Neth. J. Pl. Path. 80 (1974) 28–36

The effect of root-knot nematodes and Ethrel on Fusarium wilt of tomatoes

D. ORION and H. HOESTRA

Laboratory of Nematology, Agricultural University, Wageningen

Accepted 12 November 1973

Abstract

The reaction of two tomato varieties, 'Moneymaker' (susceptible to *Fusarium*) and 'Fortos' (resistant to *Fusarium*) to inoculation with *Meloidogyne javanica, M. incognita* and *Fusarium oxysporum* f. sp. *lycopersici* race 1 as well as to application of Ethrel (2 chloroethane phosphonic acid) – an ethylene releasing compound – was studied. With 'Moneymaker', Ethrel reduced wilt symptoms drastically and this was correlated with a reduction in the number of infected vascular bundles in the stem. Height of the stems was increased. Both nematode species counteracted the therapeutic effect of Ethrel by increasing the number of infected vascular bundles. 'Fortos' remained resistant to *Fusarium* in the presence of nematodes and/or Ethrel. Only a slight tendency toward resistance-breaking by the nematode was observed. In this variety enhancement of the pathogenic effects of the nematodes by Ethrel was evidenced by increased gall size. Histological studies indicated no difference in xylem structure among the various treatments. These results support the evidence that plant growth regulators play a role in the pathogenicity of root-knot nematodes, the *Fusarium* resistance of the plants, and the effect of nematodes on the severity of *Fusarium* wilt.

Introduction

The interaction of the root-knot nematode, *Meloidogyne* spp. and *Fusarium* wilt of tomatoes was recently discussed by Powell (1971a and b) and Bergeson (1972) in reviews on nematode-fungus associations. The conclusion drawn is that in a number of cases the root-knot nematode enhances the susceptibility of the tomato plants to *Fusarium* and can also serve as a resistance breaking agent. Bergeson (1972) attributed the influence of the root-knot nematode in enhancing *Fusarium* wilt to pathological changes in the normal balance of plant growth regulators and to quantitative and qualitative changes in root exudate which are induced by the nematodes and stimulate the germination, growth, and reproduction of fungal propagules in the rhizosphere. Brueske and Bergeson (1972) found that synthesis of cytokinins and gibberellin was interrupted in galled tomato root systems. Increase of auxin (indole 3 acetic acid) and related compounds in galls induced by various species of root-knot nematode was reported by Viglierchio and Yu (1968). Conversely, Setty and Wheeler (1968) did not find differences in auxin content between galled and healthy roots per unit of weight; but as galled root systems are heavier than healthy ones, the total quantity of auxin produced by galled roots is proportionally higher. Similar results were found by Orion (1973) concerning ethylene production in galled and healthy tomato roots. This finding agrees with the observation by Viglierchio and Yu (1968) as ethylene synthesis in plants is induced by auxin (Burg 1973). Ethrel (2 chloroethane phosphonic acid), an

ethylene releasing agent was found to enhance the pathological effects of the root-knot nematode, suggesting, that ethylene might be involved in the pathogenicity of the nematode (Orion and Minz, 1969). The goal of the present work was to compare the effect of two species of root-knot nematodes and Ethrel both separately and in combination, on *Fusarium* wilt of tomatoes.

Materials and methods

Experiment A. Tomato seedlings in cotyledon stage of the varieties 'Moneymaker' (susceptible to *Fusarium*) and 'Fortos' (resistant to *Fusarium*) were planted in 600 ml clay pots in Wageningen sandy-loam previously treated with chloropicrin. Prior to planting 3 glass tubes were placed in each pot to a depth of 10 cm, through which the plants could be inoculated with the fungus and the nematodes without causing root injury (Porter and Powell, 1967). The following treatments with 10 replicates were conducted for each tomato variety: 1, control; 2, Ethrel (Ethrel was applied as a soil drench at a dosage of 10 mg/plant in 50 ml aquous solution 12 days after planting); 3, *M. javanica* (20 g of tomato roots heavily infested with *M. javanica* were chopped for 5 min. in a Waring blendor, brought to a volume of 1000 ml and 25 ml of this suspension containing 1000–1200 eggs and larvae were applied to each pot through the glass tubes one week after planting); 4, *M. javanica* + Ethrel (a combination of treatment 2 and 3); 5, *M. incognita* (as treatment 3 but with *M. incognita* infested roots); 6, *M. incognita* + Ethrel (a combination of treatment 2 and 5). All the plants were inoculated with *Fusarium oxysporum* f. sp. *lycopersici* race 1 (IPO isolate No. 8) by 50 ml of suspension containing $5–7.10^6$ spores as well as mycelium obtained from 4-week-old cultures to each pot through the glass tubes 2 weeks after planting. The plants were kept in a greenhouse at a temperature of 24 ± 2 °C. The experiment was arranged in a randomized block design.

One month following the *Fusarium* inoculation, all the plants were harvested and root gall index (0 = no galls, 5 = 75–100% of root area covered with galls) was recorded. *Fusarium* incidence was determined from free hand stem cross sections taken 10 cm above soil level; they were placed on a microscope slide in a moist chamber for 24 h, and examined under a dissecting microscope for xylem vessels infected with *Fusarium* hyphae. The number of infected bundles was calculated as a percentage of the total number of vascular bundles.

To investigate the possibility of anatomical differences in xylem vessel structure among the plants in the various treatments, free hand cross and longitudinal stem sections were stained in cotton blue lactophenol solution and examined in lactophenol. Stem pieces (1 cm long) from just above the soil level were fixed in FAA, dehydrated and embedded in paraffin following the conventional procedure. Longitudinal sections, 10 μm thick were stained either by Cassons' or Ehrlichs' hematoxylin methods (Conn et al., 1960).

Experiment B. This experiment was similar to experiment A. Differences with experiment A are indicated in Table 1. *Fusarium* incidence in the tomato stems was determined by taking sections 5, 25 and 45 cm above soil level from each stem; they were placed on cherry agar plates and the development of *Fusarium* colonies was recorded after 48 h.

Table 1. Differences between experiments A and B.

	Exp. A	Exp. B
Volume of a single pot	600 ml	1500 ml
Stage of tomato plants at planting time	2 leaves	4 leaves
Time of nematode inoculation	7 d.a.p.	14 d.a.p.
Ethrel dosage per plant (active material)	10 mg	20 mg
Time of Ethrel application	12 d.a.p.	16 d.a.p.
Time of *Fusarium* inoculation	14 d.a.p.	26 d.a.p.
End of experiment	45 d.a.p.	'Moneymaker' 78 d.a.p. 'Fortos' 105 d.a.p.
Experimental design	blocks at random	blocks at random for each variety separately

d.a.p. = days after planting

Tabel 1. Verschillen in opzet en uitvoering tussen de proeven A en B.

Experiment c. Petri dishes containing 0, 40, 80, 160 and 320 ppm Ethrel in cherry agar were prepared (5 replicates). The Ethrel solutions were sterilized by filtration through a millipore 'Swinny' microsyringe adaptor, and then added to the cooled molten agar. *Fusarium* inoculum obtained from reisolations in experiment B was placed in the middle of the dishes. The diameter of each colony was measured daily to determine the fungal development.

Results

Experiment A. Two to four days following Ehtrel application the treated plants responded with leaf epinasty, and, a few days later with the appearance of aerial roots along the stem. Such responses are characteristic of ethylene (Zimmerman and Wilcoxon, 1935) or Ethrel application (Orion and Minz, 1969). Two weeks following *Fusarium* inoculation differences in the height of the plants became noticeable. The average height of the tomato plants at the end of the experiment is given in Fig. 1. In the nematode-inoculated treatments the two varieties reacted differently to Ethrel application: Whereas in 'Moneymaker' Ethrel application increased stem growth (significantly with *M. javanica*), in the case of 'Fortos' it reduced the stem height (significantly with *M. incognita*). Fig. 1 also shows the damaging effect of the nematode infestation on variety 'Moneymaker' (treatments without Ethrel application). In these same treatments with 'Moneymaker' there also was about 50% reduction in percentage of flowering plants at the end of the experiment.

One month following *Fusarium* inoculation several plants of 'Moneymaker' wilted and were on the verge of collapse. At that time all the plants were harvested. Gall index in both tomato varieties was 0 in the nematode-free treatments, 2.5 in the *M. javanica* treatments and 5 in the *M. incognita* treatments.

Fusarium incidence within the vascular tissue of stems of variety 'Moneymaker' showed an increase of susceptibility to *Fusarium* in the presence of *M. incognita* or *M. javanica*, in both Ethrel and non-Ethrel treated plants (Fig. 2). With Ethrel the

Fig. 1. Experiment A. Stem height of *F. oxysporum* infected tomato plants as effected by *M. javanica*, *M. incognita* and Ethrel. Columns indicated by different superscript differ significantly at the 0.05% level, according to Duncans' multiple range test.

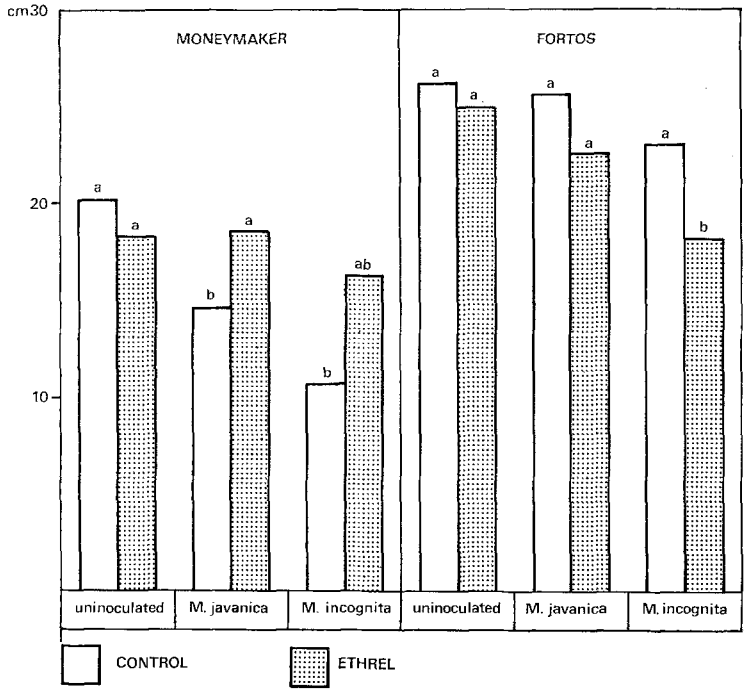

Fig. 1. Proef A. Het effect van M. javanica, M. incognita en Ethrel op de lengte van met Fusarium besmette tomateplanten. Significante verschillen zijn aangeduid door het plaatsen van letters bij de kolommen; kolommen zonder gemeenschappelijke letters verschillen significant van elkaar.

level of infestation was markedly lower than in plants not treated with Ethrel. *Fusarium* resistance of 'Fortos' is really complete with hardly any disease breaking by the root knot nematode.

In the histological study of the anatomical structure of xylem vessels, no difference among the various treatments was observed.

Experiment B. The results of this experiment confirmed the findings of the previous one. The percentage of *Fusarium* reisolations from the tomato stems (45 cm above soil level) is given in Fig. 3. As in the data of Fig. 2 'Moneymaker' is shown to be susceptible, whereas Ethrel application reduced the percentage of *Fusarium* reisolations, and both nematode species counteracted the effect of Ethrel. 'Fortos' again proved to be *Fusarium* resistant. The effect of the nematodes as resistance breakers is weak in spite of the high gall index (next paragraph) and the long exposure to both pathogens.

Although the root gall index of all *M. javanica* and *M. incognita* inoculated plants was 5, the galls of Ethrel-treated plants were larger. The effect of Ethrel on the weight

Fig. 2. Experiment A. Percentage of *F. oxysporum* infected xylem vessels in the tomato stems as effected by *M. javanica*, *M. incognita* and Ethrel.

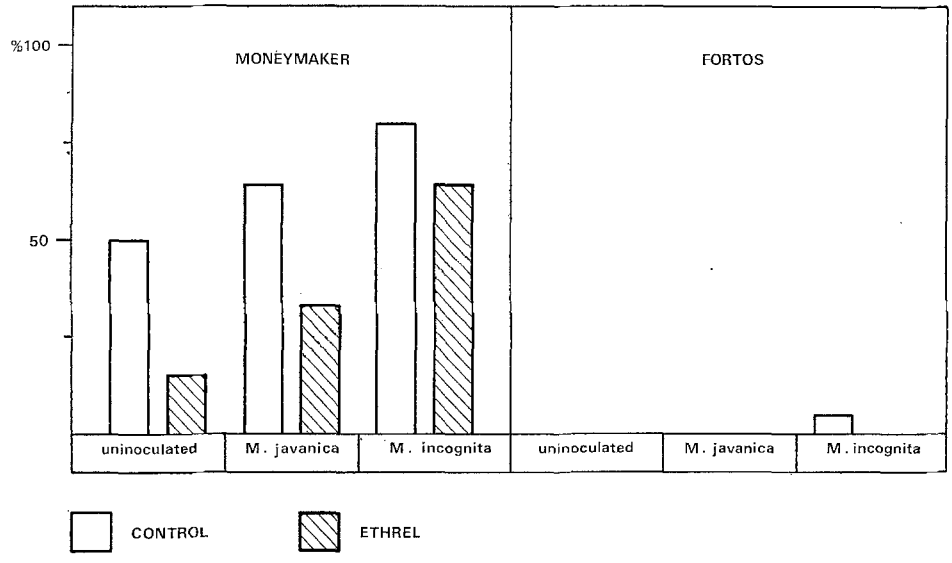

Fig. 2. Proef A. De invloed van M. javanica, M. incognita en Ethrel op het percentage xyleemvaten in de stengel dat met Fusarium is besmet.

of the root systems in shown in Fig. 4. In 'Fortos' this phenomenon is more pronounced than in 'Moneymaker', probably due to the later harvesting of 'Fortos' (Table 1).

Experiment C. Ethrel had hardly any effect on *Fusarium* development in vitro. Even at the highest concentration in the agar plates (320 ppm) there was only a slight inhibitory effect, which can not be associated with the depressive effect which so clearly occurred in the living plants, the more so since the concentration in these plates was 20 times higher than in the pot experiments.

Discussion

The results obtained in this work apply to several systems of host-parasite relationships, namely: plant-nematode-*Fusarium*, plant-*Fusarium*-ethylene, plant-nematode-ethylene.

Both *M. javanica* and *M. incognita* increased the susceptibility of 'Moneymaker' to *Fusarium* (Fig. 2 and 3), a phenomenon which is well documented (Powell 1971a and b, Bergeson 1972). On the other hand, although *Fusarium* developed to a little extent in heavily galled 'Fortos' plants *Fusarium* resistance was not broken. In his monograph on *Fusarium* wilt of tomato, Walker (1971) gives contradictory evidences of the role of the root-knot nematodes as resistance breakers. There may be several mechanisms of resistance, some of which can be broken by the nematodes, and some which can not.

The assumption that enhancement of the *Fusarium* infection in nematode infected

Fig. 3. Experiment B. The effect of *M. javanica*, *M. incognita* and Ethrel on the percentage of *F. oxysporum* reisolations from tomatoes.

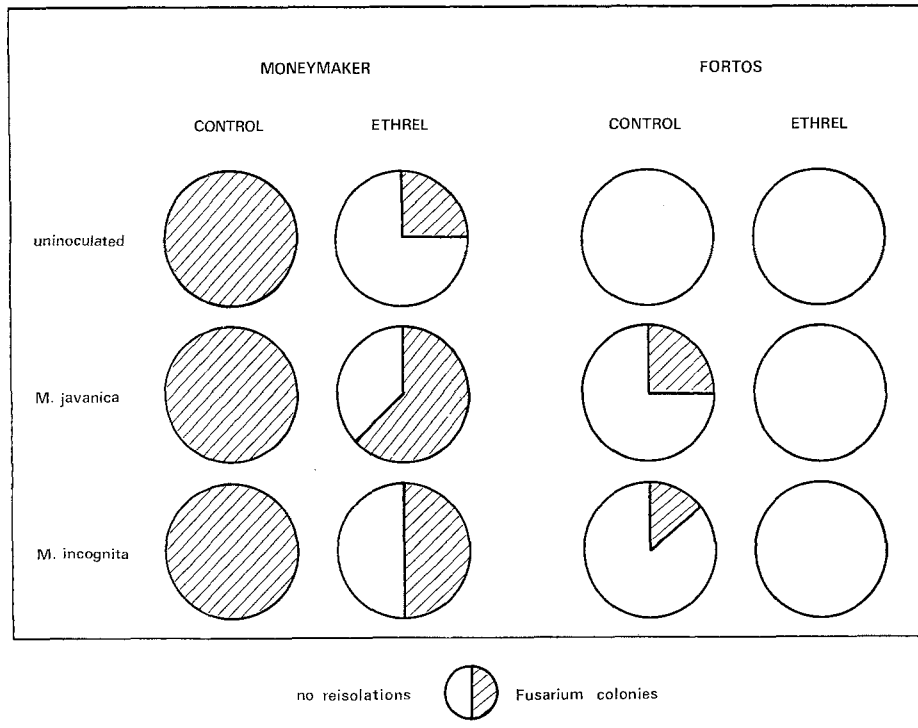

Fig. 3. Proef B. Het effect van M. javanica, M. incognita, en Ethrel op het percentage stengels waaruit Fusarium werd geherisoleerd.

plants is caused by ethylene overproduction in the galled roots, proved to be untrue. On the contrary, ethylene released from Ethrel applied to the rhizosphere was shown to have a depressive effect on *Fusarium* development in the plants (Fig. 2 and 3). This effect appeared mainly in 'Moneymaker' since in 'Fortos' there was hardly any fungus infection. Still, the slight *Fusarium* infection in 'Fortos' was completely suppressed by Ethrel application.

The effect of ethylene on *Fusarium* in the plant could not be attributed to its direct effect on the fungus. These results agree with prevous work in which *Fusarium* resistance in tomato was induced by plant growth regulators (Davis and Dimond, 1953).

The histological examination for a *Fusarium* resistance mechanism by means of barriers, as was recently reported by Beckman et al. (1972), gave negative results. Neither the resistance of 'Fortos' nor the *Fusarium* depressive effect of Ethrel could be explained by tyloses or similar structural formations within the tomato vascular system. These observations support the view that there are more *Fusarium* resistance mechanisms in tomato.

The pathological effects of the root-knot nematodes were particularly evident in 'Fortos' since in 'Moneymaker' the effects of *Fusarium* became predominant. Ethrel

Fig. 4. Experiment B. The effect of Ethrel on root system fresh and dry weight (line at the bottom of the column) of tomatoes. Columns indicated by different superscript differ significantly at the 0.05% level, according to Duncans' multiple range test. N.S. = not significant.

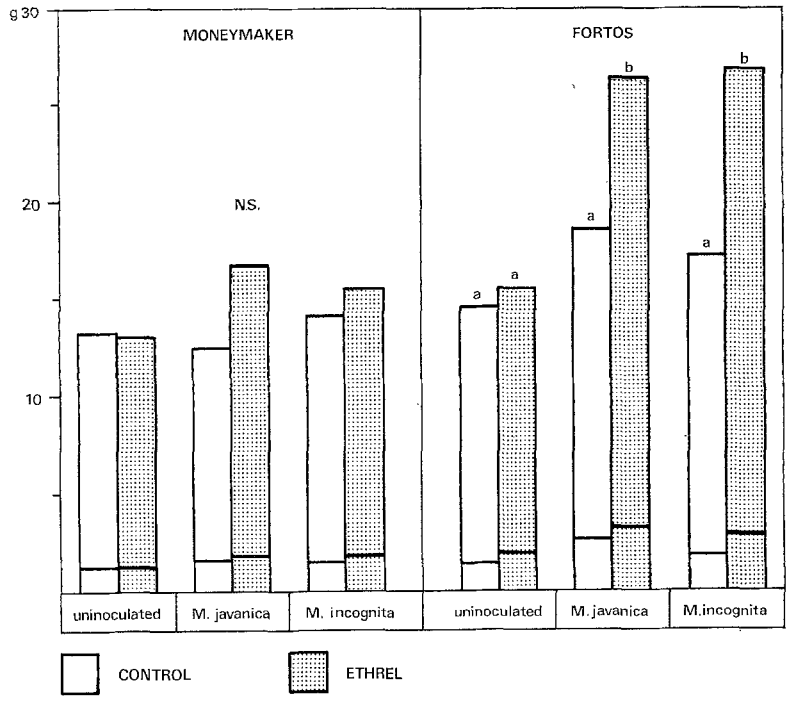

Fig. 4. Proef B. Het effect van Ethrel op vers en drooggewicht van de wortels. Dwarsstreep in de kolommen geeft het drooggewicht aan. Significante verschillen zijn aangeduid door het plaatsen van letters bij de kolommen; kolommen zonder gemeenschappelijke letters verschillen significant van elkaar. N.S. = niet significant.

application enhanced the effects of the nematodes and increased gall size (Fig. 4). These findings agree with previous reports (Orion and Minz, 1969; Orion, 1973). No difference between the responses of tomato infected with *M. javanica* and *M. incognita* to Ethrel application were observed. The apparent differences between the two species such as indicated in Fig. 2 should be attributed to the difference in rate of galling (2.5 for *M. javanica* and 5 for *M. incognita*) rather than to differences in species response to Ethrel.

This work supplies further evidence that the plant growth regulatory system is involved in host-parasite relationships of *Fusarium* and *Meloidogyne* in tomatoes. Part of the pathological effects of the root-knot nematodes might be atrributed to the production of ethylene; however, this mechanism is apparently not enhancing the *Fusarium* development in the plant. Though Ethrel and root-knot nematodes seem to counteract each other with regard to the development of *Fusarium*, the net effect might be a result of quite different mechanisms.

Samenvatting

De invloed van aantasting door wortelknobbelaaltjes en van behandeling met Ethrel op de Fusarium-verwelkingsziekte bij tomaat

Bestudeerd werd de reactie van twee tomatecultivars, 'Moneymaker' (gevoelig voor *Fusarium*) en 'Fortos' (resistent tegen *Fusarium*) op inoculatie met *Meloidogyne javanica*, *M. incognita* en *Fusarium oxysporum* f. sp. *lycopersici* ras 1, en op behandeling met Ethrel (2 chloorethaan fosfonzuur) een verbinding waaruit ethyleen vrijkomt. Bij 'Moneymaker' resulteerde behandeling met Ethrel in een sterke afneming van verwelkingssymptomen gecorreleerd met een vermindering van met *Fusarium* geïnfecteerde vaatbundels in de stengel (Fig. 2 en 3) en een grotere lengte van de planten (Fig. 1). Beide aaltjessoorten hadden het omgekeerde effect en verhoogden het aantal met *Fusarium* geïnfecteerde vaatbundels (Fig. 2 en 3).

Bij 'Fortos' was er slechts een zeer geringe mate van verlies van resistentie tegen *Fusarium* onder invloed van de aanwezigheid van wortelknobbelaaltjes. Ethrel elimineerde de geringe *Fusarium* infectie geheel. Bij deze cultivar werd het schadelijk effect van de aaltjes duidelijk versterkt door Ethrel in correlatie met een sterke toeneming van de omvang der gallen (toeneming wortelgewicht, Fig. 4). Ethrel had geen rechtstreeks effect op *Fusarium* in reincultures.

Histologische waarnemingen gaven geen aanwijzingen voor het optreden van veranderingen in de structuur van het xyleem (thyllen) onder invloed van de behandelingen.

De resultaten ondersteunen de opvatting dat groeiregulatoren een rol spelen bij de pathogeniteit van wortelknobbelaaltjes, de *Fusarium*-resistentie van de plant, en de invloed van aaltjes op de ontwikkeling van *Fusarium* in de plant.

Acknowledgments

The senior author is indebted to Dr M. Oostenbrink, head of the Department of Nematology, for supplying all facilities needed and to the International Agricultural Centre (IAC), Wageningen, for granting him a fellowship for his work. Both authors wish to thank Drs J. M. and V. R. Ferris, and Dr G. B. Bergeson, Lafayette, Indiana, USA, for their help in preparing the manuscript.

References

Beckman, C. H., Elgersma, D. M. & Machardy, W. E., 1972. The localization of Fusarial infections in the vascular tissue of single dominant gene resistent tomatoes. Phytopathology 62: 1256–1260.

Bergeson, G. B., 1972. Concepts of nematode-fungus associations in plant disease complexes: A Review. Expl. Parasit. 32: 301–314.

Brueske, C. H. & Bergeson, G. B., 1972. Investigation of growth hormones in xylem exudates and root tissue of tomato infected with root-knot nematodes. J. exp. Bot. 23: 14–22.

Burg, S. P., 1973. Ethylene in plant growth. Proc. natn. Acad. Sci. U.S.A. 70: 591–597.

Conn, H. J., Darrow, M. A. & Emmel, N. V., (ed.) 1960. Staining procedures. The Williams & Wilkins Company, Baltimore, Md.

Davis, D. & Dimond, A. E., 1953. Inducing disease resistance with plant-growth regulators. Phytopathology 43: 137–140.

Orion, D., 1973. Studies on plant root-knot nematode interrelationsships. EPPO Bull. 9: 67–71.

Orion, D. & Minz, G., 1969. The effect of Ethrel (2-Chloroethane phosphonic acid) on the pathogenicity of the root-knot nematode *Meloidogyne javanica*. Nematologica 15: 608–614.
Porter, D. M. & Powell, N. T., 1967. Influence of certain *Meloidogyne* species on *Fusarium* wilt development in flue cured tobacco. Phytopathology 57: 282–285.
Powell, N. T., 1971a. Interaction of plant parasitic nematodes with other disease causing agents. In: Zuckerman, B. Mai, W. F. & Rohde, R. A. (ed.), Plant parasitic nematodes. Vol. II. Academic Press, London & New York.
Powell, N. T. 1971b. Interaction between nematodes and fungi in disease complexes. A. Rev. Phytopath. 9: 253–274.
Setty, K. G. H. & Wheeler, A. W., 1968. Growth substances in roots of tomato (*Lycopersicon esculentum*, Mill.) infected with root-knot nematodes (*Meloidogyne* spp.). Ann. appl. Biol. 61: 495–501.
Viglierchio, D. R. & Yu, P. K., 1968. Plant growth substances and plant parasitic nematodes. II. Host influence on auxin content. Exp. Parasitol. 23: 88–95.
Walker, J. C., 1971. *Fusarium* wilt of tomato. Monograph no. 6. The American Phytopathological Society, St. Paul, Minn.
Zimmerman, P. W. & Wilcoxon, F., 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. Contr. Boyce Thompson Inst. Pl. Res. 7: 209–230.

Addresses

D. Orion: Division of Nematology, Institute of Plant Protection, Agricultural Research Organization, The Volcani Center, P.O.Box 6, Bet-Dagan, Israel.
H. Hoestra: Laboratorium voor Nematologie, Binnenhaven, Wageningen, the Netherlands.

Publications received

The publications marked with an asterisk will be reviewed in due time.

* K. F. Baker, E. B. Cowling & G. A. Zentmyer: Annual Review of Phytopathology 11 (1973). Annual Reviews Inc. Palo Alto, California, USA.
C. J. A. Barel: Studies on dispersal of *Adoxophyes orana* F. v. R. in relation to the population sterilization technique. Meded. Landbouwhogeschool, Wageningen 73 (7), 1973: 1–107.
* G. H. Boerema & M. M. J. Dorenbosch: The *Phoma* and *Ascochyta* species described by Wollenweber and Hochapfel in their study on fruit-rotting. Studies in Mycology 3 (1973): 1–50.
W. R. Simons: Nematode survival in relation to soil moisture. Meded. Landbouwhogeschool, Wageningen 73 (3), 1973: 1–85.