Influence of *Fusarium oxysporum* f. sp. *glycines* on the invasion and development of *Meloidogyne incognita* on soybean

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SUMMARY

The root-knot nematode, *Meloidogyne incognita* and the wilt fungus, *Fusarium oxysporum* f. sp. *glycines* were inoculated simultaneously onto growing seedlings of soybean resistant to the fungus. Nematode invasion of roots was not affected but giant cells were invaded by the fungus and destroyed. The overall effect was to reduce the number of females and increase the proportion of males.

RÉSUMÉ

Influence de Fusarium oxysporum *f. sp.* glycines *sur la pénétration et le développement de* Meloidogyne incognita *dans le soja*

Meloidogyne incognita et le champignon agent du wilt, *Fusarium oxysporum* f. sp. *glycines*, ont été inoculés simultanément à des plantules de soja résistant au champignon. La pénétration du nématode dans les racines n'a pas été affectée, mais les cellules géantes ont été envahies par le champignon, et détruites. Le résultat consiste en une diminution du nombre des femelles et une augmentation de celui des mâles.

The interaction between the form species of *Fusarium oxysporum* and the root-knot nematodes has been studied on tomatoes by Fattah and Webster (1983), on tobacco by Melandez and Powell (1967) and on cotton by Hillocks (1985). All the authors suggest that the nematode predisposes the host plants to *Fusarium* wilt fungi and Fattah and Webster found that the fungus may initiate early breakdown of the giant cells thus affecting nematode development.

The objective of the present experiments was to study the influence of *F. oxysporum* f. sp. *glycines* on the invasion and development of *Meloidogyne incognita* in soybean.

Materials and methods

Experiments were done using two soybean cultivars, Ware and Coll, resistant to *F. oxysporum* : the cultivar Ware was moderately resistant to *M. incognita* and Coll was susceptible to the root-knot nematode. Seedlings were grown in modified " dispo " pouches (Preiser, Babu & Haidri, 1981) which had been sterilised for 10 min at 120° in autoclave. 10 ml of sterilised 0.05 % water agar was poured into each pouch the top of which was sealed with adhesive tape. A 5 mm incision was made in the centre of the top of each pouch to enable the seedling to be placed in position, both the fungus inoculum and the nematode being introduced into the pouches through the incision.

In the first experiment *F. oxysporum* f. sp. *glycines* was introduced as a 10 ml suspension containing 3×10^6 conidia per ml and 2 000 sterilised second stage juveniles of *M. incognita* were inoculated into each pouch. Treatments were fungus plus nematode and nematode alone, replication being four-fold : samples were taken at 3, 5, 7, 10 and 30 days after inoculation.

On each sampling date the roots were washed free of agar, chopped up and boiled for 3 min in a solution containing equal parts by volume of glycerol, lactic acid and distilled water containing 0.5 % acid fuchsin (Bridge, Page & Jordan, 1982) : they were then cleared in a 50:50 solution of glycerol and distilled water. The stained roots were cut into smaller pieces and comminuted using a Silverson laboratory homogeniser at full speed for two periods of 15 sec, a treatment which releases most of the nematodes from roots without excessively damaging them. Macerated roots were placed in a measuring cylinder, the volume of the suspension being adjusted to the nematode density : five 2 ml aliquots were taken and the numbers of each nematode stage counted in an open counting dish, the various stages being identified according to the descriptions of Triantaphyllou and Hirschmann (1960).

In a second experiment seedlings were infected in the same way as in the first experiment but samples were taken 1, 2, 3 and 4 weeks after inoculation when roots were cut into 1 cm lengths, fixed in FAA for at least 48 h and dehydrated in a series of tertiary butyl alcohols

(TBA) followed by infiltration and wax embedding. Sections 10-14 µm in thickness, stained for 4-8 h in 1 % aqueous safranin, counterstained in 1 % fast green in 95 % ethanol, were cleared in clove oil and mounted in canada balsam (Moore, 1963).

Results

The number of nematodes invading the roots of both cultivars up to ten days after inoculation was slightly greater in the fungus-free plants (Tab. 1) but the overall numbers were not significantly different in each cultivar : development to J_3/J_4 was greater in fungus-free plants of each cultivar ten days after inoculation. It was noted that the total population in the roots declined after seven days indicating that nematodes may have left the roots due to the poor nutritional status of the plants in water agar.

Table 1

The number of different developmental stages of the root-knot nematode, *M. incognita* in roots of two soybean cultivars infected with *F. oxysporum* f. sp. *glycines*

Cultivars	*Treatments*	*Days after inoculation*	*Number of nematodes/root system*			
			J_2	*Developed J_2*	*J_3 & J_4*	*Total*
COLL	Fungus and nematode	3	590	—	—	590
		5	570	75	—	645
		7	620	166	50	836
		10	575	138	45	758
	Nematode alone	3	675	—	—	675
		5	633	120	—	753
		7	725	225	90	1 040
		10	675	190	115	980
WARE	Fungus and nematode	3	380	—	—	380
		5	354	20	—	374
		7	260	20	10	290
		10	200	27	10	237
	Nematode alone	3	360	—	—	360
		5	365	20	—	385
		7	420	44	37	501
		10	375	42	28	445

Thirteen days after inoculation (Tab. 2) there was a significant decrease in the number of females on both cultivars infected with both fungus and nematode : there were fewer males in the fungus-free plants.

In the second experiment fungal hyphae were not observed in the roots of either cultivar one week after inoculation but after two-weeks-hyphae were observed in xylem vessels. Three weeks after inoculation with both pathogens, giant cells, in both the phloem and the xylem, contained large quantities of mycelium and were devoid of cytoplasm : several cell nuclei had disappeared or were disintegrating as the hyphae progressed into the giant cells four weeks after inoculation. No fungal hyphae were observed in mature females, in egg masses or in the gelatinous matrix.

Discussion

Fattah and Webster (1983) have suggested that destruction of giant cells should lead to a suppression of the overall root-knot nematode population, now confirmed in the present experiments where nematode invasion was not altered by the presence of the fungus but clearly female development and severity of galling were : production of males was increased.

Table 2

The effect of *Fusarium oxysporum* f. sp. *glycines* on the number of males and females in the roots of two soybean cultivars

Cultivars	*Treatments*	*Number of adult nematodes*	
		Males	*Females*
COLL	Fungus and nematode	32	105
	Nematode alone	17	245
WARE	Fungus and nematode	22	72
	Nematode alone	5	160

The reduction in the number of females is probably due to the invasion of the giant cells by *Fusarium* but the production of toxins such as fusaric acid (Gaümann, 1957) may also play a part. The unfavourable conditions for development caused by the fungal invasion could cause a reduction in the size of the giant cells leading to the development of more " male " nematodes.

Nematode invasion prior to the inoculation of wilt fungus is said to predispose the plant to invasion by the fungus (Melandez & Powell, 1967; Fattah & Webster, 1983; Hillocks, 1985). All these authors introduced the nematode at least three weeks before the wilt pathogen but it has now been shown that similar effects can be produced by introduction of the fungus at the same time as the nematode. Mousa (1986) has also shown that, when the nematode is introduced at the same time as the fungus, the resistance mechanism to the wilt fungus is broken down and he also found a similar rate of spread of *F. oxysporum* f. sp. *glycines* in fungal resistant soybean roots as Fattah and Webster (1983) reported for *F. oxysporum* f. sp. *lycopersici* in resistant tomato roots when the nematode was introduced three weeks before the fungus. The rate at which the fungus developed in the roots was similar to the length of time, three weeks, reported by Fattah and Webster (1983) when the nematode was introduced three weeks before the fungus.

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