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Suppression of Meloidogyne hapla by arbuscular mycorrhiza fungi (AMF) on pyrethrum in Kenya

(*Keywords: Glomus etunicatum, Glomus* sp. (Isolate KS14), interaction, *Meloidogyne hapla*, pyrethrum)

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Abstract. Two arbuscular mycorrhiza fungi (AMF) isolated from a pyrethrum-growing region in Kenya were screened for efficacy against a nematode, *Meloidogyne hapla*, in greenhouses. The fungi were identified at INVAM (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi) as *Glomus etunicatum* (Isolate KS18) and *Glomus* sp. (Isolate KS14). Isolate KS14 (*Glomus* sp.) significantly suppressed nematode population, growth and development by up to 54%, egg production by up to 75% and disease severity by up to 71%. *Glomus etunicatum* (Isolate KS18) suppressed nematode growth and development by up to 50%, egg production by up to 75% and disease severity by up to 57%. In addition, *G. etunicatum* and Isolate KS14, significantly improved top dry biomass of pyrethrum by up to 33% and 47%, respectively. *Glomus etunicatum* and *M. hapla* were mutually inhibitory as root colonization by *G. etunicatum* was significantly reduced (up to 24%) by the presence of the nematode. The presence of the nematodes, on the other hand, did not significantly affect root colonization by Isolate KS14.

1. Introduction

Pyrethrum (*Chrysanthemum cinerariefolium* Vis.), a perennial shrub that produces white flowers, is an important cash crop in Kenya, being third in importance to tea and coffee. Currently, Kenya supplies 67–80% of the world pyrethrum requirements (Pers. Comm., Pyrethrum Board of Kenya (PBK)). The dried flowers are a source of pyrethrins, which are active ingredients of natural insecticides. Pyrethrum flourishes well in high altitude areas (1500–3000 m above sea level) and requires deep, well drained fertile soils and high rainfall (>1000 mm) that is well distributed throughout the year (Muturi *et al.*, 1969). Pyrethrum is propagated vegetatively by splits (clones) from mature plants or tissue culture materials and generatively using seeds. The splits are multiplied in nurseries for 4 months before splitting them again and transplanting them in the field while seeds are raised in nursery beds for 5–6 months and then transplanted in the field. Picking of mature flowers is done at an interval of 2–3 weeks for 8–9 months in a year (Muturi *et al.*, 1969). The economic life of pyrethrum is 3–4 years.

Major constraints to pyrethrum production in Kenya include, among other pests and pathogens, parasitic nematodes (Warui *et al.*, 1991). Of considerably high importance is *Meloidogyne hapla* Chitwood, a root-knot nematode which constitutes 95% of the phytonematode populations associated with pyrethrum in Kenya (Warui *et al.*, 1991). *Meloidogyne hapla* alone has been associated with a 20–30% pyrethrum yield loss (Warui *et al.*, 1991). It also causes a decrease in flower size and pyrethrin content, wilting, stunting in young seedlings, chlorosis, and predisposes infected plants to infection by other pathogens such as root-rot fungi (Warui *et al.*, 1991). The cost, and environmental and health hazards posed by the use of nematicides in the management of *M. hapla*, the low economic value of recommended rotational crops, the long rotational periods (Wanjala, 1992) and breakdown of resistance in resistant clones through development of new pathogenic races (Triantaphyllou, 1985) make the search for alternative *M. hapla* management strategies imperative.

Components of a viable control programme should be cost effective and environmentally safe. Arbuscular mycorrhiza fungi (AMF), obligate endophytic symbionts, have the potential of suppressing disease (Lindermann, 1994; Azcon-Aguilar and Barea, 1996) and would therefore provide such an alternative. *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe, *G. macrocarpum* Tul and Tul and *G. fasciculatum* Walker and Koske, for example, are known to suppress development of *Meloidogyne incognita* (Kofoid and White) Chitwood and/or *M. hapla* and their ability to induce galls (Sikora, 1979; Kellam and Schenck, 1980). Besides suppressing disease, AMF promote plant growth and yield (Harley and Smith, 1983), enhance plant water relations (Safir *et al.*, 1971), improve soil aggregation and structure and ameliorate aluminium and iron toxicity that is typical of acidic soils (Janos, 1987). Use of AMF in the long term would thus favour an agricultural system that is both production- and protection-oriented, thus enhancing stabilization of agroecosystems.

Despite all these advantages associated with AMF, a literature search revealed that no work has been done to assess interaction between *M. hapla* and AMF originating from pyrethrum fields in Kenya. This study was therefore initiated to assess effects of two AMF isolates, KS18 (*Glomus etunicatum*) and KS14 (*Glomus* sp.) on pyrethrum growth and *M. hapla*–pyrethrum interaction. The fungi were isolated from a pyrethrum field in Kenya.

2. Materials and methods

Greenhouse tests were conducted between December 1995 and April 1996, and September 1996 and January 1997 in Bonn

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University, Germany and Kenyatta University, Kenya, respectively, to investigate the effects of fungal isolates KS14 and KS18 on pyrethrum growth and on *M. hapla*–pyrethrum interaction. Three tests were carried out, one in which both isolates were tested at the same time and in the other two, the isolates were tested separately.

2.1. *Inocula preparation*

2.1.1. Arbuscular mycorrhiza fungi. The two AMF isolates used in this study were isolated from the rhizosphere of pyrethrum plants growing in Kenyenya, Kisii (1960 m altitude, 35° 00′E, 0° 38′S), a major low altitude pyrethrum-growing area in Kenya. Several AMF isolates were isolated from soils obtained from the area of study and the two fungi used in this study were selected on the basis of their ability to rapidly infect and colonize pyrethrum roots. The fungi were isolated using a combination of wet sieving (Gerdemann and Nicolson, 1963) and sucrose centrifugation techniques (Jenkins, 1964). The fungi were characterized and identified by a mycorrhizologist, Prof. Morton of the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM), West Virginia University, USA. Isolate KS18 was identified as *Glomus etunicatum*. Isolate KS14 was placed in the genus *Glomus*. The identification to species level is in progress at INVAM. The fungi were initially cultured and maintained on *Pueraria phaseoloides* Benth. (tropical kudzu) and later on pyrethrum. They are currently being maintained as KE118 (KS18) and KE114 (KS14) at INVAM and Kenyatta University, Botany Department.

2.1.2. Meloidogyne hapla. *Meloidogyne hapla* used in this study was obtained from galled pyrethrum roots sampled from Kenyenya, Kisii, where the fungi were obtained. The nematodes were maintained on tomato (*Lycopersicon esculentum* Mill. cv. Moneymaker) growing in a 2 : 1 ratio sterile sand: soil mixture in the greenhouse.

2.2. *Inoculation procedure*

2.2.1. Arbuscular mycorrhiza fungi. A 20 g mixed inoculum obtained from a 3-month-old culture was placed in a 3-cm-deep depression made in the sterilized soil contained in a 15 cm diameter plastic pot. The soil (clay, pH 5.2) used in the studies was obtained from the field where the fungi were obtained and was deficient in plant-available P (P = 9 ppm). A mineral content analysis of the soil was done at the National Agricultural Research Laboratories (NARL), Kenya. The soil was mixed with sand (3 : 1) before autoclaving it for two separate hour periods at 121°C. The inoculum consisted of the growth medium, spores, external mycelia and infected root fragments. The pots were then planted with 6-week-old pyrethrum seedlings such that the inoculum was directly below the pyrethrum root systems. Pyrethrum variety P4, recommended for its high flower yield and pyrethrin content (Ikahu and Ngugi, 1989), was used as the test plant throughout the study. The pyrethrum seeds, procured from Pyrethrum Board of Kenya (PBK), were germinated in sterile sand–soil (1 : 4) mixture before transplanting. The plants were watered as required and supplied with 0.3% Wuxal nutrient solution. (12% N, 4% P_2O_5, 6% K_2O, 0.02% boron and 0.01% copper) monthly. Untreated plants received an equivalent amount of inoculum from non-mycorrhized plants.

2.2.2. Meloidogyne hapla. *Meloidogyne hapla* eggs were extracted from galled tomato roots using an NaOCl technique (Hussey and Baker, 1973). The emergence of second stage juveniles (J-2) was facilitated by aerating the nematode egg suspension for 10–14 days at room temperature. Plants were inoculated with a nematode suspension containing 6000 J-2 three months after inoculating with the fungi. Inoculation involved dispensing the nematode suspension onto a 3-cm-deep depression made around the plant's root system. The depression was then covered with soil. Plants without the fungi and the nematodes served as controls. The treatments were arranged in a randomized complete design with six replicates.

2.3. *Data collection*

2.3.1. Plant performance. Dry shoot and fresh root weights were obtained at the end of the experiments, 5 months after fungal inoculation. Shoot systems were dried at 80°C for 48 h before obtaining their weights. Root lengths were also determined where possible. Roots from each experimental unit were carefully washed and cut into 1 cm long segments (after assessing the gall index). Root lengths were determined using a 'Comair Root Length Scanner' (Hawker de Havilland Victoria Limited, Australia).

2.3.2. Root colonization by the fungi. After estimating their lengths, roots were thoroughly mixed before taking five 1 g fresh weight sub-samples of fine roots for fungal colonization assessment. The roots were cleared and stained using Walker's (unpublished) Cold Staining technique, a modification of Phillip and Hayman's (1970) technique. Roots were cleared in 2.5% KOH for 72 h at room temperature. The KOH was changed after every 24 h. The roots were thoroughly rinsed in running tap water before acidifying them in 1% HCl for 12 h at room temperature. The roots were stained in 0.05% trypan blue for 24 h at room temperature and destained in acidified glycerol. The roots were then assessed for colonization under a dissecting microscope (×40) using a Grid-line Intersect method (Giovannetti and Mosse, 1980). Colonized root length was expressed as a percentage of the total root length.

2.4. *Nematode disease assessment*

To assess nematode damage on pyrethrum, the following variables were measured.

(1) Galling indices as a measure of disease severity. Roots were gently washed and rated for galling using a 0–4 galling scale where 0 = no galls, 1 = 1–25%, 2 = 26–50%, 3 = 51–75% and 4 = 76–100% of root system galled (Krusberg and Nelson, 1958).
(2) Number of females within the roots. A 1 g fresh root sub-sample per experimental unit was obtained, cleared and stained using an NaOCl–Acid fuchsin technique (Byrd *et al.*, 1983). The number of females within the root segments was determined using a dissecting microscope (magnification, ×40).

(3) Number of eggs within the roots. Eggs were extracted using Hussey and Baker's (1973) technique. The egg suspension was adjusted to 50 ml and 1 ml of the suspension was taken and the number of eggs enumerated in a Hawksley's slide counter.
(4) Number of J-2 in 100 ml of thoroughly mixed growth medium. The J-2s were extracted using Jenkins' (1964) centrifuge–flotation technique and enumerated in a Hawksley's counter.

2.5. *Data analysis*

Treatment effects were assessed by Analysis of Variance (ANOVA). Treatment means were separated using Least Significant Difference (LSD).

3. Results

3.1. Glomus etunicatum *(KS18)* – Meloidogyne hapla *interaction tests*

Significant ($P<0.05$) differences were detected among the dry shoot weights ($F=75.5$, df=3; $F=15$, df=5) and root lengths ($F=3.8$, df=5) in both tests (tables 1 and 2). Significant ($P<0.05$; $F=9$, df=3) differences were detected among fresh root weights as shown in table 1. The fungus improved top dry biomass of pyrethrum by 33% (table 1) and 32% (table 2). In addition, the fungus improved top dry biomass of nematode-infected plants by 42% (table 1) and 36% (table 2). Nematodes suppressed plant performance by up to 23% as was revealed by significantly less top biomass of nematode-infected plants relative to the untreated plants (tables 1 and 2). The control and the fungus-colonized plants had the longest and the shortest roots, respectively (table 2). The presence of the fungus reduced root length of untreated and nematode-treated plants by 18% and 17%, respectively (table 2). Although plants infected by nematodes alone had significantly ($P<0.05$) longer roots than those colonized by fungus alone, they were not significantly different from those infected by both the fungus and the nematodes (table 2).

Significant ($P<0.05$) differences were detected in percentage root colonization ($F=157.5$, df=1; $F=16.2$, df=3), gall indices ($F=45$, df=1; $F=52.5$, df=2), number of eggs ($F=58$, df=1; $F=42$, df=2), and number of females ($F=20$, df=1; $F=3.7$, df=2) in both tests (tables 1 and 2). Plants treated with the fungus alone had a significantly ($P<0.05$) higher percentage root colonization than those infected with both the fungus and the nematodes (tables 1 and 2). The presence of the nematode reduced fungal root colonization by 24% (table 1) and 22% (table 2).

Plants treated with both the fungus and the nematodes had significantly smaller gall indices and number of eggs and

Table 1. Mean dry shoot weight (DSW), fresh root weight (FRW), % root colonization (%RC), gall indices (GI), eggs, females and J-2 of pyrethrum inoculated with Glomus etunicatum *(G.e) and/or* Meloidogyne hapla *(M.h)*

	Plant growth		Colonization	Disease			
Treatment	DSW (g)	FRW (g)	(%RC)	GI†	Eggs/ml	Females/g	J-2/100 ml
G.e	5.65d*	23.51bc	44.8b	—	—	—	—
G.e+M.h	4.66c	18.91a	34.2a	1.7a	294.7a	51a	1177.2
M.h	3.29a	20.92ab	—	3.7b	1189.2b	101.5b	1557.8
Control	4.25b	25.18c	—	—	—	—	—
LSD	0.33	2.72	1.9	0.66	260.9	24.97	NS

Data are means of six replicates.
*Means followed by the same letter(s) are not significantly different ($P>0.05$) using LSD.
†Gall indices based on a 0–4 gall rating scale; where 0=no galls, 1=1–25%, 2=26–50%, 3=51–75% and 4=76–100% root system galled.

Table 2. Mean dry shoot weight (DSW), fresh root weight (FRW), root length (RL), % root colonization (%RC), gall indices (GI), eggs, females and J-2 of pyrethrum inoculated with Glomus etunicatum *(G.e) or* Glomus sp. *(KS14) and/or* Meloidogyne hapla *(M.h)*

	Plant growth			Colonization	Disease			
Treatment	DSW (g)	FRW (g)	RL (m)	(%RC)	GI†	Eggs/ml	Females/g	J-2/100 ml
G.e	5.69c*	18.95	93.65a	44.33b	—	—	—	—
G.e+M.h	4.56b	21.38	95.23a	34.67a	1.5a	303.83a	57.17a	1210.5
Glomus sp.	5.38c	22.92	104.23ab	48.5b	—	—	—	—
Glomus sp.*+M.h*	5.4c	23.95	118.6b	45b	1a	289a	43.68a	589.71
M.h	3.36a	21.25	111.18b	—	3.5b	1171.34b	94b	1758.85
Control	4.32b	25.26	114.45b	—	—	—	—	—
LSD	0.65	NS	15.2	4.34	0.55	235.6	40.8	NS

Data are means of six replicates.
*Means followed by the same letter(s) are not significantly different ($P>0.05$) using LSD.
†Gall indices based on a 0–4 gall rating scale; where 0=no galls, 1=1–25%, 2=26–50%, 3=51–75% and 4=76–100% root system galled.

females in their root systems than those treated with nematodes alone (tables 1 and 2). The fungus suppressed nematode disease severity by 55% (table 1) and 57% (table 2). Also, the fungus reduced the number of eggs and females by up to 75% and 64%, respectively (tables 1 and 2). Although there were no significant differences in the numbers of J-2 extracted from the soil, there were fewer in the pots containing the fungus (tables 1 and 2).

3.2. Glomus sp. *(KS14)* – Meloidogyne hapla *interaction tests*

There were highly significant differences in dry shoot weights ($P < 0.01$; $F = 15$, df = 5; $F = 76$, df = 3) among the treatments in both tests (tables 2 and 3). Plants treated with the fungus alone had the heaviest (table 3) and third heaviest shoots (table 2). Although plants treated with *G. etunicatum* had the heaviest shoots (table 2), they did not differ significantly from those treated with *Glomus* sp. alone. Colonization of pyrethrum by KS14 alone significantly improved its growth by up to 25% (table 2) and 47% (table 3) relative to the untreated plants. Also, the fungus improved top dry biomass of nematode-treated plants by 61% (table 2) and 78% (table 3). The fungus was more effective in improving top dry biomass of nematode-treated plants than *G. etunicatum* (table 2). Although there were highly significant differences ($P < 0.01$, $F = 14$, df = 3) in fresh root weights, KS14-treated plants did not differ significantly from the control (table 3). Nematode infection caused a decrease in dry shoot and fresh root weights of pyrethrum by 22% (table 2) and 17% (table 3), respectively. Unlike in *G. etunicatum* – *M. hapla* interaction where the presence of the nematodes reduced the ability of the fungus to improve shoot growth, the nematode had no significant effect on the ability of KS14 to improve shoot growth (table 2).

Although there were significant differences in root lengths ($P < 0.05$, $F = 3.8$, df = 5) among treatments, root length of KS14-*M. hapla* treated plants did not differ significantly from the control or from those treated with KS14 or nematode alone (table 2). Plants treated with *G. etunicatum* alone or in addition to the nematodes had roots that were significantly shorter than those of KS14 – *M. hapla*-treated plants (table 2).

There were highly significant differences ($P < 0.01$) in gall indices ($F = 53$, df = 2; $F = 81$, df = 1) and the number of eggs ($F = 41.7$, df = 2; $F = 47$, df = 1) extracted from roots in both tests (tables 2 and 3). Significant differences ($P < 0.05$; $F = 3.7$, df = 2) in the number of females within the roots were observed in one test only (table 2). Plants treated with both *Glomus* sp. and *M. hapla* had the least disease severity, fewest eggs and females in their roots and fewest J-2 in the soil (tables 2 and 3). The fungus was more effective in reducing disease severity, number of eggs, females and J-2 than *G. etunicatum* (table 2). The fungus reduced the disease severity, number of eggs, females and J-2 by up to 71%, 75%, 54% and 66%, respectively (tables 2 and 3). Unlike in *G. etunicatum*-treated plants where the presence of the nematode significantly reduced root colonization (tables 1 and 2), the nematode had no significant effects on root colonization by KS14 (tables 2 and 3).

4. Discussion

The ability of *G. etunicatum* and KS14 to significantly improve pyrethrum top biomass (tables 1, 2 and 3) confirms previous reports on the ability of AMF to enhance plant growth. As with other AMF, the fungi might have enhanced plant growth through enhanced mineral nutrient uptake and synthesis of plant growth promoting hormones (Allen *et al.*, 1980, 1982; Harley and Smith, 1983) and/or improved water uptake (Safir *et al.*, 1971). An analysis of mineral content of the soil used in the study revealed that the soil was deficient in plant available P (P = 9 ppm). Improved nutrient uptake results from increased absorptive surface of the root system by AMF external mycelia. As external mycelia proliferate through the soil beyond the nutrient depletion zone they absorb and translocate mineral elements, mainly phosphorus, zinc, iron, copper, boron and molybdenum, to the root system (Harley and Smith, 1983). Improved water uptake in AMF-colonized plants results indirectly from improved plant nutritional status (Harley and Smith, 1983), increased cytokinin levels (Allen *et al.*, 1982) and increased number of vascular bundles (Daft and Okusanya, 1973). Further tests should, however, be carried out to ascertain the above speculations and establish which of the mechanism(s) is/are playing a significant role in improving pyrethrum growth.

The fact that AMF external hyphae perform functions that are comparable with those of the root systems might have compensated for the plants' need to have a long root system. This may explain the relatively short and long roots of plants treated with the fungi alone and untreated, respectively (table 2). Lateral root proliferation anterior to nematode-induced galls

Table 3. Mean dry shoot weight (DSW), fresh root weight (FRW), % root colonization (%RC), gall indices (GI), eggs, females and J-2 of pyrethrum inoculated with Glomus sp. *(KS14) and/or* Meloidogyne hapla *(M.h)*

	Plant growth		Colonization	Disease			
Treatment	DSW (g)	FRW (g)	(%RC)	GI†	Eggs/ml	Females/g	J-2/100 ml
Glomus sp.	6.1b*	24.2bc	53.5	—	—	—	—
Glomus sp.+*M.h*	6.1b	22.2ab	47	1.3a	405.5a	65.5	761.5
M.h	3.4a	20.9a	—	3.5b	1278b	96.8	1896
Control	4.2a	25.1c	—	—	—	—	—
LSD	0.87	2.61	NS	0.8	407	NS	NS

Data are means of six replicates.

*Means followed by the same letter(s) are not significantly different ($P > 0.05$) using LSD.

†Gall indices based on a 0–4 gall rating scale; where 0 = no galls, 1 = 1–25%, 2 = 26–50%, 3 = 51–75% and 4 = 76–100% root system galled.

which is typical of *M. hapla* infections (Taylor and Sasser, 1978) may account for the relatively longer roots of nematode-infected plants (table 2).

The significantly lower gall indices, fewer females and eggs in roots and J-2 in the soils of nematode – AMF-treated plants (tables 1, 2 and 3) confirm previous reports on the ability of AMF to suppress phytonematodes (Lindermann, 1994; Azcon-Aguilar and Barea, 1996). Significantly reduced egg production, gall indices and populations of *M. incognita* were reported on tomato inoculated with *Glomus intraradices* (Bagyaraj *et al.*, 1979; Suresh *et al.*, 1985), on cotton inoculated with *G. fasciculatum* (Saleh and Sikora, 1984), on *Piper nigrum* L. inoculated with *G. etunicatum* (Sivaprasad *et al.*, 1990) and on soybean inoculated with *G. margarita* (Carling *et al.*, 1989). Development of *M. incognita* on cotton and *M. hapla* on onion from J-2 to adults was delayed after inoculation with *G. intraradices* (Smith *et al.*, 1986) and *G. fasciculatum* (MacGuidwin *et al.*, 1985), respectively. Inoculating chickpea with *Glomus manihotis* suppressed reproduction of *M. javanica* (Diederichs, 1987).

Glomus etunicatum (KS18) and *Glomus* sp. (KS14) might have increased plant resistance or tolerance to *M. hapla* infection, thereby influencing nematode penetration and development. The fungi might have increased pyrethrum resistance or tolerance through improved plant growth and vigour (Lindermann, 1994; Azcon-Aguilar and Barea, 1996), increased production of phenolic compounds (Dehne and Schonbeck, 1979), phytoalexins (Morandi, 1987) and lignin (Schonbeck, 1979), increased the numbers of vascular bundles (Daft and Okusanya, 1973) and altered the chemostasis (Ratnayake *et al.*, 1978). Increases in lignin and phenols in mycorrhized roots were implicated in the reduction of *M. javanica* reproduction on tomatoes (Sikora, 1979). An increase in the number of vascular bundles might have compensated for the nematode-damaged xylem tissues and alleviated the nematode's detrimental effects on water and mineral uptake and translocation. The fungi might have, in addition, altered the chemostatic attraction of *M. hapla* to pyrethrum roots by altering the quantity and quality of root exudation (Ratnayake *et al.*, 1978). The speculated mechanisms of *M. hapla* suppression by *G. etunicatum* and KS14, however, need to be verified by further tests.

Although *G. etunicatum* improved plant growth and reduced disease severity and reproduction, the presence of *M. hapla* reduced growth and development of *G. etunicatum* and consequently its stimulative effects on pyrethrum growth (tables 1 and 2). The presence of the nematode did not, however, significantly affect root colonization by KS14 (tables 2 and 3). Nematodes may increase, decrease or have no effect on root colonization by AMF (Azcon-Aguilar and Barea, 1996). Decreased sporulation of *G. etunicatum* on soybean by *M. incognita* (Carling *et al.*, 1989) and decreased vesicle formation and mycelial growth in citrus by *Radopholus similis* (Cobb) Thorne (O' Bannon and Nemec, 1979) has been reported. Kellam and Schenck (1980) on the other hand, reported that *M. incognita* had no significant effect on soybean root colonization by *G. macrocarpum*.

It appears that in the *G. etunicatum*– nematode interaction, both organisms were mutually inhibitory. Competition for host photosynthates may account for this antagonism. AMF depend on the host for their carbohydrate requirements (Harley and Smith, 1983) while nematodes utilize their host's photosynthates for their growth and development (Bird, 1974). Whereas AMF increases photosynthesis through enhanced mineral translocation, water uptake and synthesis of phytohormones, root-knot nematodes decrease photosynthesis through the reverse mechanisms, reduced uptake and translocation of mineral salts and water (Bird, 1974) and inhibition of phytohormone production and translocation (McClure, 1977).

In addition to decreasing photosynthesis, nematodes act as metabolic sinks (McClure, 1977). The increased metabolic activities of giant cells stimulate mobilization of photosynthates from shoots to roots where they are removed and utilized by the feeding nematodes. By diverting photosynthates, altering nutrient flow patterns in the plant tissue and retarding root growth, nematodes enhance competition with the fungus, thereby affecting its growth and development. This may account for decreased growth and development of *G. etunicatum* and its decreased stimulative effects on pyrethrum growth in nematode-fungus treated pyrethrum. These speculations, however, need to be verified by further work.

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