Supplement to the Journal of Nematology 27(4S):649–653. 1995.

Co-infection of Wilt-Resistant Chickpeas by *Fusarium oxysporum* f. sp. *ciceri* and *Meloidogyne javanica*

T. Uma Maheshwari,[1] S. B. Sharma,[1] D. D. R. Reddy,[2] and M. P. Haware[1]

Abstract: *Fusarium oxysporum* f.sp. *ciceri* and *Meloidogyne javanica* are important pathogens of chickpea. Interrelationships between *F. oxysporum* f.sp. *ciceri* and *M. javanica* were investigated on 53 Fusarium wilt-resistant chickpea genotypes in pot experiments. All of the genotypes were susceptible to *M. javanica*. Fusarium wilt resistance in one genotype (ICC 12275) was ineffective in the presence of *M. javanica*, and all the plants completely wilted. Resistance in four genotypes (ICCs 11319, 11322, 12254, 12272) was reduced in the presence of the nematode. Vascular discoloration above the collar region of the plants, an indication of susceptibility to the fungus, was observed. Wilt resistance in 48 genotypes was not modified by *M. javanica*. The effects of interactions between the pathogens on shoot and root weights, gall index, and galled area of root were significant only on 10–28% of the genotypes. Presence of the fungus reduced the adverse effects of nematodes on growth of 15% of the genotypes. Appraisal of wilt-resistant chickpea genotypes for their reactions to combinations of the two pathogens would help to identify and develop chickpea cultivars with wilt resistance stable in presence of *M. javanica*.

Key words: *Cicer arietinum*, interaction, *Fusarium oxysporum* f.sp. *ciceri*, *Meloidogyne javanica*, nematode, root-knot nematode, wilt resistance.

Chickpea (*Cicer arietinum*) is an important grain legume in the cropping systems of subsistence farmers in the Indian subcontinent, West Asia, and North Africa. Chickpea wilt, caused by *Fusarium oxysporum* f.sp. *ciceri*, is a serious soilborne disease (4). Sources of resistance to Fusarium wilt are available in the chickpea gene bank at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Andhra Pradesh, India. Cultivation of wilt-resistant cultivars is the most commonly recommended management option to protect chickpea yields. Root knot, caused by *Meloidogyne* spp., is a serious disease of chickpea and *M. javanica* is one of the most damaging root-knot nematode species in the warmer regions (7).

Meloidogyne javanica and *F. oxysporum* f.sp. *ciceri* occur together in many chickpea growing regions, and wilt-susceptible cultivars die earlier from wilt when co-infected with *M. javanica* (7). Two reports indicated that the *Meloidogyne* spp. break Fusarium wilt resistance in certain chickpea genotypes (10,11). The loss of Fusarium wilt resistance can have serious implications for management of chickpea wilt. Commercial chickpea cultivars lack resistance to *M. javanica* (6), and the reactions of wilt-resistant genotypes to *M. javanica* have not been reported. The purpose of this investigation was to study the interrelationships between *M. javanica* and *F. oxysporum* f.sp. *ciceri* on wilt-resistant chickpea genotypes.

Materials and Methods

Nematode inoculum: An isolate of *M. javanica* race 1, which does not attack groundnut or pepper (8), collected from the ICRISAT research farm was increased on tomato (*Lycopersicon esculentum*) cv. Rutgers in 25-cm-d pots containing an autoclaved mixture of sand, black cotton soil (44% sand, 16% silt, 40% clay), and farmyard manure (2:1:1, v/v). Second-stage juveniles (J2) were obtained from egg masses incubated at 28 C.

Fungus inoculum: Fusarium oxysporum f.sp. *ciceri* was isolated from roots of an infected chickpea plant. A single-spore isolate of the fungus was maintained on potato dextrose agar (PDA) and stored at 25

Received for publication 29 July 1994.

[1] Research Scholar, Scientist (Nematology), and Senior Scientist (Pathology), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India.

[2] Professor and Head, Department of Entomology, Andhra Pradesh Agricultural University, Rajendernagar, Hyderabad, Andhra Pradesh, India.

The authors thank Mr. Prabhakar, Statistics Unit, ICRISAT, India, for his help in data analysis.

C in an incubator. A sand-chickpea flour medium was prepared from chickpea flour (10 g) mixed with sand (90 g) in a 250-ml conical flask containing 20 ml distilled water. The flask was autoclaved for 1 hour, inoculated with the fungus from an actively growing culture on PDA, and incubated at 25 C for 15 days (4). Inoculum from one flask was mixed with 2 kg soil to fill a 15-cm-d pot. The number of *Fusarium* propagules, estimated from 20 mg soil spread on modified Czapek Dox medium (3), was between 1,250 and 1,500/g soil. Soil in pots was kept moist for 2 days and then infested with nematodes and sown with chickpea seed.

Assessment of effects of nematode and fungus: Seeds of 53 wilt-resistant chickpea genotypes, obtained from the Genetic Resources Unit of ICRISAT, were sown in 12.5-cm-d pots containing an autoclaved mixture of sand, black cotton soil (44% sand, 16% silt, 40% clay), and farmyard manure (1:2:1; v/v). A wilt-susceptible cultivar (JG 62) was included as a check. Four treatments for each genotype were used: 1) 2,500 *M. javanica* J2/pot (nematode-alone); 2) 50 g/pot fungus inoculum (fungus-alone); 3) 2,500 J2 + 50 g fungus inoculum (fungus + nematode); and 4) uninoculated control. Each treatment was replicated five times.

The treatments were arranged in randomized complete blocks. Observations on fresh and dry shoot weight, fresh root weight, number of galls, percentage galled area of the root, and gall size were recorded. Number of galls were rated on a 1-to-9 scale: 1 = 0 galls; 3 = 1–10 galls; 5 = 11–30 galls; 7 = 31–50 galls; and 9 = >50 galls. Percentage galled area of root was rated on a 1-to-9 scale based on visual assessment of root area covered by galls to the total root area as follows: 1 = 0–10% galled area; 3 = 11–20%; 5 = 21–30%; 7 = 31–50%; and 9 = >50% galled area (5). Gall size was visually rated as very small, small, medium, big, and very big (5). Number of egg masses was rated on the same scale developed for number of galls. Data collection on Fusarium wilt reaction included days taken for leaf drooping, leaf chlorosis, and vascular discoloration. Genotypes with vascular discoloration above the collar region were considered wilt-susceptible. Fusarium infection in any genotype was confirmed in repeat tests. To verify infection by Fusarium, roots were washed free of soil, and the regions below and above the collar region were split open and examined for internal discoloration. Transverse sections of the root portion showing vascular discoloration were surface-sterilized with 2.5% aqueous solution of NaOCl for 3 minutes and placed on PDA. Three days later, the tissues were observed for the presence of *F. oxysporum* f.sp. *ciceri.*

The data were subjected to analysis of variance. Means were separated by LSD values calculated when *F*-tests indicated significant ($P = 0.05$) treatment effects.

Results

Wilt incidence: None of the wilt-resistant chickpea genotypes in the fungus-infested soil showed symptoms. Forty-eight genotypes in the fungus + nematode-infested soil showed no wilt symptoms. All the plants of the wilt-susceptible check cultivar JG 62 completely wilted. The presence of *M. javanica* had a distinct effect on wilt reaction of five genotypes (ICCs 11319, 11322, 12254, 12272, and 12275). Plants of these genotypes had 10–20% chlorotic leaves in the fungus-alone treatment, but no vascular discoloration above the collar region was observed. Concomitant infection by *M. javanica* and *F. oxysporum* f.sp. *ciceri* increased the chlorosis of leaves from 10% to 34% in ICC 11319, 15% to 20% in ICC 11322, 10% to 40% in ICC 12254, 10% to 25% in ICC 12272, and 20% to 100% in ICC 12275 compared with the effect of the fungus alone. Vascular discoloration above the collar region was found in 13% of the plants of ICC 11319, 40% of ICC 11322, 60% of ICC 12254, 13% of ICC 12272, and 100% of ICC 12275. The fungus + nematode treatment also resulted in 10–50% chlorotic leaves on five

genotypes (ICCs 11315, 11317, 12270, 12429, and 12430) compared with the uninoculated control, but no vascular discoloration was observed.

Plant growth: Shoot weights of 23 genotypes were reduced, shoot weight of one genotype was increased and shoot weights of 28 genotypes were unaffected by nematode infection (P = 0.05, Table 1). The nematode reduced the root weights of 12 genotypes and increased the root weights of 17 genotypes. Shoot weights of ICCs 11313, 11315, 11317, 12236, 12244, 12247, 12248, 12270, 12429, 12466, 12468, and ICCC 4 were not affected by nematode infection despite severe galling of the roots.

Shoot weights of ICCs 12234, 12235, 12251, and 12275, and root weights of ICCs 12253, 12254, and 12270 increased in the fungus-infested soil (P = 0.05). Root weights of ICCs 12236, 12249, 12468, Radhey, BDN-9-3, Jyothi, and ICCC 4, and shoot weights of ICCs 11320, 12253, and 12254 significantly declined. Shoot weights of 45 and root weights of 42 genotypes were not influenced by the fungus.

The presence of both pathogens caused greater reductions in shoot weight than

TABLE 1. Effects of *Meloidogyne javanica* and *Fusarium oxysporum* f.sp. *ciceri* alone and together on shoot weight of chickpea genotypes.

Genotype ICC no.[a]	Uninoculated control	Fungus alone (F)	Nematode alone (N)	Nematode and fungus (N + F)	LSD (P = 0.05)		
					F	N	F × N
			Dry shoot weight (g)				
11316	4.0	4.0	3.5	3.6	NS	0.21	NS
11318	3.8	3.5	2.8	3.2	NS	0.23	NS
11319	3.9	3.8	2.2	3.2	NS	0.46	NS
11320	3.5	1.7	3.2	1.5	0.58	NS	NS
12234	4.1	4.5	3.3	3.8	0.33	0.33	NS
12235	3.9	4.4	3.6	3.9	0.39	NS	NS
12237	4.2	4.6	3.5	4.1	0.35	0.35	NS
12240	2.8	2.7	3.6	3.2	NS	0.42	NS
12242	3.9	3.8	3.3	3.5	NS	0.32	NS
12246	3.8	3.6	3.2	3.2	NS	0.32	NS
12249	3.3	3.7	1.6	2.6	NS	0.49	NS
12250	3.3	3.8	2.5	2.8	NS	0.51	NS
12251	3.3	4.6	2.1	2.9	0.45	0.45	NS
12252	3.7	3.9	1.8	2.8	0.40	0.40	0.57
12253	4.5	4.0	3.3	2.7	0.49	0.49	NS
12254	4.9	3.9	4.1	2.2	0.42	NS	0.42
12255	3.7	3.6	3.3	2.4	NS	0.56	NS
12256	4.3	4.3	3.4	3.2	NS	0.68	NS
12258	3.2	3.4	2.5	2.8	NS	0.44	NS
12259	3.6	3.9	2.8	2.8	NS	0.58	NS
12267	3.9	3.5	2.4	3.1	NS	0.48	NS
12275	2.9	4.1	2.6	2.2	0.32	NS	0.45
12430	3.3	3.6	1.7	2.6	NS	0.58	NS
12432	3.3	3.3	1.4	1.8	NS	0.39	NS
12433	2.9	3.7	1.7	2.1	NS	0.65	NS
12434	5.0	5.0	4.5	4.3	NS	0.43	NS
12464	5.6	6.4	5.1	5.2	NS	NS	0.68
12466	5.5	3.3	5.0	4.9	NS	0.54	NS
12468	8.9	4.6	9.7	4.8	1.37	NS	NS
Annigiri	5.2	5.5	4.6	4.3	NS	0.31	NS
BDN-9-3	5.9	6.1	4.9	5.5	NS	0.58	NS

Data are average of 20 plants per treatment. Shoot weights of ICCs 11311, 11313, 11315, 11317, 11322, 11324, 12236, 12239, 12241, 12243, 12244, 12245, 12247, 12248, 12270, 12272, 12429, 12465, 12467, 12468, Radhey, Jyothi, and ICCC 4, and root weight of ICCs 11313, 11316, 11320, 11322, 12237, 12256, 12429, 12433, 12434, 12464, and 12467 were not affected by any treatment. NS = Not significant at P = 0.05.

[a] International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) chickpea germplasm accession number.

did the fungus alone. The effects of both pathogens, compared with either alone, on shoot weight were significant only on four genotypes (ICCs 12252, 12254, 12275, and 12464) (Table 1); the effect differed with the genotype. On ICC 12252, the nematode reduced the shoot weight by 49% compared with uninoculated control; the fungus tended to increase the shoot weight, whereas co-infection reduced the shoot weight (P = 0.05). On 12254, the fungus reduced the shoot weight, the nematode did not, and co-infection markedly reduced shoot weight. The adverse effect of co-infection was greater than that of either of the pathogens alone, or sum of the reductions caused by the individual pathogens. Shoot weight of ICC 12275 was significantly increased by fungal infection and decreased by the co-infection. The nematode effect was not significant. On ICC 12464, co-infection had an adverse effect on shoot weight. Shoot weights of 23 genotypes were unaffected by infection with either or both pathogens.

Co-infection significantly affected root weights of 15 genotypes. The effects differed with genotype. For example, only the interaction and not the individual pathogens affected eight genotypes (ICCs 11311, 11315, 11317, 11318, 12240, 12255, 12272, and 12432). The root weights of ICC 12239 and ICC 12252 were increased by nematode infection and unaffected by the fungus; however, the adverse effect of nematode on these genotypes was diminished in presence of the fungus. Individual pathogens and their interactions were significant (P = 0.05) on root weights of only five of the tested genotypes (ICC 12270, ICC 12430, Radhey, Jyothi, and Annigiri).

Root-knot disease: All the chickpea genotypes except ICC 11311 had gall indices between 6 and 9. Ratings of percentage galled area of the root ranged between 1 and 9 (Table 2). The fungus significantly influenced the gall number on eight genotypes, and on all these genotypes except one (ICC 12253) it had an inhibitory effect. *Meloidogyne javanica* produced egg masses on all 53 chickpea genotypes, and the number of egg masses was similar in nematode-alone and nematode + fungus-infested soils (data not included). The fungus significantly reduced the galled area of root of three (ICCs 11317, 12245, and 12464) and increased it on three (ICCs 11311, 12253, and 12466) genotypes. Seven genotypes had small galls, 33 had

TABLE 2. Effect of *Meloidogyne javanica* alone and in combination with *Fusarium oxysporum* f.sp. *ciceri* on gall index and percentage galled area of root on chickpea genotypes.

Genotype ICC no.[c]	Gall index[a]			% Galled area[b]		
	Nematode	Nematode and fungus	LSD (P = 0.05)	Nematode	Nematode and fungus	LSD (P = 0.05)
11311	4.4	5.1	NS	1.2	2.7	0.9
11317	9.0	7.8	NS	9.0	6.4	0.3
11324	8.0	6.6	1.3	9.0	8.2	NS
12245	8.9	8.5	NS	7.7	6.2	1.0
12253	8.0	8.9	0.8	7.8	9.0	0.7
12464	8.0	7.2	0.2	7.6	7.0	0.4
12466	8.8	8.9	NS	8.4	8.9	0.4
12468	8.6	7.2	0.7	8.5	7.8	NS
Radhey	8.7	7.4	0.7	9.0	9.0	NS
Annigiri	9.0	7.5	0.6	8.0	7.2	NS
BDN-9-3	8.8	7.5	0.7	8.2	8.6	NS
ICCC 4	9.0	7.8	0.7	8.8	8.4	NS

Data, recorded 8 weeks after inoculation, are means of 20 plants. Gall indices and percentage galled area of root of other 41 chickpea genotypes were not influenced by the presence of *F. oxysporum* f.sp. *ciceri*.

[a] Gall index: 1 = 0 galls, 3 = 1–10 galls, 5 = 11–30 galls, 7 = 31–50 galls, 9 = >50 galls.

[b] Percentage galled area of root: 1 = 0–10% galled area, 3 = 11–20%, 5 = 21–30%, 7 = 31–50%, 9 = >50% galled area.

[c] International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) chickpea germplasm accession number.

medium, and 11 had big to very big galls. Size of galls was unaffected by the fungus.

Discussion

Nonuniformity of host response to co-infection by the two pathogens was perceived as a reflection of genotype-specific interactions in the *M. javanica–F. oxysporum* f.sp. *ciceri–C. arietinum* system. *Meloidogyne javanica* increased the susceptibility of some, but not of all, of the Fusarium wilt-resistant chickpea genotypes. Breakdown of the resistance mechanism in ICC 12275 is evidence of conditioning of gene expression for resistance to Fusarium wilt by the presence of *M. javanica* genes for parasitism on ICC 12275. Sidhu and Webster (9) reported that in tomato infected with *M. incognita,* the gene for resistance to Fusarium became ineffective, and expression of the gene for resistance to Fusarium was modified by the presence or absence of genes for resistance to the root-knot nematode. The moderate regulatory effect of the nematode population on wilt resistance in ICCs 11319, 11322, 12254, and 12272, and lack of any perceptible effect on resistance in other genotypes, is indicative of quantitative as well as qualitative differences in genes that confer wilt resistance in chickpea. ICCs 11319, 11322, 12254, and 12272 may show wilt after 90 days or near physiological maturity; we studied the reaction of the genotypes 60 days after sowing. Bergeson (1) found that the ability of *M. incognita* to break Fusarium wilt resistance varied among muskmelon cultivars, and resistance was most easily broken in a cultivar with incomplete resistance to the fungus. Similar cultivar-specific interactions between root-knot nematodes and Fusarium on summer squash were reported (2).

We found that shoot weights of 23 genotypes were not adversely affected by individual or co-infection by the pathogens, and this evidence of tolerance to *M. javanica* should be examined further in multi-location trials in areas naturally infested with both pathogens.

Though wilt resistance in more than 90% of the tested genotypes was not affected by the presence of *M. javanica,* evaluation of the reaction of wilt-resistant chickpea genotypes to blends of populations of the two pathogens would help in identifying promising genotypes and in eliminating genotypes with unstable wilt resistance in presence of the nematode.

Literature Cited

1. Bergeson, G. B. 1975. The effect of *Meloidogyne incognita* on the resistance of muskmelon varieties to Fusarium wilt. Plant Disease Reporter 59:410–413.

2. Caperton, M., R. D. Martin, and J. L. Starr. 1986. Effects of fusarium inoculum density and root-knot nematodes on wilt resistance in summer squash. Plant Disease 70:207–209.

3. Haware, M. P., Y. L. Nene, and R. Rajeshwari. 1978. Eradication of *Fusarium oxysporum* f.sp. *ciceri* transmitted in chickpea seed. Phytopathology 68: 1364–1367.

4. Nene, Y. L., M. P. Haware, and M. V. Reddy. 1981. Chickpea disease. Resistance-screening techniques. ICRISAT Information Bulletin No. 10:10.

5. Sharma, S. B., P. Remanandan, and D. McDonald. 1993. Resistance to *Meloidogyne javanica* and *Rotylenchulus reniformis* in wild relatives of pigeonpea. Supplement to the Journal of Nematology 25:824–829.

6. Sharma, S. B., O. Singh, R. P. S. Pundir, and D. McDonald. 1993. Screening of wild *Cicer* spp and chickpea lines for resistance to *Meloidogyne javanica.* Nematologia Mediterranea 21:165–167.

7. Sharma, S. B., D. H. Smith, and D. McDonald. 1992. Nematode constraints of chickpea and pigeonpea production in the semi-arid tropics. Plant Disease 76:868–874.

8. Sharma, S. B., D. H. Smith, and D. McDonald. 1995. Host races of *Meloidogyne javanica* with preliminary evidence that the "groundnut race" is widely distributed in India. International Arachis Newsletter 15: in press.

9. Sidhu, G., and J. W. Webster. 1977. Genetics of resistance in tomato to root-knot nematode–wilt fungus complex. Journal of Heredity 65:153–156.

10. Thakar, N. A., H. R. Patel, B. K. Patel, and C. C. Patel. 1986. Breaking of resistance to *Fusarium* wilt in resistant chickpea by root-knot nematode. Madras Agricultural Journal 73:410–411.

11. Upadhyay, K. D., and K. Dwivedi. 1987. Root-knot nematode *Meloidogyne javanica* breaks wilt resistance in chickpea variety Avrodhi. Current Science 56:915–916.