Suppressive effect of the root-knot nematode on *Fusarium* wilt of muskmelons [1]

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SUMMARY

The reaction of two muskmelon *(Cucumis melo)* cultivars, one *Fusarium*-susceptible ('Ein Dor') and one resistant ('Hemed'), to inoculation with *Meloidogyne javanica* and with *Fusarium oxysporum* f. sp. *melonis* race O, was studied. All plants of cv. 'Ein Dor' wilted 10-20 days following inoculation with *Fusarium* alone, but cv. 'Hemed' did not show any wilt symptoms. Inoculation with the nematode alone caused severe damage to both cultivars, as expressed by stunting and heavy galling. In plants inoculated with the nematode fourteen days prior to *Fusarium*, no wilting occurred in cv. 'Hemed'; moreover, *ca* 40% of the 'Ein Dor' plants did not wilt and *Fusarium* could not be isolated from their stems. The *Fusarium*-suppressing effect was achieved by a rather high nematode-inoculation level. Ethephon (an ethylene-releasing agent) applied as a soil drench had an effect similar to that of *M. javanica* on *Fusarium* wilt of muskmelons.

RÉSUMÉ

Suppression de la fusariose du melon due aux effets du nématode Meloidogyne javanica

On a étudié les réactions à l'inoculation avec *M. javanica* et *Fusarium oxysporum* f. sp. *melonis* race O de deux cultivars de melon *(Cucumis melo)*, l'un sensible au *Fusarium* (Ein Dor), l'autre résistant (Hemed). En présence du seul *Fusarium*, 100% des plantes du cultivar Ein Dor étaient fanées dix à vingt jours après l'inoculation alors que celles du cultivar Hemed ne présentaient aucun symptome. L'inoculation du seul nématode a causé, chez les deux cultivars, l'apparition de galles et une diminution de la croissance. Quand le nématode était inoculé quatorze jours avant le *Fusarium*, aucune fanaison n'était constatée chez les plantes du cultivar Hemed ; chez le cultivar Ein Dor, 40% des plantes n'étaient pas affectées et le *Fusarium* ne pouvait être isolé de leurs tiges. Cet effet de suppression de la fusariose était obtenu avec un inoculum de nématodes assez important. Un effet similaire à celui du nématode était obtenu par arrosage du sol avec un produit libérant de l'éthylène : l'Ethephon.

The role the root-knot nematodes, *Meloidogyne* spp. play in a complex with *Fusarium oxysporum* has been studied on a few hosts. The most extensive studies to determine the mechanism of interaction have been carried out with cotton, tobacco and tomato (Powell, 1970), while cucurbits have received less attention, even though most species of cucurbits are quite susceptible to *Meloidogyne* spp. and to *Fusarium* wilt. Sumner and Johnson (1973) found that watermelon plants were more affected when *M. incognita* was present and *Fusarium* wilt severity was significantly correlated with the initial population of the second stage larvae. Recently, Netzer, Galun and Niego (1976) released a muskmelon cultivar, "Hemed", resistant to *F. oxysporum* f. sp. *melonis* race *O. Meloidogyne* species are common in Israel and previously were found to break *Fusarium* wilt resistance in Tomato (Cohn & Minz, 1960). Since muskmelons are often grown on soil infested with root-knot nematodes and *Fusa*-

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rium, we conducted a study to determine whether the root-knot nematodes could affect the resistance of the new cultivar.

Materials and methods

A standard experimental unit was used throughout the work. Ten seeds each of cv. "Ein Dor" (*Fusarium* susceptible) and of cv. "Hemed" (*Fusarium* resistant) were sown in air-dried heat-sterilized sandy loam in four rows of five seeds per row, in 4-liter plastic trays (Fig. 1). The trays were placed in Wisconsin tanks and kept at 26° ± 1°. *Fusarium* inoculum was derived from a culture (No. 498) isolated from a wilted luskmelon plant. The liquid medium used for growing cultures and preparing the inoculum has been described elsewhere (Netzer, Niego & Galun, 1977). The *Fusarium* inoculum consisted of a liquid medium, fungal hyphae, microconidia and macroconidia. Pre-planting inoculation was carried out by mixing 100 ml of the inoculum with 3 kg of soil. Post-planting inoculation was carried out by pouring 25 ml/row of the inoculum into 2 cm-deep furrows along the side of the rows ten days after seed germination.

Crude nematode inoculum was prepared by chopping 20 g of *M. javanica*-infected excised tomato roots cultured *in vitro* (Dropkin & Boone, 1966), for five-minutes in 500 ml of water in a blender. This nematode inoculum included 20 000-40 000 eggs and second stage larvae per 100 ml of suspension. A quantitative inoculum was prepared by dissolving egg masses by agitating 100 g of infected excised tomato roots in aqueous solution of 1% sodium hypochloride for ten minutes, pouring the solution over a 500-mesh screen. The egg suspension thus obtained was immediately diluted with tap water. The desired concentration was obtained by diluting the original egg suspension. Inoculation was carried out by pouring 25 ml/row of the inoculum into furrows in the soil, as described above.

Stem sections of wilted plants were placed on potato-dextrose agar (PDA) plates for isolation of *Fusarium*. Nematode infection was estimated using a gall index from 0 to 5, where 0 = no galls, 1 = 1 to 10% of the root area covered with galls, 2 = 11 to 25%, 3 = 26 to 50%, 4 = 51 to 75%, and 5 = 76 to 100% of the root area covered with galls.

Ethephon, (2-chloroethyl) phosphonic acid, was applied to the soil in an aqueous solution as a drench. Plant growth was determined by periodical stem-height measurements, visual evaluation of wilting, and weighing of the plant at the end of the experiments.

Five sets of experiments were carried out :

A — Four treatments, 40 replicates : 1 : pre-planting soil inoculation with *Fusarium* (F) ; 2 : Pre-planting inoculation with *M. javanica* (N) ; 3 : F + N ; 4 : Uninoculated control (C).

B — Six treatments, 40 replicates : 1 : Pre-planting soil inoculation with 30 000 *M. javanica* eggs/tray (N) ; 2 : Pre-planting soil inoculation with *Fusarium* (F-pre) ; 3 : Soil inoculation with *Fusarium* ten days after seed germination (F-post) ; 4 : N + F-pre. 5 : N + F-post ; 6 : Uninoculated control (C).

C — Eight treatments, 30 replicates : Pre-planting soil inoculation with four levels of *M. javanica* eggs : 0, 7 500, 30 000 and 120 000 eggs/tray, at two soil temperatures : 25° and 30°. All the trays were inoculated with *Fusarium* ten days after seed germination.

D — Three treatments, 40 replicates : Applition of ethephon at 0,20 and 100 mg/tray seven days after seed germination. All the trays were inoculated with *Fusarium* ten days after germination.

E — Nine treatments, 30 replicates : 1 : Pre-planting soil inoculation with 30 000 *M. javanica* eggs/tray (N) ; 2 : Soil inoculation with *Fusarium* ten days after seed germination (F) ; 3 : Ethephon application of 100 mg/tray, seven days after seed germination (E) ; 4 : N + E ; 5 : N + F ; 6 : Pre-planting soil inoculation with 30 000 *M. hapla* eggs/tray + F ; 7 : E + F ; 8 : N + E + F ; 9 : Uninoculated control (C).

Results

A — Twelve days after seed germination, *Fusarium* wilt symptoms appeared on cv. "Ein Dor" inoculated with *Fusarium* and *Fusarium* + nematodes, and one week later all of these

Fig. 1. The effect of pre-plant soil inoculation with *Fusarium oxysporum* f. sp. *melonis* on susceptible ('Ein Dor') and *Fusarium*-resistant ('Hemed') cultivars (F left), as compared with an uninoculated control (C right).

plants were completely wilted. On the other hand, cv. "Hemed" plants showed no *Fusarium* wilt symptoms (Fig. 1). The plants in the nematode-inoculated treatments were considerably stunted (Fig. 2). The root-galling index of the roots of both cultivars was 4.

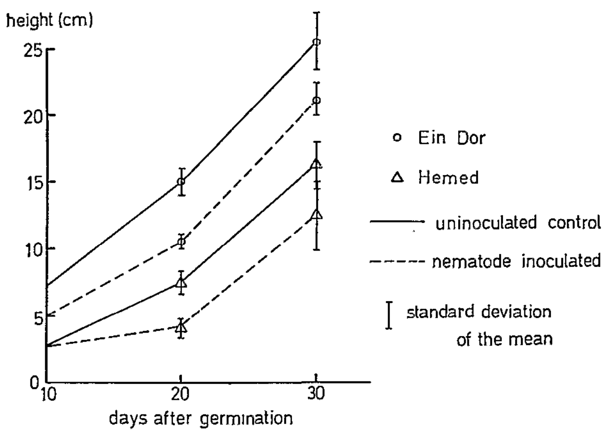

Fig. 2. The plant height of two muskmelon cultivars as affected by pre-plant soil inoculation with *Meloidogyne javanica.*

B — This experiment was designed to examine whether *M. javanica* might alter the *Fusarium* resistance when "Hemed" plants were predisposed to the fungus by the nematode. The results of this experiment confirmed the results of the previous one in regard to the high susceptibility of both cultivars to *M. javanica*, the susceptibility of cv. "Ein Dor" to *Fusarium*, and the fact that the nematode did not alter "Hemed" resistance to *Fusarium.* An unexpected result of this experiment was that the percentage of wilted "Ein Dor" plants in the pre-planting nematode inoculation + *Fusarium* post-planting inoculation treatment was only 60%, compared with 100% wilt in the *Fusarium* pre-planting, *Fusarium* post-planting, and nematode + *Fusarium* pre-planting inoculations treatments. The percentage of wilting plants and *Fusarium* re-isolations in the various treatments two and four weeks after seed germination is presented in Table 1.

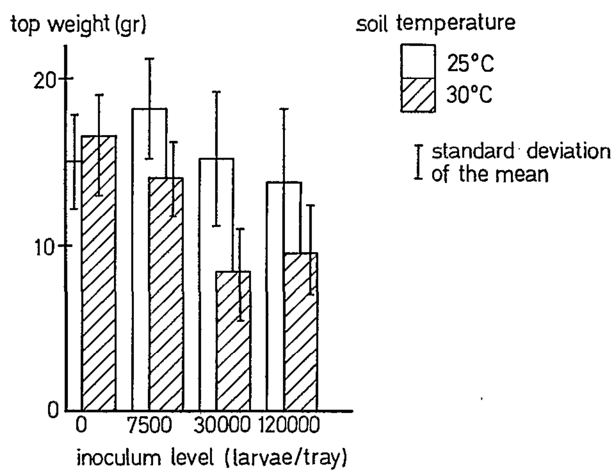

Fig. 3. The top weight of cv. 'Hemed' muskmelons inoculated with four levels of *Meloidogyne javanica* aud growu at two soil temperatures.

Table 1

Percent of wilting plants in resistant ('Hemed') and susceptible ('Ein Dor') muskmelon cultivars subjected to inoculation with *M. javanica* and F. *oxysporum* f. sp. *melonis*

Treatment	*Cultivar*	*Disease incidence %* 14 *days after inoculation*	28 *days after inoculation*
None (uninoculated control)	'Hemed'	0	0
	'Ein Dor'	0	0
M. javanica	'Hemed'	0	0
	'Ein Dor'	0	0
Fusarium pre-planting	'Hemed'	0	0
	'Ein Dor'	100	100
M. javanica + *Fus.* pre-pl.	'Hemed'	0	0
	'Ein Dor'	100	100
Fusarium post-planting *	'Hemed'	0	0
	'Ein Dor'	0	95
M. javanica + *Fus.* post-pl.	'Hemed'	0	0
	'Ein Dor'	0	60

* Inoculation ten days after seed germination.

Table 2

The effect of ethephon on wilting and isolations from resistant ('Hemed') and susceptible ('Ein Dor') muskmelon cultivars

Ethephon dosage * *(mg/tray)*	*Percent of wilted plants and fungus isolation* 'Hemed' *wilt*	'Hemed' *isolation*	'Ein Dor' *wilt*	'Ein Dor' *isolation*
0	0	0	100	96
20	0	0	100	93
100	0	0	60	69

* Applied as a soil drench seven days after seed germination.

C — The result of this experiment confirmed those of experiments A and B. The extent of *M. javanica* infection as expressed in above-ground fresh weight, of the plants was generally correlated to the inoculum level and to soil temperature (Fig. 3). The galling index of the same inoculum level was increased by a soil temperature 30° compared with the lower soil temperature, at the 7 000 and 30 000 eggs-per-tray inoculum levels (Fig. 4). The percentage of wilting plants was hardly affected by the low nematode-inoculum level, but the higher level reduced the percentage of the wilting plants, as shown in Fig. 5.

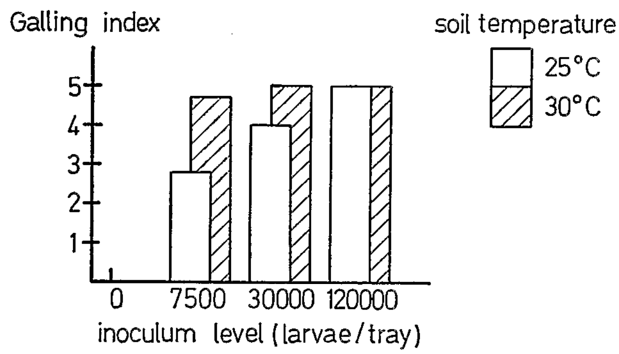

Fig. 4. Galling indices of cv. 'Hemed' muskmelons inoculated with four levels of *Meloidogyne javanica* and grown at two soil temperatures.

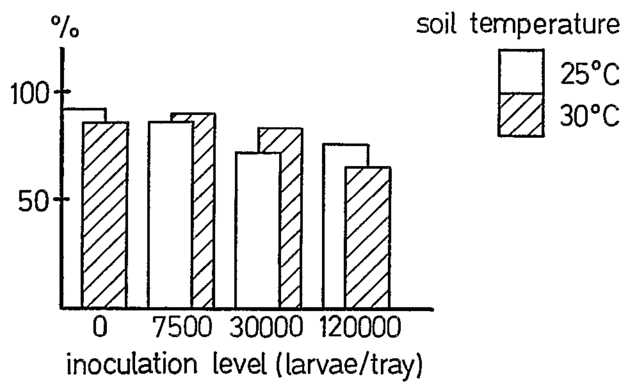

Fig. 5. The percentage of wilted plants of cv. 'Ein Dor' muskmelon at different *M. javanica* inoculation levels and two soil temperatures.

D — The results of the effect of ethephon on *Fusarium* in both cultivars of muskmelons are presented in Table 2. The lower rate of ethephon had hardly any effect on *Fusarium*, but the higher rate reduced the percentage of wilt symptoms and *Fusarium* isolated from the stems of "Ein Dor" plants.

Table 3

The effect of *M. javanica*, *M. hapla* and ethephon (100 mg/tray) on the percentage of wilted plants and isolation of *F. oxysporum* f. sp. *melonis* from wilt-resistant ('Hemed') and wilt susceptible ('Ein Dor') muskmelon cultivars

Treatments	*Cultivars*	*% wilted plants*	*% isolations*
None (uninoculated control)	'Hemed'	0	0
	'Ein Dor'	0	0
M. javanica	'Hemed'	0	0
	'Ein Dor'	0	0
Ethephon	'Hemed'	0	0
	'Ein Dor'	0	0
Fusarium	'Hemed'	0	0
	'Ein Dor'	96	100
M. jav. + Ethephon	'Hemed'	0	0
	'Ein Dor'	0	0
M. jav. + *Fusarium*	'Hemed'	0	0
	'Ein Dor'	70	76
M. hapla + *Fusarium*	'Hemed'	0	0
	'Ein Dor'	63	70
Ethephon + *Fusarium*	'Hemed'	0	0
	'Ein Dor'	74	90
M. jav. + Ethephon + *Fusarium*	'Hemed'	0	0
	'Ein Dor'	30	54

E — The effects of *M. javanica*, *M. hapla*, *Fusarium* and ethephon, in various combinations, on muskmelon wilt symptoms and *Fusarium* isolations, are shown in Table 3. The results of this experiment agree with the above. *M. javanica* and *M. hapla* suppressed *Fusarium* wilt to some extent. Ethephon had an effect similar to that of the nematodes, and when applied in conjonction with *M. javanica*, it increased its suppressive effect on *Fusarium* wilt.

Discussion

The effect of the *Meloidogyne-Fusarium* complex varies in different plant hosts and conditions. *M. incognita* increased the severity of *Fusarium* wilt in cotton and tobacco (Powell, 1971). In tomato the available information on this subject is rather contradictory. There are reports of *Meloidogyne* spp. breaking the resistance to *Fusarium* (Cohn & Minz, 1960 ; Jenkins & Coursen, 1957 ; Harrison & Young, 1941 ; McClellan & Christie, 1949 ; Pitcher, 1974 ; Sidhu & Webster, 1974, 1977) while others report that the resistance to *Fusarium* wilt was not altered by the nematode (Binder & Hutchinson, 1959 ; Jarvis, Dirks, Johnson & Thorpe, 1977 ; Jones, Overman & Crill, 1976 ; Kawamura & Hirano, 1967 ; Orion & Hoestra, 1974). *M. incognita* did not affect cabbage yellows caused by *F. oxysporum* f. *conglutinans* (Fassuliotis & Rau, 1969). Our research indicated a unique phenomenon in which *M. javanica* and ethephon somewhat suppressed *Fusarium* wilt in *Fusarium* susceptible muskmelons. In previous work, Orion and Minz (1969) found that ethephon (as ethrel) had similar effects and enhanced symptoms caused by *M. javanica*, in tomatoes. The inhibitory effect of ethephon on the *Fusarium* symptoms and isolations in the susceptible cv. "Ein Dor", is similar to that of ethephon on *Fusarium* in susceptible tomato (cv. "Moneymaker") which was found by Orion and Hoestra (1974). In that work they showed that ethephon did not have any direct effect on *F. oxysporum* cultured *in vitro*. An inhibitory effect of application of various plant growth regulators on *Fusarium* wilt of tomatoes was also reported by Davis and Dimond (1953). It was later found that the activity of auxin and related compounds which induced resistance to *Fusarium* were connected to ethylene metabolism. (Burg, 1973). The result of the present work concerning the partial supression of *Fusarium* wilt of muskmelons by the root-knot nematode gave additional support to the assumption that the effect of *Meloidogyne* spp. on the host plant and indirectly on the *Fusarium* wilt is related to ethylene activity. As to the effect of the nematode and *Fusarium* alone on the muskmelon seedlings, our research clearly indicated that in all phases of our experiments, using various levels of nematode inoculum at two different soil temperatures, the resistance of the newly bred cv. "Hemed" to *Fusarium* wilt remained unchanged while "Ein Dor" collapsed within three weeks after inoculation. The "Ein Dor" and "Hemed" cultivars were both highly susceptible to the root-knot nematodes *M. javanica* and *M. hapla*. However, as

"Hemed" had a slower growth rate than "Ein Dor", *M. javanica* effected the former more severely.

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REFERENCES

BINDER, E. & HUTCHINSON, M.T. (1959). Further studies concerning the effect of the root-knot nematode *Meloidogyne incognita acrita* on susceptibility of the Chesapeake tomato to *Fusarium* wilt. *Pl. Dis. Reptr*, 43 : 972-978.

BURG, S.P. (1973). Ethylene in plant growth. *Proc. natn. Acad. Sci. U.S.A.*, 70 : 591-597.

COHN, E. & MINZ, G. (1960). [Nematodes and resistance to *Fusarium* wilt in tomatoes.] *Hassadeh*, 40 : 1347-1349.

DAVIS, D. & DIMOND, A.E. (1953). Inducing disease resistance with plant growth regulators. *Phytopathology*, 43 : 137-140.

DROPKIN, V.H. & BOONE, W.R. (1966). Analysis of host parasite relationships of root-knot nematodes by single larva inoculation of excised tomato roots. *Nematologica*, 12 : 225-236.

FASSULIOTIS, G. & RAU, G.J. (1969). The relationship of *Meloidogyne incognita acrita* to the incidence of cabbage yellows. *J. Nematol.*, 1 : 219-222.

JARVIS, W.R., DIRKS, V.A., JOHNSON, P.W. & THORPE, H.J. (1977). No interaction between root-knot nematode and *Fusarium* foot and root rot of greenhouse tomato. *Pl. Dis. Reptr*, 61 : 251-254.

JENKINS, W.R. & COURSEN, B.W. (1957). The effect of root-knot nematodes, *Meloidogyne incognita acrita* and *M. hapla* on *Fusarium* wilt of tomato *Pl. Dis. Reptr*, 41 : 182-186.

JONES, J.P., OVERMAN, A.J. & CRILL, P. (1976). Failure of root-knot nematode to affect *Fusarium* wilt resistance of tomato. *Phytopathology*, 68 : 1339-1341.

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HARRISON, A.L. & YOUNG, P.A. (1941). Effect of root-knot nematode on tomato wilt. *Phytopathology*, 31 : 749-752.

HIRANO, K. & KAWAMURA, T. (1971). Changes in the parastism of *Fusarium* spp. in plants exposed to root-knot nematodes and *Fusarium* complex. *Tech. Bull. Fac. Hortic., Chiba Univ.* No. 19 : 29-38. [Cited from : *Rev. Pl. Path.*, (1973) 52 : No. 381.]

KAWAMURA, T. & HIRANO, K. (1967). [Studies on the complex disease caused by root-knot nematode and *Fusarium* wilt fungus in tomato seedlings. I. Development of wilt symptoms and difference in disease incidence in tomato varieties.] *Tech. Bull. Fac. Hort., Chiba Univ.* No. 15, pp. 7-19. [Cited from *Helminth. Abstr.* (1969) 38 : No. 1143.]

McCLELLAN, W.D. & CHRISTIE, J.R. (1949). Incidence of *Fusarium* infection as affected by root-knot nematode. *Phytopathology*, 39 : 568-571.

NETZER, D., GALUN, E., NIEGO, S., ZUTA, Z. & DAVIDI, C. (1976). Locally bred muskmelon cultivars resistant to *Fusarium* wilt. *Phytoparasitica*, 4 : 208.

NETZER, D., NIEGO, S. & GALUN, E. (1977). A dominant gene confering resistance to *Fusarium* wilt in cucumber. *Phytopathology*, 67 : 525-527.

ORION, D. & HOESTRA, H. (1974). The effect of root-knot nematodes and Ethrel on *Fusarium* wilt of tomatoes. *Neth. J. Pl. Path.*, 80 : 28-36.

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.

PITCHER, J.R. (1974). Reduction of resistance of tomato to *Fusarium oxysporum* f. sp. *lycopersici* by *Meloidogyne javanica*. *J. Nematol.*, 6 : 148.

POWELL, N.T. (1971). Interaction between nematodes and fungi in disease complexes. *Ann. Rev. Phytopath.*, 9 : 253-274.

SIDHU, G. & WEBSTER, J.M. (1974). Genetics of resistance in the tomato to root-knot nematode wilt fungus complex. *J. Hered.*, 65 : 153-156.

SIDHU, G. & WEBSTER, J.M. (1978). Predisposition of tomato to the wilt fungus *(Fusarium oxysporum lycopersici)* by the root-knot nematode *(Meloidogyne incognita)*. *Nematologica*, 23 (1977) : 436-442.

SUMNER, D.R. & JOHNSON, A.W. (1973). Effect of root-knot nematodes on *Fusarium* wilt of watermelon. *Phytopathology*, 63 : 857-861.