Infection by *Meloidogyne artiellia* Does Not Break Down Resistance to Races 0, 1A, and 2 of *Fusarium oxysporum* f. sp. *ciceris* in Chickpea Genotypes

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ABSTRACT

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Fusarium oxysporum f. sp. *ciceris*, and the root-knot nematode *Meloidogyne artiellia*, coinfect chickpea crops in several countries of the Mediterranean Basin. The influence of root infection by *M. artiellia* on the reactions of chickpea genotypes with different reaction to infection with *F. oxysporum* f. sp. *ciceris* races 0, 1A, and 2 was investigated under controlled environmental conditions. Results demonstrated that coinfection of chickpea genotypes resistant to specific fungal races by *M. artiellia* did not influence the Fusarium wilt reaction of the plant, irrespective of the *F. oxysporum* f. sp. *ciceris* race assayed. However, in some of the assayed combinations, coinfection by both pathogens significantly affected the level of colonization by the fungus or reproduction of the nematode in the root system. Thus, coinfection of chickpea plants with *Foc*-0 and *M. artiellia* significantly decreased the level of colonization of the root system by *F. oxysporum* f. sp. *ciceris* in genotypes 'CA 336.14.3.0' and 'PV 61', but not in 'ICC 14216 K' and 'UC 27'. Similarly, the nematode reproduction index was also significantly reduced by coinfection with *Foc*-0 in the four chickpea genotypes tested and inoculated with this race. Conversely, coinfection of chickpea plants with *Foc*-1A and *M. artiellia* significantly increased colonization of the root system by the fungus in all genotypes inoculated with this race, except for line BG 212. Altogether, we confirmed the complete resistance phenotype of 'UC 27' and 'ICC 14216 K' to *Foc*-0, and of 'ICC 14216 K' to *Foc*-1A and *Foc*-2, and demonstrated that this resistance was not modified by coinfection of the resistant plant with *M. artiellia*.

Additional keywords: *Cicer arietinum*, fungus–nematode interaction, race-specific resistance.

The use of resistant cultivars is widely recognized as the most practical and cost-efficient management strategy for most soilborne plant diseases, including Fusarium wilts caused by *formae speciales* of the species complex *Fusarium oxysporum*. However, the efficacy of that strategy can be curtailed by the occurrence of pathogenic variability in the pathogen population as well as by coinfection of a plant by more than one single pathogen (2,16,31).

Fusarium wilt of chickpea, caused by *F. oxysporu*m Schlechtend. emend W. C. Snyder. & H. N. Hans. f. sp. *ciceris* (Padwick) W. C. Snyder & H. N. Hans., is the most important soilborne disease limiting chickpea production worldwide, but mainly in the Mediterranean Basin and the Indian Subcontinent (23,55). Wide pathogenic variation exists in *F. oxysporum* f. sp. *ciceris*, which includes two symptom types (pathotypes) and eight pathogenic races (races 0, 1A, 1B/C, 2, 3, 4, 5, and 6). The two pathotypes are identified based on the distinct yellowing or wilting syndromes that they cause in susceptible chickpeas (55). The yellowing pathotype induces progressive foliar yellowing with vascular discoloration, whereas the wilting one induces severe and fast chlorosis, flaccidity, and vascular discoloration (55). The eight pathogenic races can be identified by the disease reactions on a set of differential chickpea cultivars (20,25). Races 0 and 1B/C belong to the yellowing pathotype but all other races are of the wilting one (29). The eight races have also a distinct geographic distribution: races 2, 3, and 4 have only been reported in India so far (20), whereas races 0, 1B/C, 5, and 6 are found mainly in the Mediterranean Basin and California (17,25,29). Unlike the other races, race 1A is more widespread and has been reported in India, California, and the Mediterranean Basin (20,29).

Several race-specific sources of resistance to Fusarium wilt have been identified (48) and exploited in several chickpea breeding programs (25,45,48) to develop a fair number of resistant chickpea germplasm lines that are operative against specific pathogen races. Although pathogenic races are well established in *F. oxysporum* f. sp. *ciceris*, the genetics of resistance to individual races has not been fully clarified yet. Whereas resistance against most of the pathogen races is either monogenic or oligogenic and of complete phenotype, the genetics of resistance to races 1B/C and 6 is yet to be determined (48,49,50,54). In addition to that, a late wilting phenotype characterized by a delay in the onset of disease symptoms in partially resistant genotypes was found to be a monogenic trait and controlled by three independent genes (49), each of which delays that onset (51,52,59). Furthermore, a combination of any of the two late-wilting genes is required for complete resistance to race 1 (51,52,58).

Coinfection of the host plant by races of *F. oxysporum* f. sp. and root-knot nematodes may risk valuable race-specific resistance to the interacting fungus and increase disease severity in susceptible cultivars (7,10,16,19,38,47). In chickpea, several studies have shown that interactions between *F. oxysporum* f. sp. *ciceris* and *Meloidogyne* spp. can lead to breakdown of resistance against certain races of the Fusarium wilt pathogen (7,57,58). In the Mediterranean Basin, the cereal and legume root-knot nematode, *Meloidogyne artiellia*, is the root-knot nematode species prevalent in chickpea within *Meloidogyne* spp. (5,7,13). *M. artiellia* popu-

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lations in field soils may reach high levels of infestations ranging from 0.05 to 1.8 second-stage juveniles (J2s) and eggs per cubic centimeter (5,15) and severe attacks by this root-knot nematode have been reported in that region and North Africa (7,12,13). Consequently, attention should be paid to the possibility that breakdown of resistance might occur as a result of coinfection of plants by *F. oxysporum* f. sp. *ciceris* and *M. artiellia* which would compromise the use of valuable Fusarium wilt-resistant germplasm.

Research in our laboratories have developed chickpea lines with either complete or partial resistance to *F. oxysporum* f. sp. *ciceris* race 5 (42), which is the most virulent race of the wilting pathogen present in the Mediterranean Basin and California (25,27). Also, our studies demonstrated that infection of chickpea genotypes partially resistant to *F. oxysporum* f. sp. *ciceris* race 5 with *M. artiellia* significantly increased severity of Fusarium wilt irrespective of inoculum density of the fungal pathogen. On the contrary, when chickpea genotypes harboring complete resistance to *F. oxysporum* f. sp. *ciceris* race 5 were infected by this race and *M. artiellia*, this resistance was overcome in some genotypes but not in others, irrespective of inoculum density of *F. oxysporum* f. sp. *ciceris* race 5 (7). Whether such an apparent chickpea genotype-specific resistance breakdown by *M. artiellia* infection occurs for other pathogenic races of *F. oxysporum* f. sp. *ciceris* was not known. Therefore, the objective of this research was to determine the effects of coinfections by *M. artiellia* and *F. oxysporum* f. sp. *ciceris* races 0, 1A, and 2 on development of Fusarium wilt on chickpea lines and cultivars with complete or partial resistance to these races, including effects on final nematode reproduction and root colonization by the fungus.

MATERIALS AND METHODS

Inocula of pathogens. Monoconidial isolates Foc 7802, Foc 7989, and Foc 8605 of *F. oxysporum* f. sp. *ciceris* race 0 (*Foc*-0), 1A (*Foc*-1A), and race 2 (*Foc*-2), respectively, were used in this study. These isolates were collected from infected chickpeas in Cañete de las Torres, Córdoba province, southern Spain, (Foc 7802), and Hyderabad (Foc 7989) and Kanpur (Foc 8605), India, and they were characterized to race in previous studies (26,29,36). *F. oxysporum* f. sp. *ciceris* isolates were stored in sterile soil at 4°C until use. Active cultures were obtained by placing small aliquots of the stored material onto a plate of fresh potato-dextrose agar (PDA) (250 g of unpeeled potatoes, 20 g of agar, and 20 g of glucose per liter of distilled water) and incubating for 7 days at 25°C with a 12-h photoperiod of fluorescent and near-UV light at 36 μE m^{-2} s^{-1}. Fungal inoculum for experiments consisted of chlamydospores produced in a soil mixture. For that, each fungal isolate was increased in cornmeal-sand (CMS) and incubated as described above for 2 weeks (36,56). Then, the infested CMS was thoroughly mixed (1:12, wt/wt) with an autoclaved (121°C, 1 h, twice) soil mixture (sand/loam 1:1, vol/vol), distributed in 1,000-ml Erlenmeyer flasks (400 g each) and incubated at 25 ± 1°C in darkness for 6 weeks. Production of chlamydospores by the fungus in the infested soil was confirmed by microscopic observations. Soil with chlamydospores of *Foc*-0, *Foc*-1A, and *Foc*-2 isolates were then stored until use, for a maximum period of 4 weeks at 4°C. The number of chlamydospores of *F. oxysporum* f. sp. *ciceris* isolates per gram of infested soil mixture was determined by dilution-plating on V8 juice-oxgall-PCNB agar (VOPA), a *Fusarium*-selective medium (36,40), just before sowing. The infested soil was thoroughly mixed with an autoclaved (121°C, 1 h, twice) soil mixture (sand/clay loam, 2:1, vol/vol) in the appropriate proportion to obtain the desired inoculum density for experiments.

The *M. artiellia* isolate used in this study was collected from infected chickpea roots in a field at Castel del Monte, Bari province, southern Italy, and maintained in chickpea cultivar UC 15. For that, a single *M. artiellia* egg-mass was removed from a gall containing a single nematode female in an infected chickpea root. The egg mass was surface-disinfested with 1% NaOCl for 4 min, washed four times in sterile distilled water, and then placed onto roots of a 2 to 3-week-old 'UC 15' chickpea plant grown in the autoclaved soil mixture. The inoculated plants were incubated at 21 ± 1°C, 60 to 90% relative humidity, and a 14-h photoperiod of fluorescent light at 360 ± 25 μ$Em^{-2}s^{-1}$ for 3 to 4 months. This procedure was repeated several times to increase the nematode population. Nematode inocula for experiments consisted of eggs that were increased using the same procedures and conditions as above. Eggs were extracted according to the NaOCl procedure (21). Briefly, *M. artiellia*-infected chickpea roots were thoroughly washed in tap water, cut into 1- to 2-cm-long segments and agitated in 1% NaOCl for 3 min. Then, the suspension was screened consecutively through 75- and 5-μm-pore sieves. Eggs retained on the 5-μm-pore sieve were washed several times and resuspended in sterile distilled water. The number of eggs in the suspension was determined from 1-ml aliquots of a serial dilution of the suspension and the nematode inoculum density was adjusted with sterile distilled water.

Growth chamber experiments. Four experiments (I to IV) were conducted in a growth chamber adjusted to 25 ± 1°C, 60 to 90% relative humidity, and a 14-h photoperiod of fluorescent light at 360 ± 25 μE m^{-2} s^{-1}. These environmental conditions are optimal for development of Fusarium wilt in chickpea (36,41) and favorable for reproduction of *M. artiellia* in chickpea roots (11). Chickpea seeds were surface-disinfested with 2% NaOCl for 3 min and germinated on sterile, moistened filter paper at 25 ± 1°C in darkness for 48 to 72 h. Germinated seeds, selected for uniformity (length of radicle = 1 to 2 cm), were sown into 15-cm-diameter clay pots (1 seed per pot) filled with 0.5 liter of the autoclaved soil mixture infested or noninfested with the pathogens. Plants in pots were watered as needed and fertilized with 100 ml of a 0.1%, 20-5-32+micronutrients hydro-sol fertilizer (Haifa Chemicals Ltd., Haifa, Israel) solution every week.

In experiment I, we studied the development of Fusarium wilt on chickpea lines CA 336.14.3.0 and ICC 14216 K, and cultivars. PV 61 and UC 27, which showed complete resistance to *Foc*-0 (Table 1). Plants were inoculated with a high inoculum density of 20,000 chlamydospores per gram of soil of *Foc*-0 because of the low virulence of this race (40,41), either singly or jointly with inoculum of *M. artiellia*, as well as with *M. artiellia* alone. Germinated seeds were sown in the autoclaved soil infested with 0 or 20,000 chlamydospores of *Foc*-0 per gram of soil, and with 0 or 20 eggs + J2s per cubic centimeter of soil. The four treatments in the experiment were as follows: (i) noninfested soil; (ii) soil infested with *Foc*-0; (iii) soil infested with *M. artiellia*; and (iv) soil infested with *M. artiellia* and *Foc*-0. The nematode inoculum in 10 ml of sterile distilled water was added onto a germinated seed at sowing. The same amount of sterile distilled water was added in treatments without the nematode.

In experiment II, inoculations performed as described for experiment I were carried out to determine the reaction of *Foc*-1A-resistant chickpea lines BG 212, CA 334.20.4, WR 315, CA 336.14.3.0, and ICC 14216 K (Table 1) to inoculation with this race and *M. artiellia*. Plants were inoculated with a moderate inoculum density of *Foc*-1A of 3,000 chlamydospores per gram of soil either singly, jointly with 20 eggs + J2s per cubic centimeter of soil of *M. artiellia*, or only with the nematode inoculum. Treatment combinations in this experiment were the same as those for experiment I, except that an isolate of *Foc*-1A was used.

In experiment III we studied the effects of infection by *M. artiellia* on Fusarium wilt development in *Foc*-2-susceptible chickpea line BG 212, *Foc*-2-resistant line ICC 14216 K, and cv. UC 27 in which the reaction to *Foc*-2 was unknown (Table 1). Plants were inoculated with 3,000 chlamydospores of *Foc*-2 per gram of soil, 20 eggs + J2s per cubic centimeter of soil, or jointly with the inoculum of the two pathogens. Treatment combinations in this

experiment were the same as for experiment I, except that fungal isolate consisted of *Foc*-2. Inoculations were conducted as described for experiment I.

Finally, experiment IV was conducted to confirm results obtained on cv. UC 27 and lines CA 334.20.4 and ICC 14216 K in experiments I through III, as well as to determine the effect of infection by *M. artiellia* on the reaction of the Fusarium wilt universally susceptible 'P 2245' chickpea to infection by *Foc*-0, *Foc*-1A, and *Foc*-2. Inocula consisted of 20,000 chlamydospores of *Foc*-0 per gram of soil, 3,000 chlamydospores per gram of soil of *Foc*-1A or *Foc*-2, and 20 eggs + J2s per cubic centimeter of soil of *M. artiellia*. Inoculation and treatment combinations were as indicated for experiments I through III.

For all the four experiments, treatments were arranged in a completely randomized design with 10 replicates per treatment, each consisting of a single potted plant. The experiments lasted 70 to 72 days after inoculation.

Disease assessment and data analysis. The incidence and severity of Fusarium wilt were assessed at 2- to 3-day intervals up to the end of the experiments. The severity of symptoms on individual plants was rated on a 0 to 4 scale according to the percentage of foliage with yellowing or necrosis (0 = 0%, 1 = 1 to 33%, 2 = 34 to 66%, 3 = 67 to 100%, and 4 = dead plant) (30). The incidence of foliar symptoms (*I,* 0, or 1) and severity data (*S*, rated from 0 to 4) were used to calculate a disease intensity index (DII) (6,7,36,40,41) with the equation: DII= $(I \times S)/4$. Disease progress curves were obtained from the accumulated DII over time in days from the date of sowing. Fusarium wilt development was characterized by three variables associated with disease progress curve: (i) IP = the incubation period for disease development, established as the time in days taken for DII > 0; (ii) the final disease intensity (DII observed at the final date of disease assessment); (iii) the standardized area under the DII progress curve (SAUDPC) calculated by trapezoidal integration standardized for the duration of disease development in days (37).

Upon termination of the experiments, isolations were made from the second, fifth, and eighth stem internodes of symptomless plants to determine the occurrence of vascular infections by *F. oxysporum* f. sp. *ciceris* races. Chickpea stem sections (1 cm long) were surface-disinfested in 0.4% NaOCl for 1 min, blotted dry on sterile filter paper, plated onto VOPA, and incubated at the same conditions described previously for the assessment of the fungal inoculum density in infested substrates. Also, at the end of the experiment, nematode population densities in both soil and chickpea roots and fungal population density in roots were assessed for each replicate. Nematodes were extracted from 100-cm^3 soil samples by the centrifugal-flotation method (8). Briefly, soil was washed through a 710-µm sieve, the filtered water collected in a beaker, mixed thoroughly with 4% kaolin by volume, and the mixture was centrifuged at 1,500 × *g* for 4 min. Pellets were resuspended in 250 ml of $MgSO_4$ (d = 1.16) and the suspension was centrifuged at 1,500 × *g* for 3 min. Supernatants were sieved through a 5-µm sieve, and the nematodes collected on the sieves were washed with tap water, transferred to petri dishes, and counted under a stereomicroscope (8). For nematode and fungal population density in roots, the complete root system of each plant was washed free of soil and cut into 1 to 2 cm segments. Nematodes were extracted from a 5-g sample by the maceration-centrifugation method. Root tissues in 250 ml of a 1% NaOCl solution in water were homogenized with a Waring blender at 6,300 rpm for 1 min and homogenates were centrifuged and extracted as described above. Soil and root population densities were used to determine the nematode reproduction index (Rf = final population/initial population). The severity of nematode galling in roots was not assessed because infection by *M. artiellia* did not cause visible symptoms on chickpea roots (7). To estimate the root population density of *Foc*-0, *Foc*-1A, and *Foc*-2, root tissues were homogenized as described above for nematode extraction except for the absence of NaOCl in the water suspension. The number of colony forming units (CFU) per gram of fresh root tissue was then determined on a 1-g sample by dilution-plating on VOPA and incubating at the same conditions as described for fungal isolates for 5 to 7 days (44).

Data from uninoculated control treatments as well as from treatments including Fusarium wilt-resistant lines and cultivars that showed no disease symptoms were not included in analyses of Fusarium wilt severity. Similarly, treatments without *M. artiellia* were not included for analysis of nematode reproduction, to avoid use of treatments with all zero values in analysis of variance (ANOVA) analyses. Fungal and nematode population densities were transformed by $\log_{10}(X + 1)$. All values presented in Tables and Figures are of untransformed data.

The effects of chickpea cultivar and inoculation treatment on the IP, DII, SAUDPC, root colonization by *F. oxysporum* f. sp. *ciceris*, and nematode Rf, were determined by standard ANOVA using the general linear model procedure of SAS (Statistical Analysis System, SAS Institute, Cary, NC). Means were compared using Fisher's least significant difference test at $P < 0.05$. When significant interactions between main effects occurred, orthogonal single-degree of freedom contrasts were computed to test the effect of selected experimental treatment combinations. For experiment IV, which involved different *F. oxysporum* f. sp. *ciceris* races × chickpea genotypes × inoculation treatment combination, we performed separate ANOVA analysis for each fungal race because of the occurrence of significant cultivar × treatment interactions.

RESULTS

Stability of Fusarium wilt resistance in chickpea genotypes. No symptoms developed in either the control plants uninfested with *F. oxysporum* f. sp. *ciceris* races or in chickpea genotypes

TABLE 1. Disease reaction of selected chickpea genotypes to pathogenic races 0, 1A, 2, and 5 of *Fusarium oxysporum* f. sp. *ciceris*[a]

			Foc-race			
Chickpea genotype	Source	Germplasm	0	1A	2	5
12–071/10054	Iran	Desi	S	M	R	R
BG 212	ICRISAT, India	Desi	R	R	S	S
CA 334.20.4	Spain	Kabuli	R	S	–[b]	R
CA 336.14.3.0	Spain	Kabuli	R	R	–	R
ICC 14216 K	Mexico	Kabuli	R	R	R	R
P 2245	Spain	Kabuli	S	S	S	S
PV 61	Spain	Kabuli	R	S	–	S
UC 27	California	Kabuli	R	R	–	–
WR 315	ICRISAT, India	Desi	R	R	R	R

[a] Disease was assessed on a 0 to 4 severity scale depending on the percentage of affected foliar tissue (0 = 0%, 1 = 1 to 33%, 2 = 24 to 66%, 3 = 67 to 100%, and 4 = dead plant) at 40 days after sowing in infested soil (21,24,31). Average disease reactions of <1 and >3 were considered resistant (R) and susceptible (S), respectively. Intermediate disease reactions were considered moderate susceptible (M) (17,20,27).

[b] Disease reaction unknown.

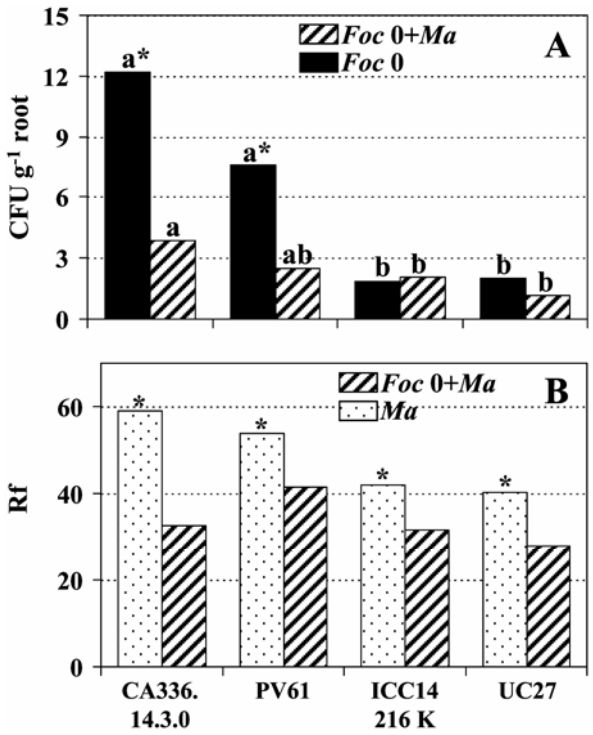

Fig. 1. Reaction of chickpea genotypes simultaneously inoculated with 20,000 chlamydospores per gram of soil of *Fusarium oxysporum* f. sp. *ciceris* race 0 and 20 eggs + second-stage juveniles per cubic centimeter of soil of *Meloidogyne artiellia*. **A,** CFU = colony forming units of *F. oxysporum* f. sp. *ciceris* per gram of fresh root tissue. **B,** Rf = nematode reproduction rate. Each bar is the mean of 14 replicates, each replicate consisting of a single potted plant. For each inoculation treatment, bars with the same letter are not different according to Fisher's least significant difference test ($P < 0.05$). For each chickpea genotype, bars with an asterisk indicate that the single infection treatment is significantly different ($P < 0.05$) from the coinfection treatment.

having complete resistance to them, in the absence of *M. artiellia* inoculum regardless the races of *F. oxysporum* f. sp. *ciceris* used in experiments I through IV (Figs. 1 to 7). An exception to this was that line BG 212 showed Fusarium wilt symptoms of moderate severity in plants infected with *Foc*-1A (Figs. 2 and 3). Also, no vascular infection of the stem by the fungus was detected by isolations from tissues of symptomless plants, irrespective of chickpea genotype and race of *F. oxysporum* f. sp. *ciceris*. Symptoms that developed in compatible plant-fungus interactions were characteristic of those corresponding to the pathotype of each isolate, i.e., yellowing symptoms for isolate Foc 7802 (*Foc*-0), and wilting symptoms for isolates Foc 7989 (*Foc*-1A) and Foc 8605 (*Foc*-2).

In experiment I, neither symptoms nor stem vascular infections developed in plants of lines CA 336.14.3.0 and ICC 14216 K, and cultivars PV 61 and UC 27 grown in soil infested with *Foc*-0, regardless the presence or absence of *M. artiellia*. Nevertheless, coinfection of chickpea plants by *Foc*-0 and *M. artiellia* significantly ($F = 10.44$; $P = 0.002$) decreased the colonization of the root system by the fungus estimated by the number of CFU per gram of fresh root tissue (Table 2; Fig. 1A). Such an effect occurred, however, in genotypes 'CA 336.14.3.0' and 'PV 61', but not in 'ICC 14216 K' and 'UC 27' (Fig. 1A), which gave rise to a significant interaction ($F = 4.43$; $P = 0.006$; Table 2) between chickpea genotypes and inoculation treatments. Colonization of roots by *Foc*-0 was highest ($P < 0.05$) in 'CA 336.14.3.0' and 'PV 61' compared with that in 'ICC 14216 K' and 'UC 27', irrespective of coinfection with the nematode. The nematode reproduction index was also significantly reduced ($F = 6.96$; $P = 0.010$; Table 2) by coinfection with *Foc*-0 in the four chickpea genotypes tested, with no significant differences among them ($F = 0.61$; $P = 0.611$; Table 2; Fig. 1B).

In experiment II, no Fusarium wilt symptoms developed in chickpea genotypes 'WR 315', 'CA 336.14.3.0', and 'ICC 14216 K' inoculated with *Foc*-1A, but moderate or severe wilt symptoms developed in lines BG 212 and CA 334.20.4, respectively (Figs. 2 and 3). In these two later chickpea genotypes, disease symptoms appeared significantly earlier ($F = 6.26$; $P = 0.018$; Table 2) in 'BG 212' compared with 'CA 334.20.4', whether plants were

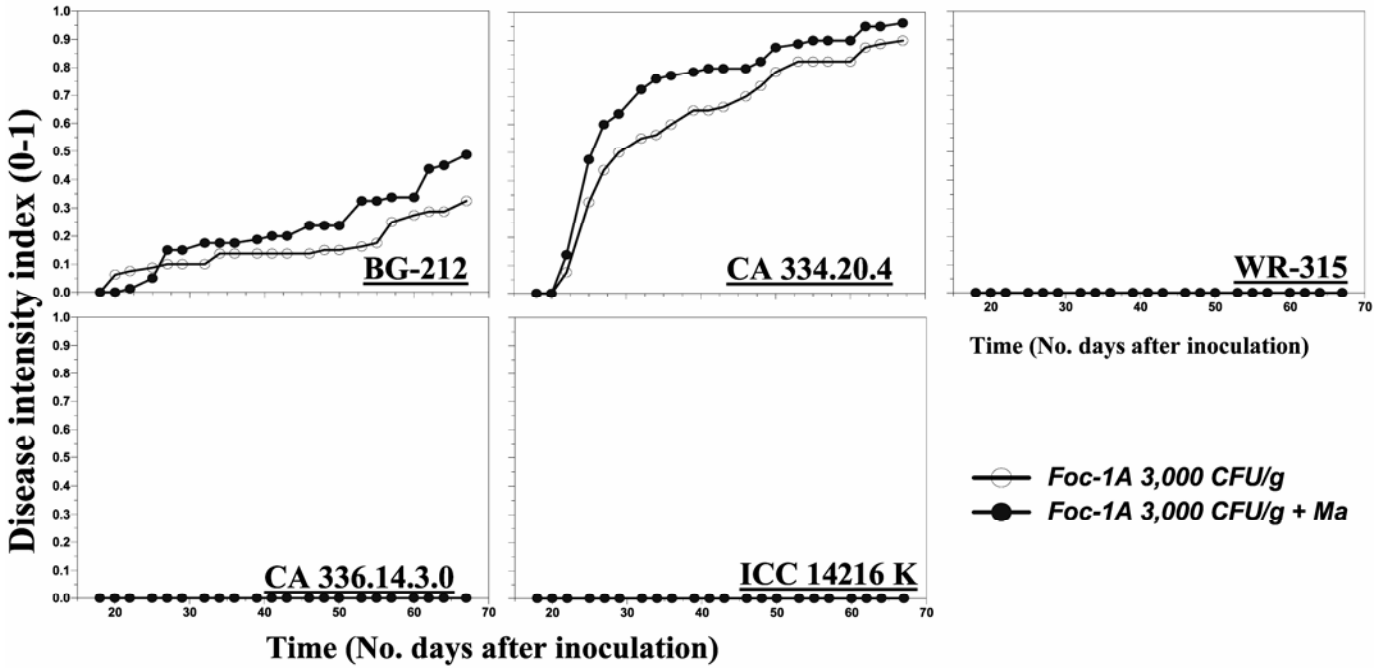

Fig. 2. Fusarium wilt disease progress curves in chickpea genotypes completely resistant ('WR 315', 'CA 336.14.3.0', and 'ICC 14216 K'), moderately susceptible ('BG-212'), and susceptible ('CA 334.20.4.0') to Fusarium wilt that were simultaneously inoculated with 3,000 chlamydospores per gram of soil of *Fusarium oxysporum* f. sp. *ciceris* race 1A and 20 eggs + second-stage juveniles per cubic centimeter of soil of *Meloidogyne artiellia* (*Ma*). Each point is the mean disease intensity index from 14 replicate plants, each replicate consisting of a single potted plant.

coinfected with *M. artiellia* or not ($F = 0.12$; $P = 0.737$; Table 2; Fig. 3A). However, the severity of Fusarium wilt, determined by the DII and SAUDPC, was significantly lower ($P < 0.05$) in line BG 212 than that in line CA 334.20.4 (Figs. 2, 3B, and C), the level of disease achieved being not significantly modified ($P < 0.05$) by coinfection with *Foc*-1A and *M. artiellia* (Figs. 2, 3B, and C; Table 2). Conversely, coinfection of chickpea plants with the two pathogens significantly ($F = 12.78$; $P = 0.001$; Table 2) increased the number of *Foc*-1A CFU per gram of root in all genotypes tested, except for line BG 212 in which no significant differences ($P \geq 0.05$) were observed between the two inoculation treatments (Fig. 3D). Comparisons among chickpea genotypes indicated that root colonization by the fungus was highest ($P < 0.05$) in 'CA 336.14.3.0' and 'BG 212', and decreased significantly ($P < 0.05$) in 'WR 315' and 'ICC 14216 K', in that order (Fig. 3D). Also, the nematode reproduction index (Rf) was not influenced significantly ($F = 2.75$; $P = 0.102$; Table 2) by coinfection of a plant with *Foc*-1A in any of the chickpea genotypes tested ($F = 0.23$; $P = 0.873$; Table 2, Fig. 3E); but Rf differed significantly ($F = 6.87$; $P < 0.001$; Table 2) among them. For the two inoculation treatments, Rf was significantly higher ($P < 0.05$) in 'WR 315', decreased ($P < 0.05$) in 'CA 336.14.3.0' and 'BG 212', and was lowest ($P < 0.05$) in 'ICC 14216 K' (Fig. 3E). Root colonization by the fungus and nematode reproduction could not be estimated in genotype CA 334.20.4 because the root system completely degenerated in severely wilted plants. The reproduction of *M. artiellia* in cv. UC 27 and line ICC 14216 K in experiment II was similar to that estimated for experiment I ($F = 1.27$; $P = 0.262$) (Figs. 1B and 3E).

In experiment III, no Fusarium wilt symptoms developed in 'ICC 14216 K' plants inoculated with *Foc*-2 alone or in combination with *M. artiellia* (Figs. 4 and 5), but severe symptoms developed in 'BG 212' and 'UC 27' plants (Figs. 4 and 5) inoculated either singly with *F. oxysporum* f. sp. *ciceris* or jointly with *M. artiellia.* However, coinfection of these two chickpea genotypes with the two pathogens did not influence ($F = 0.34$; $P = 0.562$; Table 2) the time to appearance of Fusarium wilt symptoms (IP), that depended mainly on the chickpea genotypes ($F = 11.36$; $P = 0.001$; Table 2; Fig. 5A). Fusarium wilt symptoms appeared significantly ($P < 0.05$) earlier in 'BG 212' than in 'UC 27' (Fig. 5A) in coinfected plants; but a similar level of disease, estimated by the DII and SAUDPC, developed in both chickpea genotypes ($P \geq 0.05$) whether or not they were coinfected with *M. artiellia* (Fig. 5B and C; Table 2). In the completely resistant line, ICC 14216 K, neither *Foc*-2 colonization of root tissues nor nematode reproduction in them were influenced ($P \geq 0.05$) by coinfection with the two pathogens (data not shown). Neither the nematode reproduction nor *Foc*-2 colonization of root tissues could be assessed in Fusarium wilt-susceptible genotypes because most of the root system was extensively impaired as a result of severe wilting of infected plants by the end of the experiment.

Development of Fusarium wilt in chickpea genotypes used in experiment IV was comparable to that occurring for the same inoculation treatments and chickpea genotypes in experiments I through III (Figs. 1 to 7). Thus, results confirmed that the complete resistance phenotype of 'UC 27' and 'ICC 14216 K' to *Foc*-0 was not modified by infection of a plant with *M. artiellia* (Fig. 6). In line P 2245, which is susceptible to *Foc*-0 and was not tested in the previous experiments, the severity of Fusarium wilt increased rapidly over time giving rise to death of all plants with no significant differences ($P \geq 0.05$) in the DII and SAUDPC between plants coinoculated or not with *M. artiellia* (Fig. 7B and C). However, symptoms of the disease appeared earlier ($F = 4.33$; $P = 0.048$; Table 2) in plants infected with the fungal pathogen alone compared to coinfection with both pathogens (Fig. 7A). Similarly, the complete resistance of 'ICC 14216 K' to *Foc*-1A and *Foc*-2 was confirmed, and it was not modified by coinfection

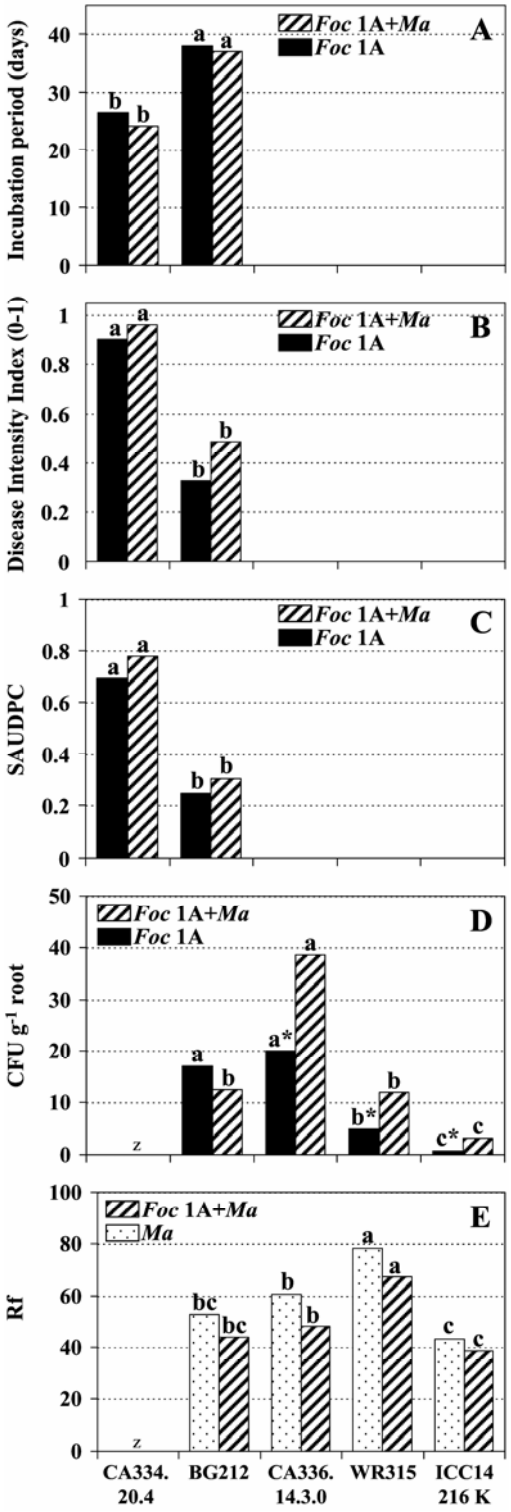

Fig. 3. Reaction of chickpea genotypes simultaneously inoculated with 3,000 chlamydospores per gram of soil of *Fusarium oxysporum* f. sp. *ciceris* race 1A and 20 eggs + second-stage juveniles per cubic centimeter of soil of *Meloidogyne artiellia.* **A,** Incubation period, estimated as the number of days until DII > 0. **B,** Disease intensity index, calculated at the last day of assessment. **C,** SAUDPC = area under disease progress curve standardized by duration time in days of the epidemic. **D,** CFU = colony forming units of *F. oxysporum* f. sp. *ciceris* per gram of fresh root tissue. **E,** Rf = nematode reproduction rate. Each bar is the mean of 14 replicates, each replicate consisting of a single potted plant. For each inoculation treatment, bars with the same letter are not different according to Fisher's least significant difference test ($P < 0.05$). For each chickpea genotype, bars with an asterisk indicate that the single infection treatment is significantly different ($P < 0.05$) from the coinfection treatment.

with *M. artiellia* (Figs. 6 and 7). As found in experiments II and III, severe Fusarium wilt developed in 'CA 334.20.4' inoculated with *Foc*-1A (experiment II) and 'UC 27' inoculated with *Foc*-2 (experiment III) whether plants were also infected with *M. artiellia* or not (Figs. 2 to 7). However, for these two genotypes the DII, SAUDPC, and IP values in plants inoculated with the fungal pathogen alone did not differ significantly ($P \geq 0.05$) from those in plants coinfected with *F. oxysporum* f. sp. *ciceris* and *M. artiellia*, except for the IP in 'CA 334.20.4' that was significantly larger ($F = 7.86$; $P = 0.009$; Table 2) in plants coinfected with *Foc*-1A and *M. artiellia* (Fig. 7A to C). The extent of root colonization by *Foc*-0 in resistant 'ICC 14216 K' and 'UC 27', as well as that by *Foc*-1A and *Foc-2* in resistant 'ICC 14216 K', were not significantly ($P \geq 0.05$) modified by coinfection with the nematode. However, colonization of 'ICC 14216 K' root tissues by *Foc*-2 was significantly reduced ($F = 4.66$; $P = 0.045$; Table 2) by coinfection with *M. artiellia* (Fig. 7D). The nematode reproduction, indicated by the Rf, was only influenced by coinfection with *Foc*-0 in 'UC 27' and 'ICC 14216 K', where significantly higher Rf values occurred in the absence of the fungus ($F = 6.52$; $P = 0.014$; Table 2; Fig. 7E). As found in the previous experiments, neither the nematode reproduction nor colonization of root tissues by *F. oxysporum* f. sp. *ciceris* could be assessed in Fusarium-wilt susceptible genotypes infected by the fungus because severe wilt led most plants to die before the end of the experiment (Fig. 6).

DISCUSSION

Earlier research demonstrated that coinfection of chickpeas by the root-knot nematodes *M. artiellia* (7) or *M. javanica* (56) can breakdown the resistance in some chickpea genotypes against race 5, or unidentified races of *F. oxysporum* f. sp. *ciceris*, respectively. In this study, we aimed to determine whether coinfection of chickpeas by any of races 0, 1A, or 2 of *F. oxysporum* f. sp. *ciceris* and *M. artiellia* would influence the Fusarium wilt reac-

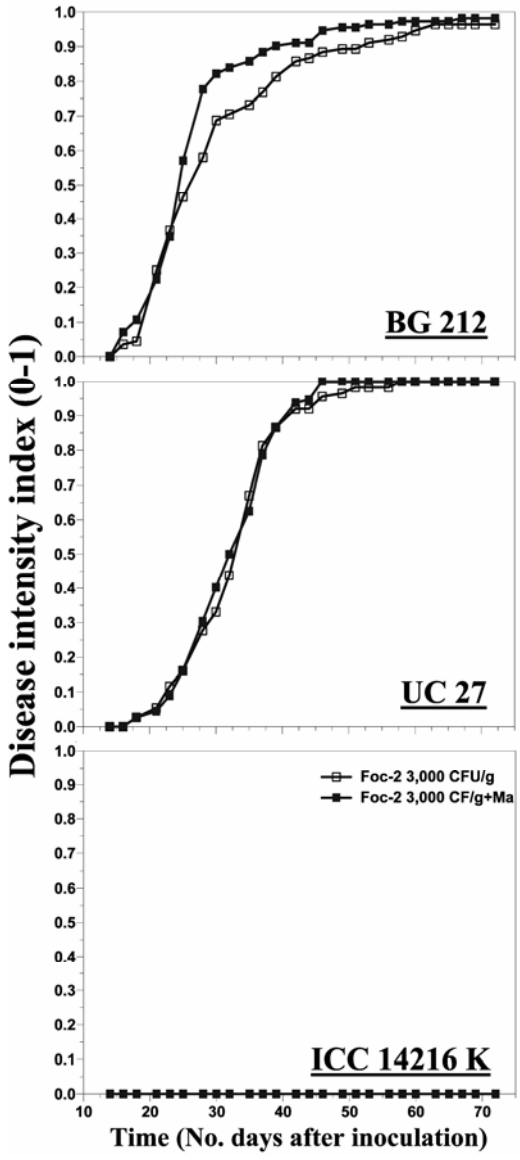

Fig. 4. Fusarium wilt disease progress curves in chickpea genotypes susceptible ('BG 212' and 'UC 27') and completely resistant ('ICC 14216 K') to Fusarium wilt simultaneously inoculated with 3,000 chlamydospores per gram of soil of *Fusarium oxysporum* f. sp. *ciceris* race 2 and 20 eggs + second-stage juveniles per cubic centimeter of soil of *Meloidogyne artiellia* (*Ma*). Each point is the mean disease intensity index from 14 replicate plants, each replicate consisting of a single potted plant.

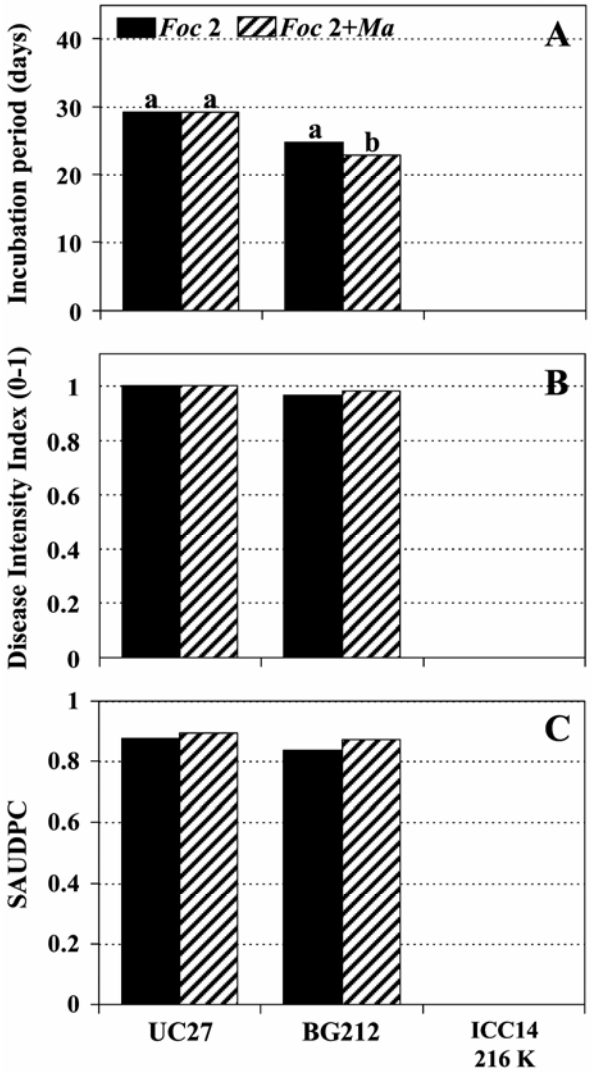

Fig. 5. Reaction of chickpea genotypes simultaneously inoculated with 3,000 chlamydospores per gram of soil of *Fusarium oxysporum* f. sp. *ciceris* race 2 and 20 eggs + second-stage juveniles per cubic centimeter of soil of *Meloidogyne artiellia*. **A,** Incubation period, estimated as the number of days until DII > 0. **B,** Disease intensity index, calculated at the last day of assessment. **C,** SAUDPC = area under disease progress curve standardized by duration time in days of the epidemic. Each bar is the mean of 14 replicates, each replicate consisting of a single potted plant. For each inoculation treatment, bars with the same letter are not different according to Fisher's least significant difference test ($P < 0.05$).

tion of chickpea genotypes with complete or partial resistance to these races of the fungus. Results from growth chamber experiments under optimum conditions for Fusarium wilt development clearly demonstrated that neither resistant nor susceptible reactions of the investigated chickpea genotypes to *F. oxysporum* f. sp. *ciceris* races 0, 1A, and 2 were modified by coinfection with *M. artiellia.* Those findings differ noticeably from results reported by Castillo et al. (7), whereby coinfection of chickpeas by *M. artiellia* and *F. oxysporum* f. sp. *ciceris* race 5 overcame the complete resistance to this race in lines CA 334.20.4 and CA 336.14.3.0 but not in line ICC 14216 K, irrespective of the fungal inoculum density, and resistance breakdown in cv. UC 27 took place at 30,000 chlamydospores g^{-1} of soil but not at 3,000 chlamydospores g^{-1} of soil. Differences in virulence among *F. oxysporum* f. sp. *ciceris* races (28,40,41) together with differences in the genetics and sources of resistance to the different *F. oxysporum* f. sp. *ciceris* races in the chickpea genotypes could account for the dissimilar response.

On the other hand, the nematode species or its population may have an important role in this phenomenon of breakdown of resistance. Although feeding behavior in root-knot and root-lesion nematodes is clearly different, similarly to this study, resistance in 'UC 27' to the highly virulent race 5 of *F. oxysporum* f. sp. *ciceris* was not overcome when plants were challenged with the root-lesion nematode *Pratylenchus thornei* (6).

When studying interactions between root-knot nematodes and soilborne fungal pathogens is generally accepted by some authors that monogenic or oligogenic resistance in plant genotypes against wilting fungi is not likely to be altered by infections with root-knot nematodes compared with the instability of polygenic resistance under coinfection by fungi and nematodes (15,38). This has been proved consistently for in planta interactions between *Meloidogyne* spp. and several formae speciales of *F. oxysporum*, including cabbage (14), chrysanthemum (30), cowpea (18,47), muskmelon (3), pea (9), summer squash (4), and tomato (1,32). However, the opposite is also true since other studies have reported that monogenic resistance against Fusarium wilt pathogens becomes ineffective in the presence of root-knot nematodes (7,15,38,50).

Studies on the genetics of resistance to Fusarium wilt in different chickpea lines and cultivars indicates that resistance against specific races of *F. oxysporum* f. sp. *ciceris* is governed by major resistance genes. In fact, at least two genes seem to be involved in the resistance against the *F. oxysporum* f. sp. *ciceris* races 0, 1A, and 2 used in this study (22,48,54). Therefore, our results would clearly agree with those reports that state the stability of monogenic Fusarium wilt resistance under coinfections with the Fusarium wilt pathogen and *Meloidogyne* spp. Indeed, the breakdown of resistance to the Fusarium wilt pathogen in chickpeas due to coinfection with root-knot nematodes should be considered an exception. Such a resistance breakdown has been reported only for certain chickpea cultivars and only for the highly virulent *F. oxysporum* f. sp. *ciceris* race 5 coinfected with *M. artiellia* (7). Interestingly, resistance against race 5 is governed by a single gene (48,54). Conversely, breakdown of resistance to Fusarium wilt in chickpeas has rarely been reported after coinfection with *M. javanica* (33,56), and to our knowledge, it has never been described following infection by *M. incognita* (35). Unfortunately, the nature of the *F. oxysporum* f. sp. *ciceris* race used in those latter studies was not indicated. More commonly, the available literature indicates that the severity of Fusarium wilt in chickpeas increases in genotypes with partial resistance to the dis-

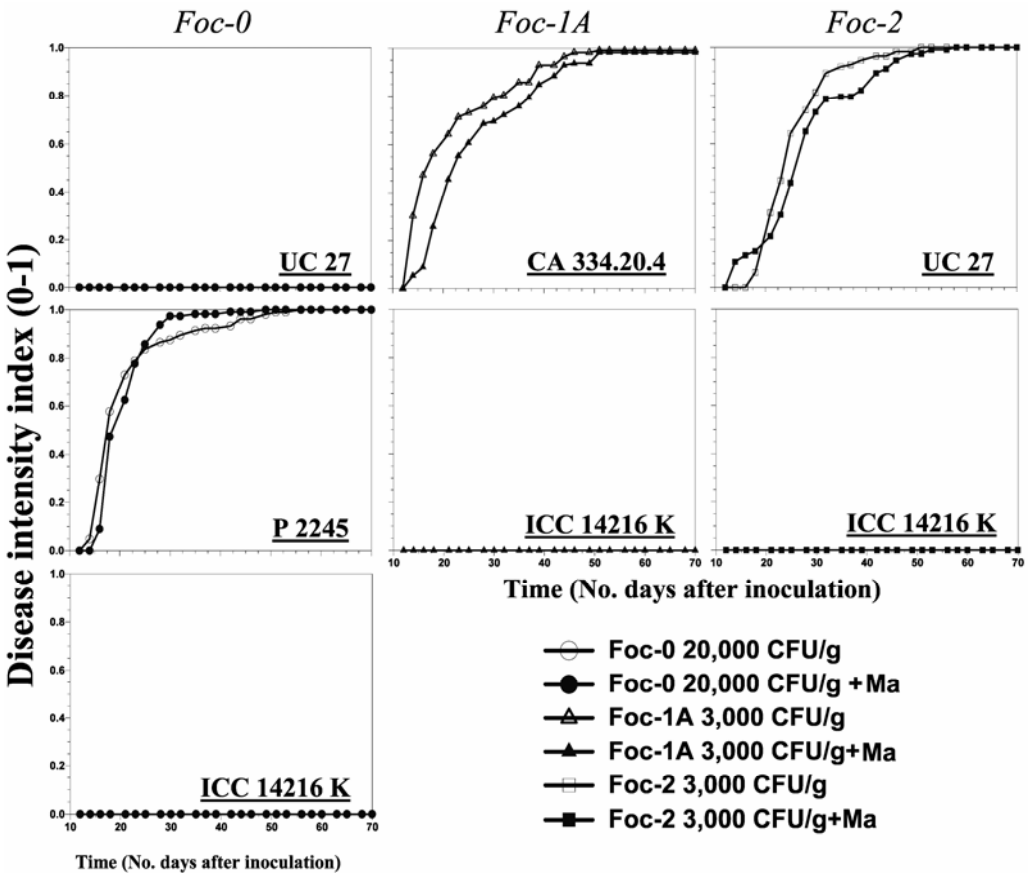

Fig. 6. Fusarium wilt disease progress curves in chickpea genotypes ('UC 27', 'CA 334.20.4', 'P 2245', and 'ICC 14216 K') with different reaction to *Fusarium oxysporum* f. sp. *ciceris* (*Foc*) races, and simultaneously inoculated with either 3,000 (*Foc*-1A and *Foc*-2) or 20,000 (*Foc*-0) chlamydospores per gram of soil of *F. oxysporum* f. sp. *ciceris* and 20 eggs + second-stage juveniles per cubic centimeter of soil of populations of *Meloidogyne artiellia* (*Ma*). Each point is the mean disease intensity index from 14 replicate plants, each replicate consisting of a single potted plant.

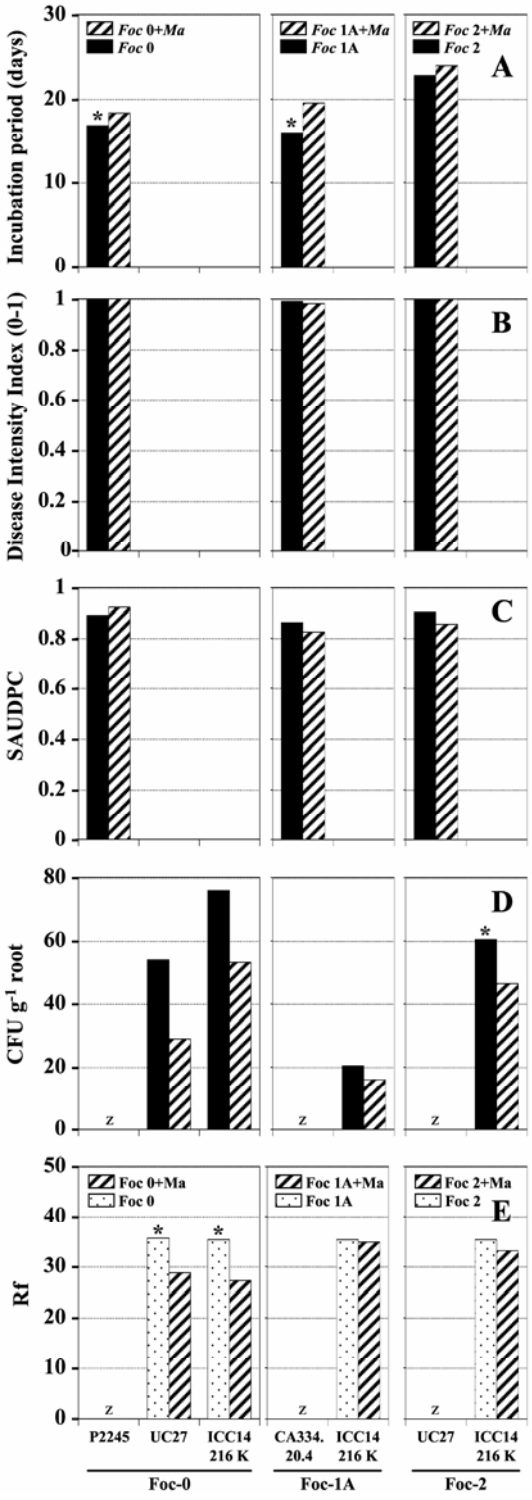

Fig. 7. Reaction of chickpea genotypes with different reaction to *Fusarium oxysporum* f. sp. *ciceris* (*Foc*) races, and simultaneously inoculated with either 3,000 (*Foc*-1A and *Foc*-2) or 20,000 (*Foc*-0) chlamydospores per gram of soil of *F. oxysporum* f. sp. *ciceris* and 20 eggs + second-stage juveniles per cubic centimeter of soil of populations of *Meloidogyne artiellia* (*Ma*). **A,** Incubation period, estimated as the number of days until DII > 0. **B,** Disease intensity index, calculated at the last day of assessment. **C,** SAUDPC = area under disease progress curve standardized by duration time in days of the epidemic. **D,** CFU = colony forming units of *F. oxysporum* f. sp. *ciceris* per gram of fresh root tissue. **E,** Rf = nematode reproduction rate. Each bar is the mean of 14 replicates, each replicate consisting of a single potted plant. For each inoculation treatment, bars with the same letter are not different according to Fisher's least significant difference test ($P < 0.05$). For each chickpea genotype, bars with an asterisk indicate that the single infection treatment is significantly different ($P < 0.05$) from the coinfection treatment.

ease when the fungal pathogen interacts with any of the *Meloidogyne* spp. referred above (7,33,35). However, in this present study neither breakdown of resistance to Fusarium wilt nor increase in wilt severity were observed, the major outcome of the interaction between *F. oxysporum* f. sp. *ciceris* and *M. artiellia* being a differential development of populations of the two pathogens in chickpea roots.

How coinfection of a host plant influences the population of each of the two pathogens involved in the interaction is a controversial issue. While some reports indicate a detrimental effect to either the nematode or fungal population in coinfected plants (53,56,57), others report no effect (7) or even an increase in the population of the Fusarium wilt pathogen with infection by the nematode (3). In our study, the final outcome of the in planta interaction between *F. oxysporum* f. sp. *ciceris* and *M. artiellia* was determined by the race and pathotype of the fungal pathogen. Thus, populations of *M. artiellia* were not affected by the presence of races 1A and 2 of *F. oxysporum* f. sp. *ciceris*, which induce the wilting syndrome, but the nematode population significantly decreased in the presence of race 0, which induces the yellowing syndrome, and that happened in chickpea genotypes either partially or completely resistant to the *F. oxysporum* f. sp. *ciceris* race 0. On the other hand, coinfection with *M. artiellia* led to an increase in the population of *F. oxysporum* f. sp. *ciceris* race 1A in the infected chickpea roots, but that coinfection was detrimental for population of *F. oxysporum* f. sp. *ciceris* races 0 and 2.

Changes in the reproduction of pathogens in some disease complexes have been attributed to several factors, including competition for infection sites by, or antagonism between, the microorganisms involved. Competition for available infection sites in chickpea roots does not seem to be the case for the interaction between *M. artiellia* and *F. oxysporum* f. sp. *ciceris* for which different infection sites were reported (24,60). Moreover, the observed decline in the population of *M. artiellia* in roots of *F. oxysporum* f. sp. *ciceris*-infected chickpeas could be due to a direct effect of the fungus on development of the nematode as described for interactions between *M. incognita* and Fusarium wilt pathogens in coffee (43), tomato (2), and soybean (39).

In summary, our results on the interactions between *F. oxysporum* f. sp. *ciceris* races and *M. artiellia* in chickpeas suggest that the specific combinations of host genotype, nematode specie, and fungal pathogenic race should be carefully considered when investigating interactions between Fusarium wilts and root-knot nematodes, as well as that the interactions should be considered of biological and physiological nature rather than physical (2,16, 34,38). Also, our results show that nematode species with closely related biology and feeding habits such as the species of *Meloidogyne* spp. may harbor differences in the ability to predispose a host plant to infection by the same fungal pathogen, suggesting that those species may induce different physiological or biochemical modifications resulting in a different degree of promotion of the infection and development of the same fungal pathogen (46). Research is in progress in our laboratory to identify different proteins/genes involved in the chickpea–*M. artiellia* interaction, and also to learn about major proteins in giant cells that might be involved in the resistance breakdown phenomenon.

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TABLE 2. Analysis of variance (ANOVA) of incubation period (IP), disease intensity index (DII), standardized area under DII progress curve (SAUDPC), root colonization by *Fusarium oxysporum* f. sp. *ciceris* (colony forming units, CFU) and *Meloidogyne artiellia* reproduction index (Rf) for Fusarium wilt-partially resistant and wilt-complete resistant chickpea genotypes inoculated with *F. oxysporum* f. sp. *ciceris* races 0, 1A, and 2 and a population of *M. artiellia* in growth chamber experiments I to IV[a]

		IP[b]		DII[c]		SAUDPC[d]			CFU g^{-1} root (×104)		Rf[e]	
	df	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	df	*F* value	*P* > *F*	*F* value	*P* > *F*
Exp. I: *F. oxysporum* f. sp. *ciceris* race 0												
Cultivar	--[f]	--	--	--	--	--	--	3	19.00	<0.0001	0.61	0.6110
Treatment	--	--	--	--	--	--	--	1	10.44	0.0017	6.96	0.0096
Cultivar × treatment	--	--	--	--	--	--	--	3	4.43	0.0056	0.22	0.8818
Exp. II: *F. oxysporum* f. sp. *ciceris* race 1A												
Cultivar	1	6.26	0.0183	32.24	<0.0001	42.37	<0.0001	3	50.09	<0.0001	6.87	0.0004
Treatment	1	0.12	0.7366	1.48	0.2317	1.03	0.3170	1	12.78	0.0006	2.75	0.1015
Cultivar × treatment	1	0.02	0.8945	0.29	0.5920	0.05	0.8233	3	5.90	0.0012	0.23	0.8732
Exp. III: *F. oxysporum* f. sp. *ciceris* race 2												
Cultivar	1	11.36	0.0014	5.09	0.0283	1.57	0.2151	…[g]	…	…	…	…
Treatment	1	0.34	0.5617	0.57	0.4556	1.30	0.2595	…	…	…	…	…
Cultivar × treatment	1	0.40	0.5321	0.57	0.4556	0.14	0.7125	…	…	…	…	…
Exp. IV: *F. oxysporum* f. sp. *ciceris* race 0												
Cultivar	–[h]	–	–	–	–	–	–	1	2.64	0.1112	0.14	0.7096
Treatment	1	4.33	0.0479	0.00	ne	2.17	0.1528	1	3.43	0.0706	6.52	0.0142
Cultivar × treatment	–	–	–	–	–	–	–	1	0.03	0.8535	0.24	0.6265
Exp. IV: *F. oxysporum* f. sp. *ciceris* race 1A												
Treatment	1	7.86	0.0094	0.35	0.5585	1.54	0.2256	1	0.36	0.5559	<0.01	0.9983
Exp. IV: *F. oxysporum* f. sp. *ciceris* race 2												
Treatment	1	0.33	0.5703	0.00	ne	1.76	0.1963	1	4.66	0.0446	0.16	0.6969

[a] Data are the mean of 14 replicate plants per treatment combination.
[b] IP established as the number of days from inoculation until DII > 0.
[c] DII calculated at the last day of assessment 67, 72, and 70 days after inoculation, for experiments II, III, and IV, respectively.
[d] SAUDPC = area under disease progress curve standardized by duration time in days of the epidemic.
[e] Rf (nematode reproduction rate) = final nematode density per plant/initial nematode population density per plant.
[f] -- Indicates no disease developed in these experimental combinations.
[g] … Indicates severe impairment of most of the root system in wilted plants made not possible to assess neither nematode reproduction nor *Foc*-5 colonization in root tissues.
[h] – Indicates there was only one level with non zero values, and therefore this term was not included in the ANOVA. Ne = not estimated because all values were identical for both treatments.

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