracts were concentrated in vacuo and fractionated on a column of silicagel with light petroleum with increasing quantities of diethylether as eluent. In this way three fractions with strong, in vitro, nematicidal activity could be obtained. By means of repeated chromatography on columns of silicagel or aluminium oxide and on thick layers of silicagel (thick TLC) two nematicidal principles could be obtained pure enough for identification.

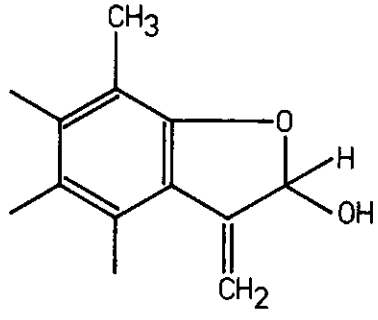
The most apolar compound with nematicidal properties was 1-tridecanene-3,5,7,9,11-pentayne (ene-pentayne) an unstable unsaturated hydrocarbon, known as '4100-pigment', common in *Compositae* (Hegnauer, 1964; Bohlmann, 1967).

$$CH_3-(C\equiv C)_5-CH=CH_2$$

1-tridecanene-3,5,7,9,11-pentayne.

The structure of this compound can be established mainly from its characteristic UV-spectrum. Catalytic hydrogenation of this pigment in absolute alcohol with hydrogen gas and finely divided platinum oxide yielded $C_{13}H_{28}$. This could be established by gaschromatographic comparison of the hydrogenated compound with an authentic sample of $C_{13}H_{28}$.

The second compound with, in vitro, nematicidal activity could finally be obtained in crystalline form after repeated chromatography on columns of silicagel and of thick TLC. Detection of the compound on columns and plates was easily done because of its fluorescence in near ultra-violet light. Recrystallization from a mixture of diethylether and pentane yielded white crystals with a melting point of 75–76.5 °C. The spectral data of the compound were in agreement with 2,3-dihydro-2-hydroxy-3-methylene-6-methylbenzofuran.



2,3-dihydro-2-hydroxy-3-methylene-6-methylbenzofuran

The IR spectrum (chloroform) indicates the presence of an OH group because of absorption bands at 3340 and 1335 cm^{-1} and an aromatic ring (1585 and 1493 cm^{-1}). The presence of a band at 866 cm^{-1} suggests the presence of a $R_2C = CH_2$ group.

The NMR spectrum (TMS internal standard) indicates the presence of an 1,2,4-trisubstituted benzoic ring because of a doublet at 3.39τ (J = 1 c/s, 1H), a double doublet at 3.28τ (J = 1 and 8 c/s, 1H) and a doublet at 2.71τ (J = 8 c/s, 1H). A singlet at 7.6τ (3H) is ascribed to a CH_3 group and two doublets at 4.76τ (J = 2 c/s, 1H) and 4.49τ (J = 2 c/s, 1H) to a $R_2C = CH_2$ group. A doublet at 6.48τ (J = 10 c/s, 1H) and a double multiplet at 3.88τ (J = 10 c/s, 1H) are in agreement with an OH group and an H atom respectively.

M^+ (found: 162.0684; calculated: 162.0680) indicates the empirical formula $C_{10}H_{10}O_2$ and the mass spectrum gave a fragmentation pattern as could be expected for this benzofuran-derivative.

The UV spectrum (hexane) with maxima at 333, 328, 318, 266, 257 and 228 nm (ε 6800, 8000, 8900, 12500, 14000 and 11000) afforded no further information on the structure.

The LD_{50}, in vitro, of 2,3-dihydro-2-hydroxy-3-methylene-6-methylbenzofuran was 3–5 ppm for *Pratylenchus penetrans*, *Ditylenchus dipsaci* and larvae of *Heterodera rostochiensis*.

The third compound from the roots of the *Helenium* hybrid with nematicidal activity could not be obtained in a pure state. The substance was present in low concentrations in the root extract and the partially purified compound showed strong absorbance near 350 nm.

The ene-pentayne and the benzofuran-derivative are the first nematicidal principles isolated from roots of a member of the genus *Helenium* (GOMMERS, 1971b). After these investigations had been finished BOHLMANN et al. (1969), in their chemotaxonomical work on *Compositae*, published the isolation of the benzofuran-derivative from roots of *Helenium mexicanum*, *H. nudiflorum* NUTT. and *H. puberolum*. They also synthetized the compound, but did not mention its nematicidal activity.

Experimental

The *Helenium* hybrid 'Moerheim Beauty' was increased by allowing young shoots to produce roots. The young plants were grown in the 'Kruidentuin' at Buitenpost. The roots were harvested in the autumn, cleaned by washing with tapwater and stored in plastic bags in a freezer until use.

5000 g of roots were chopped and extracted three times with excess quantities of a mixture of diethylether and light petroleum (b.p. 40–60 °C). After evaporation of the bulk of the solvent under reduced pressure the partially concentrated extract was dried over sodium sulphate, filtered and further concentrated in vacuo. The whole procedure was carried out in the cold room and in an atmosphere of nitrogen. Only during concentrating the extract in vacuo was the temperature raised to 30 °C. Influences of light were avoided as much as possible.

The oily concentrate was chromatographed on a column of silicagel (height 60 cm, diameter 4.2 cm) with light petroleum with increasing quantities of diethylether.

1-Tridecanene-3,5,7,9,11-pentayne was eluted from the column with light petroleum. Repeated chromatography on columns of aluminium oxide (standardized according to BROCKMANN, activity II-III) with 1% of diethylether in light petroleum (v/v) yielded the pure compound.

As the instability of the compound prevented exact operations on the balance the extinction coefficients of the UV spectrum are uncertain, but agree well with those reported in the literature (SÖRENSEN et al. 1954).

λ_{max} (hexane) 410, 378, 351, 329, 308, 286, 271, 265, 257 nm
(ε 1500, 2920, 2940, 2000, 1490, 89000, 84200, 95100, 69500)

Hydrogenation of the ene-pentayne was achieved at room temperature in an atmosphere of hydrogen with finely divided platinum oxide as a catalyst. About 10 mg of the compound in 10 ml of pentane were added to the reaction vessel containing 0.04 g PtO_2 covered with 50 ml absolute ethanol. Having filled the apparatus with hydrogen gas the reaction mixture was magnetically stirred for three hours thus allowing contact between the gas and the catalyst. After about one hour no hydrogen gas consumption could be measured. The catalyst was filtered off and 15 ml of water were added to the filtrate. This mixture was extracted three times with 20 ml of pentane. The dried solution (sodium sulphate) was concentrated in vacuo to about 0.5 ml, 50 µl of this concentrate was gaschromatographed on a column of carbowax (Chrom WAS 60/80 with 10% of Carbowax 20H; length 2 m, diameter ¼ inch) with N_2 gas (60 ml/min) as carrier. The temperature of the column was 130°C. The place of the main peak (volatile impurities were probably hydrogenated degradation or polymerisation products of the compound) coincided exactly with an authentic sample of $C_{13}H_{28}$.

2,3-Dihydro-2-hydroxy-3-methylene-6-methylbenzofuran was eluted from the column of silicagel with 14% (v/v) diethylether in light petroleum. Further purifications were carried out on thick layers of silicagel (Merck silicagel HF_{254}) with 40% of diethylether in light petroleum. Recrystallization from a mixture of diethylether and pentane finally yielded the pure compound.

5.2.2. *Echinops sphaerocephalus*

Purification of the nematicidal principles was carried out with root extracts, made up with light petroleum and diethylether, concentrated in vacuo. Chromatography of the oily concentrate on a column of aluminium oxide with light petroleum, with increasing quantities of diethylether as eluent, yielded several fractions having nematicidal properties in vitro. By means of repeated chromatography three nematicidal principles could be isolated in a pure state, thus allowing identification.

The most apolar fraction was the ene-pentayne ('4100-pigment') already known as a nematicidal compound from roots of the *Helenium* hybrid 'Moerheim Beauty'. In contrast with the observations of BOHLMANN et al. (1965) and BOHLMANN and HINZ (1965) this compound is present in low concentrations in the roots of *Echinops sphaerocephalus*.

The second purified nematicidal compound was the blue-fluorescing α-terthienyl (2,2'-5'2''-terthienyl). Identification was straightforward, the UV and IR spectra being in accordance with those given in the literature (UHLENBROEK and BIJLOO, 1958) and those of a synthetic sample. The melting point of the crystals did not change after mixing with authentic α-terthienyl. Elementary analysis of the compound gave figures which were in accordance with those calculated for α-terthienyl.

The third nematicidal principle that could be isolated in large quantities was 5-(3-buten-1-ynyl)-2,2'-bithienyl, one of the nematicidal principles from roots of *Tagetes* (UHLENBROEK and BIJLOO, 1959). The UV, IR and NMR spectra were in accordance with those given in literature (UHLENBROEK and

BIJLOO, 1959; BOHLMANN and HERBST, 1962; HORN and LAMBERTON, 1963). The data obtained from elementary analysis agreed with the calculated ones.

The other fractions with nematicidal properties could not be obtained pure enough, partially because of their instability, to allow definite conclusions on their structures. All these fractions possess an absorption maximum in the region 330–360 nm. The presence of sulphur could be established with the reaction of CASTELLANA. Probably thiophenic compounds are also responsible for the in vitro nematicidal activity of these fractions.

Experimental

Echinops sphaerocephalus was cultivated in the 'Kruidentuin' at Buitenpost. The roots of this perennial plant were harvested in the autumn of the first years growth. Roots were thoroughly cleaned with tapwater and stored, in plastic bags, in a freezer until use.

1400 g of roots were chopped and extracted three times for 24 hours each time with 5 l of a mixture of light petroleum (b.p. 40–60 °C) and diethylether (2/1 v/v). After evaporation of the bulk of the solvent under reduced pressure the partially concentrated extract was dried over sodium sulphate, filtered and further concentrated in vacuo. The whole procedure was carried out in the cold (+ 4 °C) and in an atmosphere of nitrogen. Only during concentration of the extract in vacuo was the temperature of the waterbath raised to approximately 30°.

The brown oily concentrate was chromatographed on a column of aluminium oxide (standardized according to BROCKMANN, activity II-III; dimensions: height 40 cm, diameter 3.4 cm) with light petroleum with increasing quantities of diethylether.

1-Tridecanene-3,5,7,9,11-pentayne was eluted from the column with 1% (v/v) of diethylether in light petroleum. Repeated chromatography on small columns of aluminium oxide (height 20 cm, diameter 2 cm) yielded this unstable compound in a pure state.

Crude 5-(3-buten-1-ynyl)-2,2′-bithienyl (800 mg) was eluted from the column with 5% (v/v) of diethylether in light petroleum. Repeated chromatography on small columns of aluminium oxide finally yielded 180 mg of the unstable colourless compound pure enough for identification

λ_{max} (hexane) 340,251 nm (ε 27 800, 9750)
IR: – C ≡ C – 2190; – CH = CH_2 970,920; 2-substituted thiophene 845 cm^{-1}
elementary analysis found: C 66,65 H 3,81 S 29,71
calculated: C 66,63 H 3,71 S 29,64

Crude 2,2′-5′,2″-terthienyl (α-terthienyl) was obtained from the column by elution with 10 to 15% (v/v) of diethylether in light petroleum. Recrystallization from light petroleum yielded 160 mg pure α-terthienyl (m.p. 92–93 °C)

λ_{max} (iso-octane) 350,251 nm (ε 23 600, 9300)
elementary analysis found: C 58,16 H 3,38 S 38,83
calculated: C 58,15 H 3,25 S 38,73

5.3. INFLUENCE OF LIGHT ON THE NEMATICIDAL ACTIVITY OF α-TERTHIENYL AND RELATED COMPOUNDS[4]

We found for the first time in these experiments that daylight enhanced the nematicidal activity of 2,3-dihydro-2-hydroxy-3-methylene-6-methylbenzofuran from the *Helenium* hybrid 'Moerheim Beauty' and α-terthienyl was similarly

[4] Published in part (Gommers, 1972b; Gommers and Geerligs, 1973).

affected. Because of lack of the benzofuran-derivative this phenomenon was further studied with α-terthienyl.

5.3.1. *Influence of light*

Several species of plant parasitic and saprozoic nematodes were killed in 1–3 days when they were kept in glass covered vials with 1 ppm emulsion of α-terthienyl (prepared with Tween 20) in daylight. When the same species were stored in the dark for the same time nearly all nematodes survived. Slow nematicidal action of α-terthienyl in the dark was only observed after prolonged incubation in the dark. In two weeks, about 50% of *Ditylenchus dipsaci* was killed. The effect of daylight on the nematicidal activity of α-terthienyl was noted for the plant parasites *Ditylenchus dipsaci* (exposed for three days), *Pratylenchus penetrans* (exposed for two to three days), the entomophagous nematode *Neoaplectana carpocapsae* WEISER and the cephalobid *Panagrellus redivivus* (exposed for 3–6 hours). No indications could be found for resistance of soil-inhabiting nematode species against this combination of α-terthienyl and daylight. All nematode species as recovered from different types of soil were killed when immersed in α-terthienyl and exposed to daylight.

It could be shown with a series of glass covered Kodak Wratten filters that near ultra-violet light is the active part of the daylight spectrum (fig. 3). In this region α-terthienyl has an absorption maximum (ZECHMEISTER and SEASE, 1947; UHLENBROEK and BIJLOO, 1958). The light transmitted through filter 39 killed the nematodes immersed in α-terthienyl as efficiently as daylight. Filter 39 is transparent for wavelengths from 300–510 nm. Light transmitted through filter 2A, i.e. wavelengths above 410 nm, did not kill the nematodes in an emulsion of α-terthienyl but caused them to move spasmodically and curl up, which are also the first symptoms when nematodes in α-terthienyl are exposed to

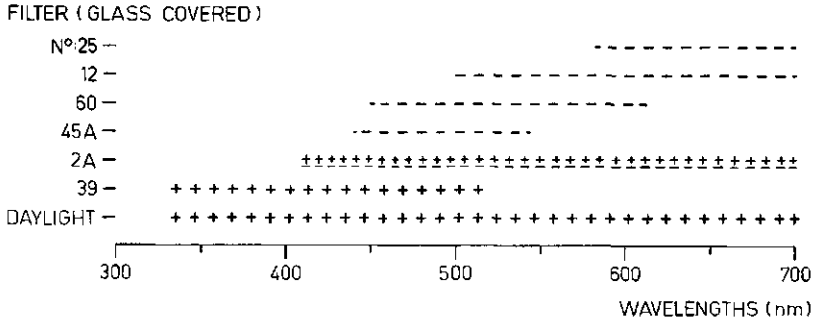


FIG. 3. Influence of daylight and parts of the daylight spectrum (obtained with the aid of Kodak Wratten filters) on the nematicidal activity of a 1 ppm emulsion of α-terthienyl on various nematodes (*Ditylenchus dipsaci* (KÜHN) FILIPJEV exposed for three days; *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN exposed for two to three days; *Neoaplectana carpocapsae* WEISER and *Panagrellus redivivus* (L.) GOODEY exposed for three to six hours).
++ nematodes killed; ± ± nematodes affected slightly; – – no effect.

lethal light. The light through the other filters used did not affect the nematodes during the incubation time.

Preliminary experiments showed that trans-1-(2-thienyl)-2-(5-chloro-2-thienyl)-ethene and trans-1-(2-thienyl)-2-(3-methyl-2-thienyl)-ethene[5] (synthetically prepared nematicides), were, like the benzofuran-derivative, more nematicidal in near ultra-violet irradiation than in the dark.

5.3.2. *Permeation and release of α-terthienyl*

Qualitative observations

For reasons of standardization further experiments were carried out with *Ditylenchus dipsaci* and *Pratylenchus penetrans* dipped in an emulsion of α-terthienyl and irradiated with a 'black light' tube (Philips: TL 20 W/8). This tube emits light of 300–400 nm and it was used at 40 cm distance. To avoid any influence of far ultra-violet which may be directly lethal for several nematode

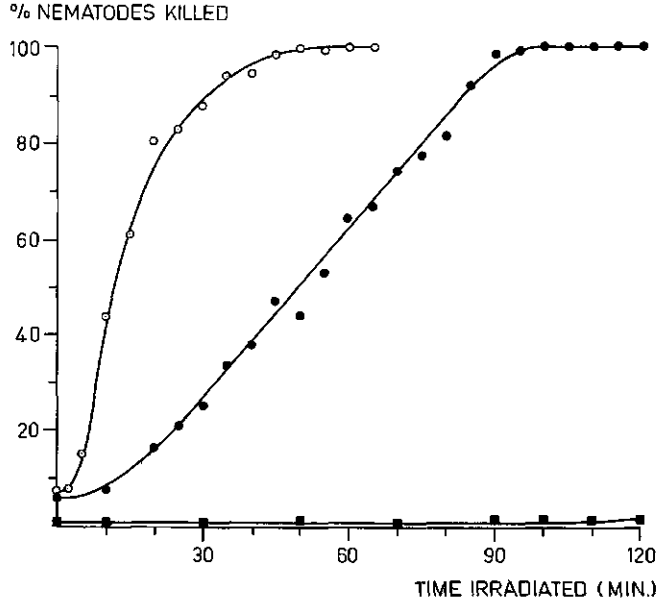


FIG. 4. Effect of irradiation with near ultra-violet light on the kill of *Ditylenchus dipsaci* (KÜHN) FILIPJEV immersed in a 1 ppm emulsion of α-terthienyl. After irradiation nematodes were stored in the dark for 4–5 days and then evaluated as dead or alive. Each point is the mean of three replicates.

●—● Nematodes immersed in α-terthienyl and irradiated immediately.

○—○ Nematodes initially exposed to 1 ppm α-terthienyl in the dark for 70 minutes and then irradiated.

■—■ Controls; no treatment with α-terthienyl; nematodes irradiated in water.

[5] Kindly supplied by Dr. M. J. Handelé.

species (GODFREY and HOSHINO, 1933; KEELING, 1960; GREEN and WEBSTER, 1965; GREEN and PLUMB, 1967) a glassfilter impenetrable by wavelengths below 330 nm was used. Red light did not induce nematicidal activity of α-terthienyl (cf. preceding paragraph) therefore nematodes in α-terthienyl were always handled in red light or in the dark before and after irradiation.

Approximately 200 *D. dipsaci* were immersed in 1 ml of a 1 ppm emulsion of α-terthienyl in small fixation dishes (SEINHORST, 1966b). After various irradiation times the fixation dishes with the nematodes were again placed in the dark. Four or five days later the nematodes, dead and alive, were counted under a dissecting microscope. Nematodes were considered to be dead when distortion of the body content was visible.

Irradiation for about 90 minutes was sufficient to kill all nematodes (fig. 4). This time could be shortened considerably by incubation of the nematodes in α-terthienyl in the dark prior to irradiation, probably because the compound

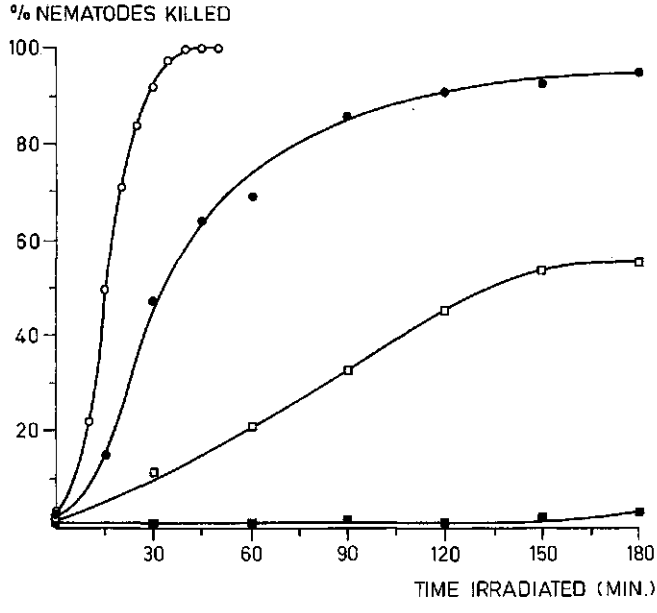


FIG. 5. Effect of near ultra-violet light on *Ditylenchus dipsaci* (KÜHN) FILIPJEV initially exposed to a 1 ppm emulsion of α-terthienyl in the dark for 3 h. After irradiation nematodes were stored in the dark for 4–5 days and then evaluated as dead or alive. Each point is the mean of three replicates.

○—○ Nematodes washed twice with acetone and water alternately and irradiated immediately.

●—● Nematodes washed as above and kept in the dark in running water for 3 h prior to irradiation.

□—□ As above but kept in running water for 20 h prior to irradiation.

■—■ Controls; no treatment with α-terthienyl; nematodes washed with acetone and water only.

accumulated in the nematodes. Incubation in the dark for 70 minutes prior to irradiation decreased the time to kill all nematodes to about 30 minutes.

As is the case with a number of other organic chemicals (HOLLIS, 1961; MARKS et al., 1968; CASTRO et al., 1970; NELMES, 1971; HAVERKATE et al., 1972; CASTRO and THOMASON, 1973) α-terthienyl could apparently be partially removed from nematodes. *D. dipsaci*, incubated for three hours in the dark in 1 ppm α-terthienyl, were twice washed with acetone followed by demineralized water (MARKS et al., 1968) and then kept in running demineralized water for different lengths of time. The acetone washings removed adsorbed α-terthienyl from the cuticle. Washing *D. dipsaci* with water considerably increased the irradiation time needed to kill the nematodes (fig. 5). When washed for three hours 100% of the nematodes were killed after about a two hours exposure. When washed for 20 hours only 50% of the nematodes were killed after 3 hours

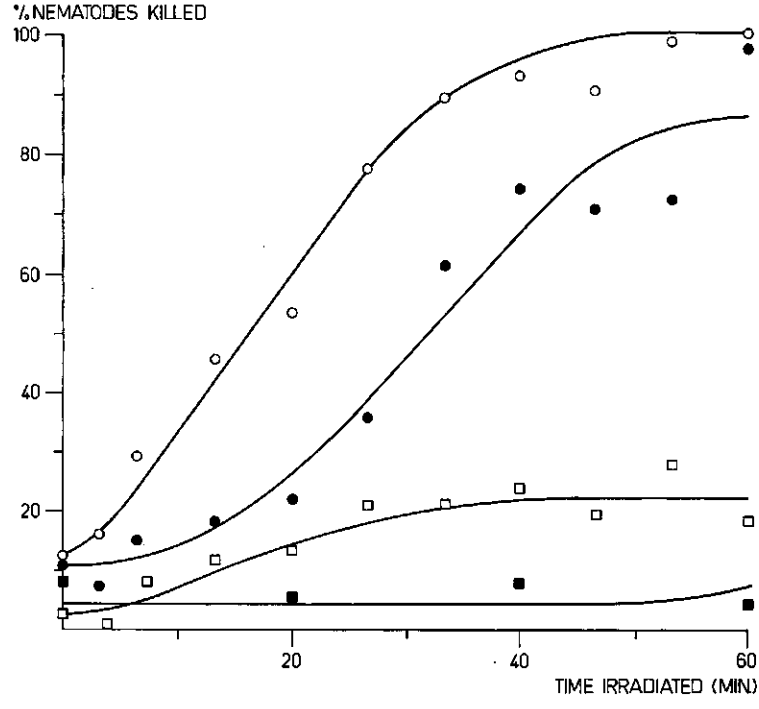


FIG. 6. Effect of near ultra-violet light on the kill of *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN in a 1 ppm emulsion of α-terthienyl and of *P. penetrans* initially treated in the dark with this emulsion. After irradiation nematodes were stored in the dark for 4 days and then evaluated as dead or alive. Each point is the mean of three replicates.

●—● Nematodes immersed in α-terthienyl irradiated immediately.

○—○ Nematodes initially exposed to 1 ppm α-terthienyl in the dark for 1 h and then irradiated.

□—□ As above but kept in water for 4 h prior to irradiation.

■—■ Controls: no treatment with α-terthienyl; nematodes irradiated in water.

of near ultra-violet irradiation. Apparently α-terthienyl is released from the nematodes slower than it is taken up.

Similar results were obtained with *P. penetrans* and α-terthienyl (fig. 6). About 80% of *P. penetrans* were killed after one hour simultaneous immersion in α-terthienyl and near ultra-violet irradiation. Initial incubation of nematodes for 60 minutes in the dark in a 1 ppm emulsion of α-terthienyl decreased the exposure time to near ultra-violet light required to kill all nematodes to 50–60 minutes. α-Terthienyl could also partially be removed from *P. penetrans*. Initial incubation in 1 ppm α-terthienyl for one hour, subsequently briefly rinsing the nematodes twice with acetone and demineralized water and finally a four hours stay in water again increased the irradiation time needed to kill these nematodes. Exposure to near ultra-violet light for 60 minutes killed only 20% of *P. penetrans*. The controls, *P. penetrans* irradiated in water, nearly all survived the experiment.

Near ultra-violet light itself may be lethal to nematodes (FULDNER, 1955), but under the circumstances of this study irradiated nematodes in water remained unaffected.

When nematodes were irradiated before they were dipped in α-terthienyl there was no effect; thus *D. dipsaci* and *P. penetrans* cannot be sensitized in advance. Irradiation of α-terthienyl prior to incubation did not affect the compound which remained non-nematicidal in the dark.

Quantitative observations[6]

The uptake of α-terthienyl by nematodes was also followed quantitatively. A suspension of *D. dipsaci* (1.5×10^4/ml) was therefore incubated in 50 ml of an emulsion of 8.5 ppm α-terthienyl made with the aid of Tween 20 (2000 ppm) at 20°C in the dark. The decrease of α-terthienyl from the emulsion was followed at 350 nm using the spectrophotometric technique described by HAVERKATE et al. (1972). After about 16 hours an equilibrium was reached between concentrations in the nematodes and in the ambient emulsion (fig. 7).

When resuspended in a 2000 ppm solution of Tween 20 nematodes did not measurably release the compound. This observation seems to be in contrast with the results of the irradiation experiments. In similar experiments with second stage larvae of *Anguina tritici* partial release of unchanged α-terthienyl in the ambient solution was apparent (BREVOORD, personal communication).

Uptake and partial release of permeated compounds was found with several nematodes. MARKS et al. (1968) recorded a rapid uptake and release of 1,2-dibromoethane and also of water and acetone by *Aphelenchus avenae*. Similar results were found with 1,3-dichloropropene, 1,2-dibromo-3-chloropropane, ethanol, methanol, n-propanol, n-butanol, cyclohexanol and phenol (CASTRO and THOMASON, 1973). Glucose, sodiumacetate and glycine on the other hand

[6] Experiments carried out by Mr. J. W. Brevoord at the 'Boekesteijn' Laboratory of Philips-Duphar B.V., 's Gravelande, The Netherlands.

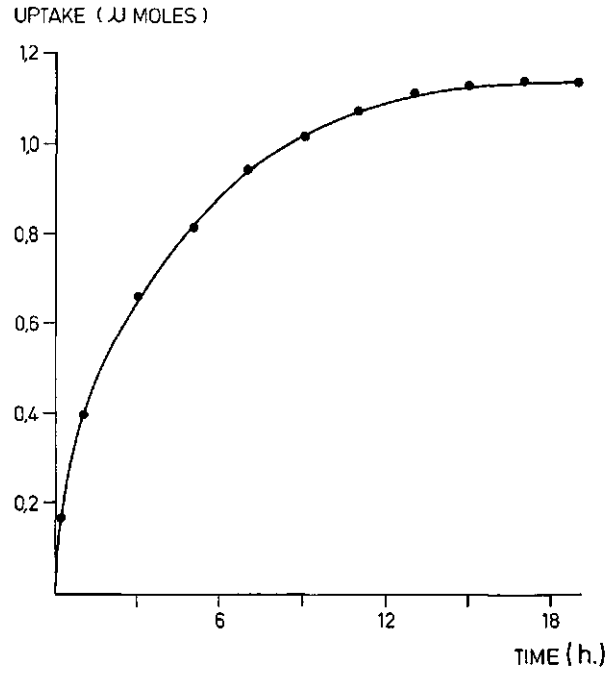


FIG. 7. Uptake of α-terthienyl by *Ditylenchus dipsaci* (KÜHN) FILIPJEV in the dark.
Initial concentration: 8.5 ppm (1.74 µM).
Nematodes: 1.5×10^4/ml.
Temperature: 20°C.

permeated very slowly or not at all (MARKS et al., 1968). The nematicide 2-chloro-6-phenyl-pyricidine was readily taken up by larvae of *Anguina tritici* and also partially released again (HAVERKATE et al., 1972). The systemic nematicide aldicarb (2-methyl-2-(methylthio)-propionaldehyde-0-(methylcarbamoyl)-oxime) also rapidly permeates *Panagrellus redivivus* and is oxidized to aldicarb-sulphoxide (NELMES, 1971).

5.3.3. *Effect of near ultra-violet light on Pratylenchus penetrans from roots of Tagetes*

Pratylenchus penetrans, treated in vitro with α-terthienyl was efficiently killed by near ultra-violet light. Therefore the effect of near ultra-violet light on *P. penetrans* recovered from roots of *Tagetes patula* was also studied.

Two weeks old seedlings of *T. patula* var. 'Rode Vlam' were planted in a glasshouse in 400 ml pots of soil containing about 200 *P. penetrans* per 100 ml. After 8–10 days penetrated nematodes were recovered from root systems. In order to minimize 'cleansing' of the nematodes during a lengthy extraction procedure they were rapidly extracted from the roots by the following technique

and irradiated with near ultra-violet light as soon as possible. After 4 days incubation in the dark nematodes, dead and alive, were counted. The whole extraction procedure was carried out in red light or in the dark.

About 0.5 g of roots were processed in a Waring Blendor at low speed for 20 seconds. Large pieces of root tissue were removed by sieving (125 μm) and the nematodes with the remaining root debris were caught on a 22 μm sieve and transferred with water to a 3 μm Millipore filter and concentrated under suction. The filter was inverted in a counting dish with water thus allowing the nematodes to move into the water. This procedure took about 5 min. and the recovery was about 30% compared with the funnel spray method (OOSTENBRINK, 1960). Approximately 25 active females were transferred with a needle to 1 ml of water in dishes. Experimental irradiations started one to two hours after extraction from the root tissues. *P. penetrans* from roots of oats which were grown in the same type of infested soil for the same time were used as controls.
To exclude the possible interference of penetration of light-sensitive substances

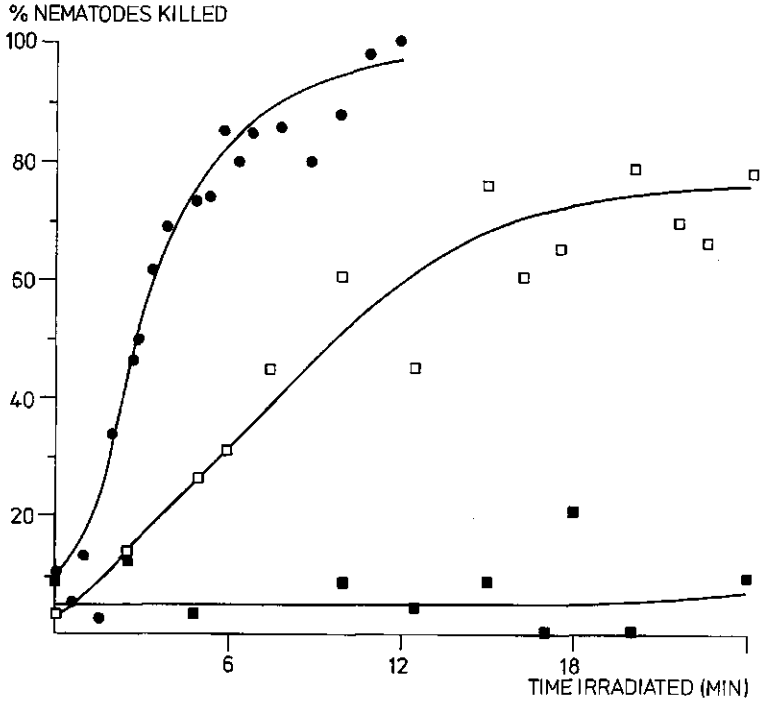


FIG. 8. Effect of near ultra-violet light on the kill of *Pratylenchus penetrans* (COBB) FILIPJEV and SCHUURMANS STEKHOVEN recovered from roots of *Tagetes patula* L. After irradiation nematodes were stored in the dark for 4 days and then evaluated as dead or alive. Each point is the mean of three replicates.
●—● Nematodes recovered from roots of *Tagetes* and irradiated after about 1½ h.
□—□ As above, but nematodes kept in water for 2 h. prior to irradiation (time between recovery and exposure approximately 1½ + 2 h.).
■—■ Controls: nematodes recovered from roots of oats.

from the ruptured root tissue of *Tagetes* into the nematodes during the extraction procedure the controls were handled as follows. Before processing roots of oats an excessive quantity of fresh nematode free *Tagetes* roots (150% by weight) were added to it. After blending the mixture was allowed to stand for one hour before sieving, thus having close contact between the nematodes and broken root tissue of *Tagetes*.

Radiation for about 12 minutes with near ultra-violet light killed the nematodes recovered from the roots of *Tagetes* (Fig. 8). Incubation of nematodes for two hours in water and subsequently rinsing them twice with acetone and water prior to exposure to near ultra-violet light increased the irradiation time needed to kill *P. penetrans*. Only 70–80% of the nematodes were killed after 24 minutes of radiation. Nearly all nematodes from the control treatments survived.

5.4. Discussion

5.4.1. *Chemical aspects*

Certain *Compositae* effectively reduced populations of *Pratylenchus penetrans*, *Meloidogyne hapla* and some other plant parasitic nematodes.

The concept of a chemical base for the suppressing effect of *Tagetes* as proposed by Uhlenbroek and Bijloo (1958; 1959) seems attractive for all *Compositae* having this effect. Suppressing properties on *P. penetrans* were mainly found in the genera *Melampodium*, *Iva*, *Ambrosia*, *Franseria*, *Milleria*, *Baeria*, *Schkuhria*, *Eriophyllum*, *Helenium*, *Gaillardia*, *Tagetes* and *Echinops* (table 11). This effect held for nearly all species of these genera tested. These genera are also chemically closely related (Romo and Romo de Vivar, 1967; Bohlmann, 1967; Hegnauer, 1964) which strengthens the supposition that the same or related nematicidal principles are responsible for the suppressing effect.

Preliminary experiments showed that after column chromatography of root extracts of *Compositae* having this suppressing effect several fractions with strong, in vitro, nematicidal activity could be separated. Because of either low concentrations, the instability of the compounds or perhaps the presence of closely related chemicals only a few of these nematicidal principles could be isolated in a pure state. All the nematicidal fractions possessed strong absorbance near 350 nm.

The nematicidal activity of the ene-pentayne isolated from roots of the *Helenium* hybrid 'Moerheim Beauty' and *Echinops sphaerocephalus* and also spectroscopically observed in root extracts of the *Compositae* is doubtful. It is not clear whether the nematicidal activity of this unstable polyine must be ascribed to the chemical itself or to degradation or polymerisation products. Aqueous solutions of ene-pentayne stored for only one hour at room temperature contain reaction products and after one week low concentrations of the ene-pentayne were still detectable. The polyine common in *Compositae* (Bohlmann and Mannhardt, 1957; Bohlmann and Sucrow, 1963; Hegnauer, 1964; Bohlmann, 1967) can be isolated, sometimes in large quantities, from roots of those *Compositae* that acted as excellent host plants for *Pratylenchus*

penetrans. For instance roots of *Xanthium strumarium* contain abundant ene-pentayne (SÖRENSEN et al., 1954) and it is notwithstanding the presence of this substance a good host plant.

α-Terthienyl and 5-(3-buten-l-ynyl)-2,2'bithienyl from roots of *Tagetes* and roots of *Echinops sphaerocephalus* and 2,3-dihydro-2-hydroxy-3-methylene-6-methylbenzofuran from roots of the *Helenium* hybrid 'Moerheim Beauty' were, in vitro, lethal to several plant parasitic and saprozoic nematodes in concentrations of 1–5 ppm. It was shown that near ultra-violet light increased the nematicidal activity of α-terthienyl (fig. 3). The nematicidal activity in the dark was low. The benzofuran-derivative and two synthetically prepared nematicides, trans-1-(2-thienyl)-2-(5-chloro-2-thienyl)-ethene and trans-1-(2-thienyl)-2-(3-methyl-2-thienyl)-ethene (HANDELÉ, 1971) were, in vitro, also more nematicidal in near ultra-violet light than in the dark.

The effect of near ultra-violet light on the nematicidal activity of α-terthienyl cannot be ascribed either to a permanent sensitization of the nematodes to the chemical or to a permanent change of the compound to a nematicidal form (par. 5.3.2.). The same conclusions can be drawn from the experiments with *Ditylenchus dipsaci* and *Pratylenchus penetrans* on the permeation and release of α-terthienyl (fig. 4,5,6). The lethal effect of α-terthienyl decreased drastically as soon as irradiation with near ultra-violet light ceased.

The experiments also show that accumulation of α-terthienyl in the nematodes shortened the irradiation time needed to kill them. Subsequent incubation of the α-terthienyl treated nematodes in water apparently removed some of the chemical from the nematodes so that the irradiation time needed to kill these nematodes increased (fig. 5,6). These phenomena demonstrate a positive relation between the concentration of α-terthienyl in the nematode and the dosage of near ultra-violet light needed to kill them. The absorption maximum of α-terthienyl at 350 nm coincides with the wavelengths of light that enhances the nematicidal activity of the compound.

Pratylenchus penetrans recovered from roots of *Tagetes patula* and *P. penetrans* treated, in vitro, with α-terthienyl behaved similarly upon irradiation with near ultra-violet light. In both cases curves of the dosage-response type with relation to near ultra-violet light were found (fig. 6,8) and washing with water increased the exposure time needed to kill the nematodes. Therefore the compounds which permeate into the nematodes during their stay in *Tagetes* roots could, like α-terthienyl, be partially removed. *P. penetrans* which had been in the roots of *Tagetes* for up to 8–10 days were killed about 5 times faster than nematodes incubated in a 1 ppm emulsion of α-terthienyl for one hour. Permeation of active compounds from ruptured root tissue of *Tagetes* during the extraction was not responsible for this effect since the controls, *P. penetrans* from roots of oats treated with broken *Tagetes* roots, survived irradiation with near-ultra-violet light (fig. 8).

These results show that the kill of *P. penetrans* from roots of *Tagetes* and *P. penetrans* treated in vitro with α-terthienyl in near ultra-violet light are governed by similar phenomena. UHLENBROEK and BIJLOO (1958; 1959) isolated, besides

α-terthienyl, 5-(3-buten-1-ynyl)-2,2'-bithienyl (λ_{max}340 nm) from roots of *Tagetes*. This compound was more nematicidal than α-terthienyl in vitro and must very probably also be activated to exert nematicidal activity. The more rapid kill of nematodes from roots of *Tagetes* compared with those treated for one hour with a 1 ppm emulsion of α-terthienyl in near ultra-violet light may be due to higher concentrations of this type of compound, or to the effects of substances more active than α-terthienyl.

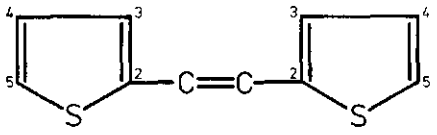
The foregoing strongly suggests that, in vitro, α-terthienyl (and some related compounds) becomes activated reversibly by near ultra-violet light to a nematicidal form. This nematicidal form probably reacts with certain sensitive sites in the nematodes. The compound regains its far less active form as soon as the irradiation with near ultra-violet light ceases. The possibility that upon irradiation with near ultra-violet lights certain sensitive sites in the nematodes become changed temporarily in such a way that α-terthienyl can react with them cannot be ruled out but is unlikely. The system that becomes inhibited or disorganized by the activated α-terthienyl is unknown. *Pratylenchus penetrans* that penetrated roots of *Tagetes* were killed (WINOTO SUATMADJI, 1969) but more slowly compared with those recovered from roots and exposed to near ultra-violet light. How this type of compound is activated in the roots or becomes activated in the nematodes inside the roots is also unknown. The activation in vivo may very probably be ascribed to a system or systems, analogous to the in vitro energy transference mediated by light.

The uptake of α-terthienyl by *Ditylenchus dipsaci* was also followed quantitatively by measuring the decrease of α-terthienyl from an emulsion (fig. 7). After about 16 hours incubation in the dark an equilibrium was reached between the concentration of α-terthienyl in the nematodes and in the ambient emulsion. Release of α-terthienyl after resuspending these nematodes in 2000 ppm solution of Tween 20 could not be measured.

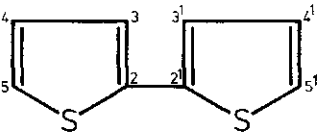
The discrepancy between the results above and the irradiation experiments, where loss of active α-terthienyl was apparent, may be due to the phenomenon that most of the α-terthienyl dissolves in the fat tissues of the nematodes or becomes irreversibly adsorbed and therefore loses its activity. The fat content of *D. dipsaci* is rather high, estimated at 33–38% (TRACY, 1958; KRUSBERG, 1967). At the other hand, larvae of *Anguina tritici* released unchanged α-terthienyl.

No evidence was found for metabolism of α-terthienyl to non-toxic products by the nematodes but this possibility was not fully investigated. NELMES (1971) has shown that metabolism of the nematicide aldicarb does take place in *Panagrellus redivivus*. Whatever the reason these results imply that low concentrations of α-terthienyl are sufficient to kill nematodes in near ultra-violet light. They also imply that permeation of α-terthienyl and its activity in near ultra-violet light are not related parameters. Similar observations were made with the permeation of the nematicide ethylenebromide in *Chaenorhabditis* (MARKS et al., 1968) and its effect on the respiratory response of this nematode (MARKS, 1971).

UHLENBROEK and BIJLOO (1960) synthetized a number of thiophenic compounds with strong in vitro nematicidal activity. The active compounds can be considered to be derivatives of 2,2'-bithienyl. Character, size, number and position of the substituents seem to be important for nematicidal activity. Substitution in a position ortho to the internuclear bond is unfavourable.



1,2-di-(2-thienyl)-ethene



2,2'-bithienyl

According to the authors, this is probably due to steric hindrance of these substituents on the mesomeric structures of these dithienyls. HANDELÉ (1971) found nematicidal activity within the dithienylethenes, in particular certain 1,2-di-(2-thienyl)-ethenes were highly nematicidal in vitro. Some 1-phenyl-2-(2-thienyl)-ethenes, 1-(2-thienyl)-2-(3-thienyl)-ethenes and 1,2-di-(3-thienyl)-ethenes also exerted in vitro nematicidal action. It was postulated that the nematicidal activity of 1,2-di-(thienyl)-ethenes involves electron acceptance by these molecules (DRENTH and HANDELÉ, 1972). Substitution in the thiophenic ring of the 1,2-di-(2-thienyl)-ethenes did not alter the nematicidal activity in general. Substitution at both 5 positions decreased the nematicidal activity considerably. The influence of light was not taken in account in these tests (BIJLOO, personal communication). Most of the active compounds possess, like α-terthienyl, an absorption maximum between 300–400 nm. Two dithienylethenes tested were also more nematicidal in near ultra-violet light than in the dark. This strongly suggests that this type of compounds must also be activated to exert nematicidal acitvity, and may explain the absence or low level of nematicidal activity of these compounds when mixed in the soil (HANDELÉ, 1971). Similar observations were made with α-terthienyl (DAULTON and CURTIS, 1963). The steric hindrance by substitution on the 3-place of 2,2'-bithienyls suggests that free rotation of the two thiophenic rings is important for nematicidal activity of these compounds. Possibly the distance between the two S-atoms plays a role. The same indication can be obtained from the results of HANDELÉ (1971), who found that cis-isomers of dithienylethenes were more nematicidal than the trans-isomers. The shortest distance of the S-atoms in these cis-isomers approximates the shortest distance of the S-atoms in 2,2'-bithienyl.

Some biological systems are known to be photoregulated, for instance processes of vision (WALD, 1968) and the uptake of light by plant pigments (KROES, 1970). Certain flavine mononucleotide containing enzymes in plants become activated or deactivated by light (SCHMID, 1970). Also a number of biological active chemicals are changed by light, in some cases reversibly, to more effective inhibitors. For comparison a few examples are given. One of the first records of such effects was that of RAAB (1900). He established that irradia-

ation with visible light markedly accelerated the killing of infusoria by acridine dyes and that the effective wavelengths were those absorbed by the dyes. KAUFMAN et al. (1968) showed that the less stable cis-isomer of p-azophenyldiphenyl-carbamyl chloride was five times more reactive an inactivator of chymotrypsin than the more stable trans-isomer. These isomers were interconvertible by light of different wavelengths. The cis-isomer was obtained by exposure of the trans-isomer to light of 320 nm. The trans-isomer could be regained by exposure of cis to light of 420 nm. The inactivation of chymotrypsin could thus be regulated using light of the appropriate wavelengths. Similarly, the enzymatic activity of acetylcholinesterase could be photoregulated through the mediation of p-phenylazophenyl-carbamyl fluoride (BIETH et al., 1969). BARTELS et al. (1971) synthetized azo-compounds with quarternary ammonium groups interacting with the cholinesterase receptor of the electrogenic membrane of the electroplax of *Electrophorus electricus*. A trans-isomer appeared to be a potent activator whereas the cis-form was practically devoid of activity. The cis-isomers were obtained from trans by exposure to 330 nm. Another type of light mediated inactivation is photo-affinity labeling. KIEFER et al. (1970) for instance irreversibly inactivated acetylcholinesterase and acetylcholine receptor with certain azides. These azides were converted by light to highly reactive nitrenes.

5.4.2. *Biological aspects*

The behaviour of α-terthienyl and related compounds in near ultra-violet light indicates that the suppressing effect of *Tagetes* (and other *Compositae*) on certain nematode populations cannot simply be ascribed to the presence of nematicidal principles in the roots or to release of these compounds into the soil.

WINOTO SUATMADJI (1969) found differences in suppressing properties of *Tagetes* species with respect to *Pratylenchus penetrans*. The suppressing effect decreased in the order *Tagetes patula*, *T. erecta* and *T. minuta*. SEINHORST (1966a) reported similar results and found reproduction of *Pratylenchus penetrans* on *Tagetes erecta*. Nevertheless α-terthienyl and the bithienyl-derivative could be isolated from these three *Tagetes* species (UHLENBROEK and BIJLOO, 1958; 1959; BOHLMANN and HERBST, 1962; HORN and LAMBERTON, 1963; ATKINSON et al., 1964). *Flaveria repanda* acts as a host plant for *Pratylenchus penetrans* (table 11) but the roots contain α-terthienyl (BOHLMANN and KLEINE, 1965). *Eclipta alba*, *E. prostrata* and *E. erecta* (*Helianthinae*) contain α-terthienyl and the 2,2′-bithienyl derivative (BOHLMANN et al., 1964; BOHLMANN and ZDERO, 1970). *E. alba* is nevertheless readily infected by *Meloidogyne javanica* (COLBRAN, 1958). From roots of the *Gorteriinae Berkheya adlami* and *B. radula* (BOHLMANN et al, 1967) also both nematicidal principles could be isolated and *B. macrocephala* probably contains the 2,2′-bithienyl derivative (SÖRENSEN, 1961). The species *B. gracilis* WELW. et HOFFM. (JACK, 1943), *B. subulata* Harv. (MARTIN, 1958; 1959) are recorded as host plants for *Meloidogyne*. It is likely that these related species also contain the nematicidal polythienyls.

Since nothing is known about concentration and distribution of the nematicidal compounds in the root tissues nor about the mode of action of these chemicals in vivo and in vitro, these records need not be inconsistent with the hypothesis that α-terthienyl and related compounds exert nematicidal activity in vivo.

The behaviour of *Meloidogyne hapla* on a number of *Compositae* differs from that of *Pratylenchus penetrans*. *Iva xanthiifolia* and two *Baeria* species acted as rather good host plants for *M. hapla*. MAAS et al. (1972) tested a number of *Compositae* with suppressing properties on *Pratylenchus penetrans* on their ability to suppress *M. hapla*. Three isolates originating from different sources built up high populations on *Tagetes*. One of these isolates was effectively suppressed by *Baeria californica* whereas the other two built up high populations on this plant species. This demonstrates that in the case of *M. hapla* races or biotypes may exist which probably are resistant against the nematicidal principles in these plant species.

The picture becomes even more complicated because reasons for the suppressing properties of the *Compositae* on *Meloidogyne* other than these chemical ones cannot be ruled out completely. DROPKIN and NELSON (1960), for instance, ascribed the resistance of certain soybean varieties to *Meloidogyne* to an incomplete or aberrant development of the giant cells. WINOTO SUATMADJI (1969) also found in some cases a delayed development of the giant cells in roots of *Tagetes* infested with *Meloidogyne hapla*. Whether the delayed development is a feature of *Tagetes* or must be ascribed to early death of the nematode, stopping development of a giant cell, is not clear. Microtome sections of a number of *Compositae* with suppressing properties on *Meloidogyne* showed that if giant cells were present, they were normal. FASSULIOTIS (1970) ascribed the resistance of certain *Cucumis* species to *Meloidogyne* to delayed a development, a hindrance of the larval development beyond the second stage and a stimulation to maleness. No observation on this subject were made with the *Compositae* and *Meloidogyne hapla*.

Tough cellwalls which might prevent the penetration of nematodes into the roots could be excluded as reason for suppressing properties in the case of *Pratylenchus penetrans*. Excessive lesion formation by *Pratylenchus penetrans* proposed to be a reason for the suppressing properties of *Tagetes* (SEINHORST and KLINKENBERG, 1963) was not observed. Density-dependant relations between *P. penetrans* and a number of *Compositae* are also not the reason for the killing effect of these *Compositae* (Table 9).

5.4.3. *Release of nematicidal principles from roots into the soil.*

There are no indications that nematicidal compounds released into the soil play a role in the suppressing properties of the *Compositae* as suggested by CHRISTIE (1959), WALLACE (1963) and ROHDE (1965) for *Tagetes* and also stated by MILNE (1972). The definition of BERGÉ (1971) that nematicidal plants release nematicidal substances in the soil probably does not hold for the *Compositae* used in this study. These views are also rejected by the results of OMIDVAR

(1961; 1962), HESLING et al. (1961) and KOEN (1966). These investigators could not find inhibiting or killing effects of root diffusates of *Tagetes* on *Heterodera* and *Meloidogyne*. WINOTO SUATMADJI (1969) also found no rhizosphere nematicidal effects of *Tagetes* on nematode populations in the soil. The poor nematicidal effect of α-terthienyl in the soil (DAULTON and CURTIS, 1963) and in the dark also makes nematicidal activity of this type of compound, if released at all by plant roots, unlikely.

The results of the present field experiments and glasshouse trials also give no evidence for release of active nematicidal substances in the soil by the *Compositae* with suppressing properties on *Pratylenchus penetrans*. Populations of saprozoic nematodes are not significantly changed by this type of *Compositae*. If nematicidal principles were released one would also expect a decrease in numbers of this group and of the ectoparasitic nematodes.

As summarized in table 12, populations of ectoparasites behave differently on different *Compositae*. Some ectoparasites multiply vigorously on some *Compositae*, whereas other populations are hardly affected. Possibly certain nematode species feed from cells or tissues which are free from nematicidal principles or contain sublethal concentrations. In these cases the maximum density of a certain nematode species will be the equilibrium density or the nematode/plant relationship can be considered as a non-host relation. The first relationship was found with *Tylenchorhynchus dubius* and *Baeria californica* and *Tagetes patula*. The non-host relationship was found with *Tylenchorhynchus dubius* and the *Helenium* hybrid 'Moerheim Beauty', *Schkuhria pinnata* and *Milleria quinqueflora* (fig. 2).

6. SUMMARY

A wide range of *Compositae* and some other plant species were tested for their ability to suppress densities of plant parasitic nematodes. Experiments were carried out in the field and in glasshouses. *Pratylenchus penetrans* was used as test organism and *Digitalis purpurea* (a good host plant), *Tagetes patula*, the *Helenium* hybrid 'Moerheim Beauty', the *Gaillardia* hybrid 'Burgunder' (poor hosts) and fallow served as references. Data were also collected on the behaviour of populations of other plant parasitic nematodes on these plant species.

Suppressing effects on populations of *P. penetrans* were found within the genera *Melampodium, Silphium (Melampodiinae), Iva, Ambrosia, Franseria, Parthenium (Ambrosiinae), Milleria (Milleriinae), Baeria, Schkuhria, Eriophyllum, Helenium, Gaillardia (Heleniinae), Tagetes (Tagetinae)* and *Echinops (Echinopinae)*. With a few exceptions all species tested of the aforementioned genera suppressed populations of *P. penetrans* equally as well as did *Tagetes* species (table 11).

The suppression of *P. penetrans* by *Tagetes patula, Milleria quinqueflora, Ambrosia trifida, Schkuhria pinnata, Baeria californica* and the *Helenium* hybrid 'Moerheim Beauty' was not due to density-dependent relations, since all initial densities used were suppressed to zero or nearly to zero (table 9).

The inability of *P. penetrans* to invade the roots of *Compositae* that suppress them, is also not the explanation for the suppressing properties of these plants. *P. penetrans* penetrated roots of 6 selected *Compositae* to the same extent and with the same speed as roots of the good host *Avena sativa* (fig. 1).

Compositae with suppressing properties on *P. penetrans* acted differently on populations of other plant parasitic nematodes. Certain nematode species were suppressed or kept at the same level of infestation whereas others built up high populations (table 12). It is shown that in the case of *Tylenchorhynchus dubius* and 5 such *Compositae* two different relationships exist: a density-dependent relationship found with *Tagetes patula* and *Baeria californica* and a non-host relationship with *Milleria quinqueflora, Schkuhria pinnata* and the *Helenium* hybrid 'Moerheim Beauty' (fig. 2).

Tagetes patula, the *Helenium* hybrid 'Moerheim Beauty', *Ambrosia trifida, Schkuhria pinnata, S. senecioides* and *Milleria quinqueflora* hardly supported *Meloidogyne hapla*. A few larvae developed to maturity and few root knots and males were found after cultivation of these *Compositae* (table 10). Serial sections of these rootknots showed a structure similar to root knots of good host plants. Two *Baeria* species and *Iva xanthiifolia* acted as host plants for *M. hapla*.

Root extracts of a number of *Compositae* with suppressing properties on *Pratylenchus penetrans* contained, after fractionation on columns of silicagel, different fractions with, in vitro, nematicidal activity. Nearly all nematicidal fractions absorbed strongly in the near ultra-violet region.

The '4100-pigment', 1-tridecanene-3,5,7,9,11-pentayne, a polyine common in *Compositae*, exerted nematicidal activity. The killing effect is probably not due to the chemical itself but to degradation or polymerisation products.

The previously known nematicidal principles from *Tagetes*, α-terthienyl and 5-(3-buten-1-ynyl)-2,2'-bithienyl, were also isolated from roots of *Echinops sphaerocephalus*. Besides these compounds other unindentified sulphur containing nematicidal principles were found in the roots of this plant.

Root extracts of the *Helenium* hybrid 'Moerheim Beauty' contained at least three fractions with nematicidal properties. The most apolar fraction was 1-tridecanene-3,5,7,9,11-pentayne. The second nematicidal principle has been identified as 2,3-dihydro-2-hydroxy-3-methylene-6-methylbenzofuran. The LD_{50} in vitro was 3–5 ppm. The third compound could not be obtained pure enough to permit identification.

It was found that daylight enhanced the in vitro nematicidal activity of α-terthienyl, 2,3-dihydro-2-hydroxy-3-methylene-6-methylbenzofuran and of two synthetic nematicidal thiophenes. Near ultra-violet light was shown to be the active part of the daylight spectrum (fig. 3). This is precisely the region where these compounds strongly absorb.

Exposure of nematodes in a 1 ppm emulsion of α-terthienyl to near ultra-violet light for different lengths of time gave curves of the dosage-response type if irradiation times were plotted against percentages of nematodes killed. 100% effect was reached after about $1\frac{1}{2}$ hour of irradiation (fig. 4,6). Incubation of nematodes in 1 ppm α-terthienyl in the dark decreased the irradiation time needed to kill them. Rinsing the α-terthienyl treated nematodes with water before irradiation increased the irradiation time required for 100% kill (fig. 5,6). Apparently α-terthienyl is released from the nematodes but more slowly than it is taken up. Permanent senzitization of α-terthienyl or of the nematodes by near ultra-violet light could be excluded. Neither irradiation of the nematodes before placing them in α-terthienyl nor irradiation of an emulsion of α-terthienyl (before putting nematodes in it) had any effect. There were also no indications that α-terthienyl is broken down or converted to inactive forms.

P. penetrans which had been in the roots of *Tagetes* for up to 8–10 days were also rapidly killed when exposed to near ultra-violet light.

Curves of the dosage-response type were also found and washing these nematodes with water also increased the time needed to kill them (fig. 8).

Apparently α-terthienyl and/or related compounds permeate from root tissues of *Tagetes* into the nematodes. Neither the way these compounds are activated, or become activated, in vivo and in vitro, nor the mode of action of this type of compound is known.

7. SAMENVATTING

Verscheidene *Compositae* en een aantal plantesoorten uit andere families werden in veld- en kasproeven getoetst op hun vermogen populaties van planteparasitaire nematoden te verlagen. *Pratylenchus penetrans* werd als toetsorganisme gebruikt; de goede waardplant *Digitalis purpurea*, de slechte waardplanten *Tagetes patula*, de *Helenium* hybride 'Moerheim Beauty' en de *Gaillardia* hybride 'Burgunder' en braak dienden als referenties. Ook werden gegevens verzameld over het gedrag van andere planteparasitaire nematoden bij de teelt van deze plantesoorten.

Na beperking van het onderzoek tot de groep van de in dit verband meest werkzame *Compositae* werd deze groep uitvoeriger onderzocht.

Populaties van *P. penetrans* werden sterk gereduceerd door het telen van soorten van de volgende geslachten: *Melampodium, Silphium (Melampodiinae), Iva, Ambrosia, Franseria, Parthenium (Ambrosiinae), Milleria (Milleriinae), Baeria, Schkuhria, Eriophyllum, Helenium, Gaillardia (Heleniinae), Tagetes (Tagetinae)* and *Echinops (Echinopinae)*. Enkele uitzonderingen daargelaten, brachten alle getoetste plantesoorten van de genoemde geslachten populaties van *P. penetrans* terug tot hetzelfde lage niveau als *Tagetes* soorten (tabel 11).

Het populatieverlagend effect van *Tagetes patula, Milleria quinqueflora, Ambrosia trifida, Schkuhria pinnata, Baeria californica* en de *Helenium* hybride 'Moerheim Beauty' kan niet toegeschreven worden aan dichtheidsafhankelijke relaties (tabel 9) noch aan het feit dat *P. penetrans* wortels van dit type plant niet of nauwelijks zou kunnen binnendringen. *P. penetrans* penetreerde wortels van zes zulke *Compositae* in dezelfde mate en met dezelfde snelheid als wortels van de goede waardplant *Avena sativa* (fig. 1).

Compositae welke populaties van *P. penetrans* sterk reduceerden hadden verschillende effecten op populaties van andere planteparasitaire nematoden. Populaties van sommige nematodesoorten werden sterk teruggedrongen of bleven op het uitgangsniveau, terwijl andere soorten zich sterk konden vermeerderen (tabel 12). Tussen *Tylenchorhynchus dubius* en vijf van deze *Compositae* werden twee typen relaties gevonden: een dichtheidsafhankelijk verband bleek te bestaan bij *Tagetes patula* en *Baeria californica* en een 'niet-waardplant' verband bij *Milleria quinqueflora, Schkuhria pinnata* en de *Helenium* hybride 'Moerheim Beauty' (fig. 2).

Tagetes patula, de *Helenium* hybride 'Moerheim Beauty', *Ambrosia trifida, Schkuhria pinnata, S. senecioides* en *Milleria quinqueflora* reduceerden vrij effectief populaties van *Meloidogyne hapla*. Er vormden zich slechts enkele wortelknobbels en in de grond werden weinig mannetjes aangetroffen (tabel 10). Met een microtoom gesneden coupes van deze wortelknobbels vertoonden in grote lijnen dezelfde structuren als werden gevonden in wortelknobbels van waardplanten. Twee soorten van het geslacht *Baeria* en *Iva xanthiifolia* bleken waardplanten te zijn voor *M. hapla*.

Over silicagel gefractioneerde wortelextracten van *Compositae* die populaties van *P. penetrans* reduceerden, bleken een aantal fracties te bevatten die in vitro een sterke nematicide werking vertoonden. Bijna al deze fracties absorbeerden sterk in het langgolvig ultraviolet. Het '4100-pigment', 1-tridecaneen-3,5,7,9,11-pentayne, een onverzadigde koolwaterstof die in veel *Compositae* gevonden wordt, vertoonde nematicide activiteit. Waarschijnlijk moet deze activiteit niet aan de verbinding zelf maar aan afbraak- of polymerisatieproducten worden toegeschreven.

α-Terthienyl en 5-(3-buten-1-ynyl)-2,2'-bithienyl, twee verbindingen met nematicide werking uit *Tagetes* wortels, werden ook geisoleerd uit wortels van *Echinops sphaerocephalus*. Naast deze beide verbindingen zijn andere, niet geïdentificeerde, zwavelhoudende nematicide verbindingen in wortels van deze plant aanwezig.

Extracten van wortels van de *Helenium* hybride 'Moerheim Beauty' bevatten minstens drie fracties met nematicide werking. De meest apolaire van de drie was 1-tridecaneen-3,5,7,9,11-pentayne. Een tweede verbinding werd geïdentificeerd als 2,3-dihydro-2-hydroxy-3-methyleen-6-methylbenzofuran. In vitro kwam de LD_{50} overeen met 3–5 ppm. De derde verbinding kon niet zuiver genoeg geisoleerd worden voor identificatie.

Daglicht verhoogde de nematicide werking van α-terthienyl, 2,3-dihydro-2-hydroxy-3-methyleen-6-methylbenzofuran en twee synthetische thiofenen. Langgolvig ultraviolet licht, het gebied waar deze verbindingen een absorptiemaximum hebben, bleek verantwoordelijk voor dit effect (fig. 3).

Bestraling van nematoden in een 1 ppm emulsie van α-terthienyl met langgolvig ultraviolet licht gedurende verschillende tijden resulteerde in curven van het dosis-respons type indien de bestralingsduur uitgezet werd tegen het percentage gedode nematoden. Honderd procent doding werd bereikt na 1½ uur bestralen (fig. 4,6). Incubatie van nematoden in 1 ppm α-terthienyl in het donker voor de bestraling verminderde de bestralingstijd die nodig was om 100% doding te verkrijgen. Werden in α-terthienyl geïncubeerde nematoden voor de bestraling met water gewassen dan was weer meer tijd nodig voor 100% doding (fig. 5,6). Aangetoond werd dat α-terthienyl door nematoden afgegeven wordt, maar langzamer dan het wordt opgenomen. Het letale effect van langgolvig ultraviolet licht mag niet toegeschreven worden aan een permanente sensibilisatie van α-terthienyl of van de nematode want bestraling van een α-terthienyl emulsie of van nematoden vooraf had geen enkel effect. Er zijn eveneens geen aanwijzingen dat α-terthienyl afgebroken wordt of wordt omgezet in inactieve verbindingen.

Nematoden van de soort *P. penetrans* die maximaal 8–10 dagen in *Tagetes* wortels hadden verbleven werden snel gedood in langgolvig ultraviolet licht. In dit geval werden eveneens dosis-respons curven gevonden en met water wassen verlengde de bestralingstijd die nodig was om deze nematoden te doden (fig. 7). Deze resultaten worden toegeschreven aan het feit, dat α-terthienyl en/of verbindingen met soortgelijke werking vanuit wortelweefsels van *Tagetes* de nematoden binnendringen.

Op welke wijze deze verbindingen zijn of worden geactiveerd in vivo en in vitro is niet bekend, evenmin waar en hoe zij hun werking uitoefenen.

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