Temperature Response of Chickpea Cultivars to Races of *Fusarium oxysporum* f. sp. *ciceris*, Causal Agent of Fusarium Wilt

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ABSTRACT

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Use of resistant cultivars and adjustment of sowing dates are important measures for management of Fusarium wilt in chickpeas (*Cicer arietinum*). In this study, we examined the effect of temperature on resistance of chickpea cultivars to Fusarium wilt caused by various races of *Fusarium oxysporum* f. sp. *ciceris*. Greenhouse experiments indicated that the chickpea cultivar Ayala was moderately resistant to *F. oxysporum* f. sp. *ciceris* when inoculated plants were maintained at a day/night temperature regime of 24/21°C but was highly susceptible to the pathogen at 27/25°C. Field experiments in Israel over three consecutive years indicated that the high level of resistance of Ayala to Fusarium wilt when sown in mid- to late January differed from a moderately susceptible reaction under warmer temperatures when sowing was delayed to late February or early March. Experiments in growth chambers showed that a temperature increase of 3°C from 24 to 27°C was sufficient for the resistance reaction of cultivars Ayala and PV-1 to race 1A of the pathogen to shift from moderately or highly resistant at constant 24°C to highly susceptible at 27°C. A similar but less pronounced effect was found when Ayala plants were inoculated with *F. oxysporum* f. sp. *ciceris* race 6. Conversely, the reaction of cultivar JG-62 to races 1A and 6 was not influenced by temperature, but less disease developed on JG-62 plants inoculated with a variant of race 5 of *F. oxysporum* f. sp. *ciceris* at 27°C compared with plants inoculated at 24°C. These results indicate the importance of appropriate adjustment of temperature in tests for characterizing the resistance reactions of chickpea cultivars to the pathogen, as well as when determining the races of isolates of *F. oxysporum* f. sp. *ciceris*. Results from this study may influence choice of sowing date and use of chickpea cultivars for management of Fusarium wilt of chickpea.

Additional keywords: molecular markers

Fusarium wilt of chickpea (*Cicer arietinum*), caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *ciceris* (Padwick) Matuo & K. Sato, is a major limiting factor of chickpea production in the Mediterranean Basin and the Indian Subcontinent (14). Annual yield losses from this disease have been estimated to range from 10 to 15% (14,43), but Fusarium wilt epidemics can be devastating to individual crops and cause 100% loss under favorable conditions (10,32). In particular, disease attacks are devastating if they occur when the crop is under heat and water stresses during the reproductive and seed filling phases (3,28,40).

Management of Fusarium wilt can be achieved by use of resistant cultivars and adjustment of sowing dates (14,17,18,28, 31,32). However, several factors influence the efficacy of these management practices, including pathogenic variability in the fungus populations as well as abiotic factors such as temperature and moisture. *F. oxysporum* f. sp. *ciceris* exhibits significant pathogenic variability. Eight races of *F. oxysporum* f. sp. *ciceris* (races 0, 1A, 1B/C, 2, 3, 4, 5, and 6) have been described to date (6,12,15,20), which can be grouped into the wilting and yellowing pathotypes based on the disease symptoms they induce in pathogenicity tests (43). Races 0 and 1B/C are of the yellowing pathotype, while races 1A and 2 through 6 belong to the wilting pathotype (15,21,22). Races 2, 3, and 4 have only been reported in India (12,14), whereas races 0, 1B/C, 5, and 6 are found mainly in the Mediterranean and in California (10,15,21,22). Race 1A has been reported in India (12), California, and the Mediterranean (15,21,22). This diversity in phenotypes and geographic distribution of races of *F. oxysporum* f. sp. *ciceris* makes the identification of races of the pathogen in a given area of chickpea production important for resistance breeding as well as for efficient use of available resistant cultivars. Similarly, adequate characterization of the resistance of chickpea lines and cultivars to specific races of *F. oxysporum* f. sp. *ciceris* is essential for resistance deployment.

F. oxysporum f. sp. *ciceris* races 0, 1A, 5, and 6 can be identified by means of specific molecular markers in polymerase chain reaction (PCR) assays (21). However, the identification of races 1B/C, 2, 3, and 4, or new races of *F. oxysporum* f. sp. *ciceris*, as well as the characterization of resistance reactions in chickpea germ plasm, are dependent on traditional pathogenicity tests (15,37,43). While this method of resistance screening is simple conceptually, development of disease during biological pathotyping can be influenced by temperature and other factors (e.g., soil moisture and inoculum density of the pathogen) (3,9,26,30). Lack of adjustment for these sources of variability may lead to the incorrect identification of races of *F. oxysporum* f. sp. *ciceris* or incorrect assessment of the resistance of chickpea genotypes. The influence of temperature on expression of resistance or susceptibility of other crops to plant pathogens has been shown (e.g., 2,5,7,8, 11,23,24,33,41), but has not yet been reported for Fusarium wilt of chickpea.

Previous studies have indicated that the chickpea cultivar Ayala, developed at the Volcani Center in Israel, was resistant to Fusarium wilt under field conditions, and to *F. oxysporum* f. sp. *ciceris* race 0 in artificial inoculations (J. A. Navas-Cortés, J. Katan, B. Retig, and R. M. Jiménez-Díaz, *unpublished data*). However, observations of disease development on Ayala plants under greenhouse conditions in Israel suggested that the resistance in this cultivar may be temperature-dependent,

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and that warmer temperatures, associated with later sowings, may affect the disease reaction of this cultivar (J. Katan and B. Retig, *unpublished data*). The objectives of this study were: (i) to further characterize the disease reaction of the chickpea cultivar Ayala to the races of *F. oxysporum* f. sp. *ciceris* most widespread in Israel and Spain; (ii) to determine whether the resistance reaction of Ayala and other chickpea cultivars to *F. oxysporum* f. sp. *ciceris* is affected by temperature; and (iii) to characterize into races isolates Foc-8726 and Foc-9620 of *F. oxysporum* f. sp. *ciceris* by molecular and biological pathotyping.

MATERIALS AND METHODS

Fungal isolates and inoculum production. Seven *F. oxysporum* f. sp. *ciceris* monoconidial isolates were used in the study (Table 1). Isolates Foc-7802, Foc-7989, Foc-8012, and Foc-9620, representative of races 0, 1A, 5, and 6, respectively, and Foc-8726 (unknown race) were used in inoculation experiments in growth chambers. Isolates Foc-9023 and Foc-USA-3-1-JG-62 were used as internal controls of races 6 and 1B/C, respectively, in the molecular assays. All pathogen isolates were stored in sterile soil tubes at 4°C.

Cultures of *F. oxysporum* f. sp. *ciceris* isolates were obtained by placing small aliquots (approximately 100 mg) of a soil culture of each isolate onto a plate of 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 the plate for 5 days at 25°C with a 12-h photoperiod of fluorescent and near-UV light at 36 $\mu E \cdot m^{-2} \cdot s^{-1}$. Inocula for experiments were produced in an autoclaved cornmeal–sand mixture (CMS) in flasks incubated under the same conditions for 2 weeks (43). The infested CMS was mixed thoroughly with an autoclaved soil mixture (clay loam:sand:peat at 1:1:1, vol/vol/vol) at a rate of 1:12 (wt/wt). A mixture of the same ratio of noninfested CMS and autoclaved soil at the same rate served as the control. The ratio of infested CMS in the mixture ensured an inoculum density of approximately 10^5 CFU/g soil. Inoculum density of the infested soil mixtures was estimated by dilution plating, with four 1-g soil samples each suspended in 100 ml of sterile 0.1% water agar in 250-ml Erlenmeyer flasks. The suspensions were stirred in a blender for 1 min, and serial dilutions of each suspension were plated onto *Fusarium*-selective V8 juice-oxgall-PCNB agar (VOPA) (4). Plates were incubated as described above for 5 to 7 days.

Biological and molecular pathotyping of isolates. Twelve chickpea differential cultivars were used to type the race of isolates Foc-9620 and Foc-8726 of *F. oxysporum* f. sp. *ciceris*, including P-2245, JG-62, BG-212, C-104, JG-74, CPS-1, WR-315, Annigeri, Chafa, K850 3/27, 12071/10054 (PV-1), and ICCV-4 (12,15). Type isolates of races 1A (Foc-7989) and 5 (Foc-8012) were included as control treatments. The experiment consisted of a factorial treatment design, with four or six replications (pots) and four plants per pot. Replicates (pots) were arranged in a completely randomized design. The disease reactions of chickpea differentials to inoculation with type isolates of *F. oxysporum* f. sp. *ciceris* races present in Israel and Spain are shown in Table 1.

For each cultivar, chickpea seeds were surface-disinfested in 2% NaOCl for 3 min, germinated, and sown in 15-cm-diameter clay pots (four plants per pot) filled with CMS-soil mixture infested with inoculum of the appropriate isolate. Control plants were grown in a noninfested soil mixture. Plants were kept in walk-in growth chambers (Euroclima, Córdoba, Spain) adjusted to 25 ± 2°C and 60 to 90% relative humidity with a 14-h photoperiod of fluorescent light at 360 $\mu E \cdot m^{-2} \cdot s^{-1}$. The plants were watered as needed and fertilized weekly with 100 ml of 0.1% hydrosol fertilizer solution (Haifa Chemicals, Ltd., Haifa, Israel; 20-5-32 of N-P-K + micronutrients). Plants were monitored for symptoms of Fusarium wilt, as described below, for 8 weeks after sowing. The experiment was repeated twice.

Disease reactions were determined by assessing the incidence and severity of symptoms on individual plants at 2- to 3-day intervals. Symptom severity was assessed on a 0 to 4 rating scale according to the percentage of foliage with yellowing or necrosis in an acropetal progression, where: 0 = 0%, 1 = 1 to 33%, 2 = 34 to 66%, 3 = 67 to 100%, and 4 = dead plant (22,26,30). At the end of the experiment, mean severity scores <1 were considered resistant reactions, and scores >3 were considered susceptible reactions. Intermediate scores (≥1 and ≤3) were considered moderately susceptible reactions (18). After 8 weeks, isolations were made from stem segments of individual plants showing a severity score ≤2 (34 to 66% of foliage with yellowing or necrosis) to confirm the occurrence of vascular infections of the plant. This avoided incorrect assignment of low disease severity scores to noninfected plants senescing at the end of an experiment. Stem pieces were cut into 5- to 10-mm-long pieces, surface-disinfested in 0.2% NaOCl for 2 min, plated onto VOPA, and incubated for 5 to 7 days at 25°C with a 12-h photoperiod of fluorescent and near-UV light at 36 $\mu E \cdot m^{-2} \cdot s^{-1}$.

Random amplified polymorphic DNA (RAPD) and specific-PCR assays for characterization of *F. oxysporum* f. sp. *ciceris* and races 0, 1A, 5, and 6 were used for the molecular characterization of isolates. For DNA extraction, a small agar piece taken from an actively growing fungal culture was placed onto a film of sterile cellophane layered over a media plate, and incubated under the conditions described above for 4 to 6 days. Mycelia growing onto the cellophane surface were then harvested, lyophilized, and stored at –20°C. Genomic DNA of the isolates was purified using the protocol of Raeder and Broda (35) with slight modifications (21). The extracted DNA was checked for quality on an agarose gel, quantified using the Quant-iT DNA Broad Range fluorometric assay kit (Molecular Probes, Inc., Leiden, The Netherlands) with a Tecan Safire fluorospectrometer (Tecan Spain, Barcelona, Spain) according to manufacturer's instructions, and diluted in sterile water to a final concentration of 25 to 50 ng/µl.

Table 1. Isolates of *Fusarium oxysporum* f. sp. *ciceris* used in this study and reactions of differential chickpea cultivars to inoculation with type isolates of races of *F. oxysporum* f. sp. *ciceris* present in Spain and Israel, and to inoculation with isolates Foc-8726 (unassigned race) and Foc-9602 (race 6) in experiments under controlled conditions at 25°C

		Differential chickpea line[a]							
Isolate	**Race, syndrome**	**PV-1**	**JG-62**	**C-104**	**JG-74**	**CPS-1**	**ICCV-4**	**Source**	**Refs**
Foc-7802	0, yellowing	S	R	M	R	R	R	Infected chickpea stem, Spain	6, 16
Foc-7989	1A, wilting	M	S	M	R	R	R	M.P. Haware, ICRISAT, India	6, 21, 22
Foc-USA-1-JG-62	1B/C, yellowing	S	S	R/M	R	R	R	Infected chickpea stem, USA	21, 22
Foc-8012	5, wilting	R	S	S	M	M	S	Infected chickpea stem, Spain	6,16
Foc-9023	6, wilting	R	S	M	R	R	M	Infected chickpea stem, Spain	21, 22
Foc-9620	6, wilting	R/R[b]	S/S	M/M	R/R	R/R	M/R	Infected chickpea stem, Israel	21, 22
Foc-8726	Unknown, wilting	R/R	S/S	R/R	R/R	R/R	R/R	Infected chickpea stem, Spain	18

[a] Disease was assessed on a 0 to 4 severity scale based on the percentage of symptomatic foliar tissue (0 = 0%, 1 = 1 to 33%, 2 = 34 to 66%, 3 = 67 to 100%, and 4 = dead plant) at the end of the experiment (12,15). Average disease severity ratings <1 and >3 at the end of the experiments were considered resistant (R) and susceptible (S), respectively. Intermediate severity ratings (≥1 and ≤3) were considered moderately susceptible reactions (18).

[b] Results from two independent experiments are shown.

RAPD and specific-PCR reactions were performed as described elsewhere (21,22), respectively, in a Perkin-Elmer 9600 thermocycler (Perkin-Elmer, Norwalk, CT). For RAPD assays, primers OPI-09, OPF-10, OPF-12, and OPF-16 (Operon Technology, Alameda, CA) were used. Specific-PCR assays were carried out using primer pairs that produced amplicon markers of *F. oxysporum* f. sp. *ciceris* (Foc0-12f/Foc0-12r), race 0 (FocR0-M15f/FocR0-M15r), races 1A and 6 (FocR6-P18f/FocR0-M15r), race 5 (FocR5-L10f/FocR5-L10r), and race 6 (FocR6-O2f/FocR6-O2r) (21). All reactions were repeated at least twice and always included a positive control of a known template DNA and a negative control with no DNA. Amplification products were separated by electrophoresis in 1.5% agarose gels in 1× Trisacetate-EDTA (TAE) buffer, stained with ethidium bromide, and visualized under UV light. A 0.1-kb DNA ladder XIV size marker was used for electrophoresis (Roche Diagnostics, Mannheim, Germany).

Greenhouse and field experiments. Chickpea cultivars Ayala and Bulgarit were used in greenhouse and field experiments in Israel. Both cultivars are commonly used in commercial chickpea production in Israel. Ayala and Bulgarit are moderately susceptible and highly resistant, respectively, to Ascochyta blight caused by *Didymella rabiei* (39), and are resistant and susceptible, respectively, to Fusarium wilt in Israel (J. Katan and B. Retig, *unpublished data*).

An experiment was first done under controlled greenhouse conditions at Rehovot, Israel, to determine the effect of temperature on the disease reaction of cultivars Ayala and Bulgarit to Fusarium wilt. Seeds of Ayala and Bulgarit were sown in 11-cm-diameter pots (five plants per pot) containing soil naturally infested with *F. oxysporum* f. sp. *ciceris* from a field plot at Merhavya. Plants were grown in two greenhouses adjusted to temperature regimes of 24/21°C or 27/25°C (day/night) with natural light, and watered as needed. These conditions were conducive for development of Fusarium wilt (26). Five replicate pots of each treatment were arranged in a completely randomized design. The experiment lasted 12 weeks and was conducted twice. All surviving plants were examined for the presence of the pathogen as described above.

In 1998 and 1999, field experiments with five replicates were conducted to assess the influence of two sowing dates on chickpea production at each of two locations, at The Volcani Central Experimental Station in Bet Dagan, Israel, and in an experimental plot at Merhavya, Israel, that were naturally infested with *F. oxysporum* f. sp. *ciceris*. In 2000, the experiment was carried out only at Merhavya. Sowing dates were on 25 January and 2 March at Bet Dagan, and on 18 January and 20 February at Merhavya. Similar sowing dates were chosen in 1999 and 2000. Seeds were machine-sown in rows spaced 75 cm apart, and the seeds were spaced 6 to 8 cm apart within rows. Weeds in the plots were removed by hand, and the fungicide difenoconazole (Score, 25% EC; Novartis, Basle, Switzerland) was applied at a rate of 125 g a.i./ha for control of Ascochyta blight as needed.

Development of Fusarium wilt in the greenhouse experiment was assessed by recording disease incidence at 2-week intervals, and that in the field experiments by recording the incidence and severity of symptoms (assessed on a 0 to 4 rating scale as described above) at the end of the growing period.

Reaction of the chickpea cultivar Ayala to different races of *F. oxysporum* f. sp. *ciceris*. An experiment was conducted to determine the disease reaction of the chickpea cultivar Ayala to *F. oxysporum* f. sp. *ciceris* races 0 (isolate Foc-7802), 1A (isolate Foc-7989), 5 (isolate Foc-8012), and 6 (isolate Foc-9620), as well as to isolate Foc-8726 of unknown race. The differential cultivars JG-62, PV-1, and ICCV-4 were included as control treatments to determine reproducibility of the disease reactions among experiments. The experiment was carried out in a walk-in growth chamber (Euroclima, Córdoba, Spain) adjusted to 25 ± 2°C and 60 to 90% relative humidity with a 14-h photoperiod of fluorescent light at 360 $\mu E \cdot m^{-2} \cdot s^{-1}$, as described above for the biological pathotyping of isolates. The experiment consisted of a factorial treatment design with four replicate pots per treatment combination (four plants per pot) in a completely randomized design.

Incidence of foliar symptoms, I, (0 to 1 scale), and severity data, S, (rated on a 0 to 4 scale as described above) on individual plants were assessed at 2- to 3-day intervals and used to calculate a disease intensity index (DII) (26,31), where DII = $(I \times S)/4$. Thus, DII (0-1) expressed the mean disease intensity at any given time as a proportion of the maximum possible amount of disease. Average DII_{final} scores <0.25 or >0.75 were considered resistant or susceptible reactions, respectively. Intermediate scores (≥0.25 and ≤0.75) were considered moderately susceptible reactions. After 7 weeks, isolations were made from plants showing a severity score ≤2, to determine the occurrence of plants with vascular infection, as described above.

Effect of temperature and race of *F. oxysporum* f. sp. *ciceris* on the resistance reaction of chickpea cultivars. An experiment was conducted to determine the effect of temperature on the resistance reaction of the cultivars Ayala, JG-62, and PV-1 to *F. oxysporum* f. sp. *ciceris* races 0 (isolate Foc-7802), 1A (isolate Foc-7989), and 6 (isolate Foc-9620), and to isolate Foc-8726 of unknown race. The experiment was carried out as described above for the biological pathotyping of isolates, except that plants were grown in phytotrons (Sanyo Gallencamp PLC, Fitotron, Leicester, England) adjusted to a constant temperature of either 24 or 27°C and 60 to 80% relative humidity with a 14-h photoperiod of fluorescent light at 360 $\mu E \cdot m^{-2} \cdot s^{-1}$. The experiment consisted of a factorial treatment design with four replicate pots per treatment (four plants per pot) arranged in a completely randomized design. Incidence of foliar symptoms and severity data on individual plants assessed at 2- to 3-day intervals were used to calculate the DII. After 7 weeks, average DII_{final} and percentage of vascular infection were used to score plant response to pathogen races as resistant, moderately susceptible, or susceptible, as described above.

Data analyses. The nonlinear form of the Gompertz model was evaluated for goodness-of-fit to the DII progress data using nonlinear regression analysis as described previously (26,31). In the Gompertz equation, $DII(t) = K \exp[-B \exp(-r \cdot t)]$, where DII = disease intensity index, K = asymptote parameter, B = constant of integration, r = relative rate of disease increase, and t = time of disease assessment in days after sowing. Regression analyses were conducted using the least-squares program for nonlinear models (NLIN) procedure of SAS (Version 8.0; SAS Institute, Cary, NC).

Disease development was characterized by four variables associated with disease progress curves: (i) IP = the incubation period for disease development, established as the time in days to reach a DII = 0.05; (ii) the final disease intensity (DII_{final} = 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 (26,30,31); and (iv) r parameter estimates of the Gompertz model fitted to the DII progress data. The effect of chickpea cultivar, incubation temperature, and fungal isolate on the IP, DII_{final}, and SAUDPC was determined by multivariate analysis of variance (MANOVA) using the general linear model (GLM) procedure of SAS. Significant differences between temperature treatments were tested using the Wilks' lambda statistic (28). Orthogonal single degree-of-freedom contrasts were computed to test the effect of selected experimental treatment combinations (27). The standard errors of the r obtained from regression analyses were used to compare the effects of experimental treatments (30).

RESULTS

Biological and molecular pathotyping of *F. oxysporum* f. sp. *ciceris* isolates Foc-8726 and Foc-9620. Disease reactions of

six differential chickpea cultivars to *F. oxysporum* f. sp. *ciceris* isolates Foc-8726 and Foc-9620 are shown in Table 1. Reactions of the other six chickpea cultivars to these isolates were not informative for the race characterization of these isolates (*data not shown*). The low disease severity (<1.0) and incidence of plants with vascular infection (<36% and 5%) that was observed on ICCV-4, inoculated with Foc-9620 in both experiments, did not correspond to the type reaction caused by a race 6 isolate (22). The disease reactions of the remaining five chickpea differentials to Foc-9620 were characteristic of that predicted for race 6 isolates (Table 1).

Results of RAPD and specific-PCR assays confirmed isolate Foc-9620 as *F. oxysporum* f. sp. *ciceris* race 6, i.e., DNA amplification using primer OPF-12 yielded the 1.5-kb RAPD band marker of the forma specialis *ciceris,* and DNA amplification using primer OPI-09 produced the 1.3-kb DNA fragment marker of race 6 (22) (*data not shown*). Similarly, specific-PCR assays using primers Foc0-12f/Foc0-12r yielded the predicted marker of the forma specialis *ciceris*, and primers FocR6-P18f/FocR0-M15r and FocR6-O2f/FocR6-O2r yielded the amplicon markers of races 1A and 6, and race 6, respectively (*data not shown*).

Isolate Foc-8726 induced wilting symptoms on the susceptible chickpea cultivars JG-62 and P-2245 in both experiments (Table 1). However, a resistant reaction was observed on cultivars PV-1, ICCV-4, CPS-1, and JG-74 (Table 1), which are used to differentiate races 1A, 5, and 6. This made it impossible to assign isolate Foc-8726 to any of the known races of *F. oxysporum* f. sp. *ciceris*. Interestingly,

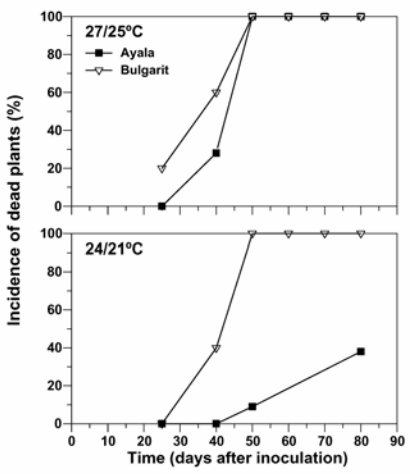

Fig. 1. Effect of temperature on development of Fusarium wilt on the chickpea cultivars Ayala and Bulgarit based on incidence of dead plants. Plants were sown in soil naturally infested with *Fusarium oxysporum* f. sp. *ciceris* (unknown race) and grown in a greenhouse adjusted to 24/21°C or 27/25°C day/night temperature regimes. Each data point is the mean of five replicate pots with five plants per pot.

typing of isolate Foc-8726 by RAPD assays using primers OPI-09, OPF-10, OPF-12, and OPF-16 yielded DNA fragment markers typical of *F. oxysporum* f. sp. *ciceris* (1.5 kb with OPF-12) and more specifically of race 5 (0.9 kb with OPF-10) (*data not shown*). Similarly, specific-PCR assays using primer pairs Foc0-12f/Foc0-12r and FocR5-L10f/FocR5-L10r produced marker amplicons of *F. oxysporum* f. sp. *ciceris* and of race 5, respectively (*data not shown*). Consequently, isolate Foc-8726 might be a variant of *F. oxysporum* f. sp. *ciceris* race 5. However, further studies need to be conducted to confirm this.

Greenhouse and field experiments. Bulgarit plants developed a typical highly susceptible reaction to *F. oxysporum* f. sp. *ciceris* at both temperature regimes, reaching 100% incidence of dead plants 50 days after sowing (Fig. 1). In contrast, a significant difference in disease development was observed on Ayala at the two temperature regimes evaluated. A susceptible reaction was observed at the higher (27/25°C) temperature regime with 100% incidence of dead plants 50 days after sowing, whereas a moderately susceptible reaction was observed at 24/21°C, with only 40% of the plants dead 80 days after sowing (Fig. 1). A similar result was obtained for the repeat experiment (*data not shown*).

The chickpea cultivar Bulgarit showed a highly susceptible reaction to Fusarium wilt at both sowing dates and both locations included in the 1998 field experiment, reaching a final disease severity score >3. Ayala was completely resistant to the disease in the earlier sowing date at each of the two locations, and in the late sowing date at Merhavya, but exhibited a mean disease severity rating of 0.81 (resistant) for the later sowing date at Bet Dagan. A similar result was obtained in 1999. In 2000, disease severity at Merhavya at the later sowing reached a mean disease severity rating of 1.2 (moderately susceptible). This indicated a reduction in expression of resistance of Ayala to the population of *F. oxysporum* f. sp. *ciceris* prevailing in the field plot.

Reaction of the cultivar Ayala to races of *F. oxysporum* f. sp. *ciceris*. Symptoms that developed in compatible interactions were characteristic of the corresponding pathotype of each isolate, i.e., the yellowing symptoms for race 0 isolate Foc-7802 and wilting symptoms for isolates Foc-7989 (race 1A), Foc-8012 (race 5), Foc-9620 (race 6), and Foc-8726. Both experimental factors, i.e., chickpea cultivar (Wilks' lambda 0.142; $P < 0.001$) and isolate of the pathogen (Wilks' lambda 0.219; $P < 0.001$), had a significant effect on development of Fusarium wilt. A significant interaction (Wilks' lambda 0.182; $P < 0.001$) was detected between chickpea cultivar and isolates of *F. oxysporum* f. sp. *ciceris* (Fig. 2, Table 2). Appropriate descriptions of DII increase over time were obtained with the Gompertz model ($R^2 \geq 0.94$, *data not shown*).

Based on disease development (determined by IP, DII_{final}, SAUDPC, and r) and incidence of infected plants, Ayala was highly susceptible to races 1A, 5, and 6 (Table 2). Symptoms started to develop 17 to 21 days after sowing, progressed at similar ($P \geq 0.05$) rates for all three isolates, and reached $DII_{final} > 0.8$ and SAUDPC > 0.6, as well as 100% of plants with vascular infection, by 47 days after sowing (Table 2). Ayala was also susceptible to isolate Foc-8726, but significantly ($P < 0.001$) less susceptible to this isolate than the former three isolates (Fig. 2, Table 2). Conversely, Ayala was relatively resistant to race 0 ($DII_{final} < 0.15$), although infection of the vascular tissues by the pathogen was observed in 46.7% of the plants by the end of the experiment.

The disease reactions of cultivars JG-62 and PV-1 to the races and isolates of *F. oxysporum* f. sp. *ciceris* evaluated were as expected. Cultivar JG-62 was resistant to race 0 and highly susceptible to races 1A, 5, and 6, and to isolate Foc-8726 (Table 2). However, rate of disease development for race 5 and isolate Foc-8726 was significantly ($P < 0.05$) slower than those for races 1A and 6 (Table 2). Cultivar PV-1 was susceptible to race 0, but resistant to races 1A, 5, and 6, and to isolate Foc-8726 (Table 2). The disease reaction caused by race 1A was less severe ($DII_{final} = 0.16$) than expected for a moderately susceptible host–pathogen interaction and resulted in a low percentage of plants (12.5%) with vascular infection. A resistant reaction to Foc-9620 developed on cultivar ICCV-4, compared ($P < 0.007$) with the highly susceptible reaction of this same cultivar to race 5 (Fig. 2, Table 2), that was similar to those observed in the biological pathotyping experiments (*data not shown*).

Effect of temperature and race of *F. oxysporum* f. sp. *ciceris* on the resistance reaction of chickpea cultivars. The disease syndromes that developed on susceptible cultivar–race treatment combinations were not influenced by incubation at 24°C versus 27°C, but corresponded with specific *F. oxysporum* f. sp. *ciceris* pathotypes (i.e., yellowing symptoms for race 0 and wilting symptoms for races 1A and 6, and isolate Foc-8726). All three experimental factors, i.e., chickpea cultivar (Wilks' lambda 0.150; $P < 0.001$), isolate of the pathogen (Wilks' lambda 0.141; $P < 0.001$), and temperature (Wilks' lambda 0.728; $P = 0.002$), had a significant effect on development of Fusarium wilt. A significant interaction (Wilks' lambda 0.299; $P < 0.001$) was detected between incubation temperatures and isolates of *F. oxysporum* f. sp. *ciceris* (Fig. 3, Table 3). Appropriate descriptions of DII increase over time were obtained with the Gompertz model ($R^2 \geq 0.94$, *data not shown*).

The disease reaction of cultivars Ayala, JG-62, and PV-1 to race 0 (i.e., isolate Foc-7802) was not influenced by incubation temperatures of 24°C versus 27°C, i.e., Ayala and JG-62 were resistant, and PV-1 was susceptible, with plants developing yellowing symptoms characteristic of infection by race 0. For plants of PV-1 inoculated and incubated at 24°C, the IP, DII_{final}, and SAUDPC were not significantly different ($P > 0.05$) from values calculated for plants grown at 27°C. However, disease symptoms were first observed 5 days later and developed at a faster rate ($P < 0.05$) at 24°C than at 27°C (Fig. 3, Table 3).

The disease reactions of cultivars PV-1 and JG-62 to wilting isolates Foc-8726 and to races 1A and 6 of *F. oxysporum* f. sp. *ciceris* were more complex than the reactions induced by the yellowing race 0 (Table 3). As observed in the previous experiment, PV-1 was resistant to isolates Foc-8726 and Foc-9620. However, the resistant reaction of PV-1 to race 1A observed at 24°C shifted to a highly susceptible reaction at 27°C in this experiment (Fig. 3, Table 3). For this race–cultivar interaction, incubation at 27°C significantly reduced ($P = 0.030$) the IP by 14 days, and increased the DII_{final} 5.2 times, the SAUDPC 5.8 times, and significantly increased ($P < 0.05$) the *r* 3.8 times, compared with the values calculated for the 24°C treatment (Table 3). Vascular infection was observed on 31.3% of the plants inoculated with race 1A and incubated at 24°C, but 100% of the plants were infected by race 1A at 27°C (Table 3).

The reaction of cultivar JG-62 to inoculation with race 1A and 6 of *F. oxysporum* f. sp. *ciceris* was not influenced by temperature (Table 3). Conversely, significantly ($P = 0.008$) less disease was observed in JG-62 inoculated with isolate Foc-8726 at 27°C compared with that at 24°C, and both of these reactions were significantly ($P < 0.001$) less severe compared with disease reactions caused by race 1A and 6 on this cultivar (Fig. 3, Table 3). Interestingly, disease development at 24°C on JG-62 inoculated with isolate Foc-8726 was similar to that observed on JG-62 plants grown at 25 ± 2°C in the experiment to test reaction of the cultivar Ayala to races of *F. oxysporum* f. sp. *ciceris* (Figs. 2 and 3). Compared with the susceptible disease reaction induced by isolate Foc-8726 on JG-62 at 24°C, incubating the inoculated plants at 27°C reduced DII_{final} and SAUDPC 2.1 and 1.9 times, respectively, but did not reduce the IP (Table 3). However, the incidence of plants with vascular infection was similar for plants grown at the two temperatures (Table 3).

The chickpea cultivar Ayala was resistant to *F. oxysporum* f. sp. *ciceris* race 0 and to isolate Foc-8726, but was susceptible to race 1A and 6. While resistance to

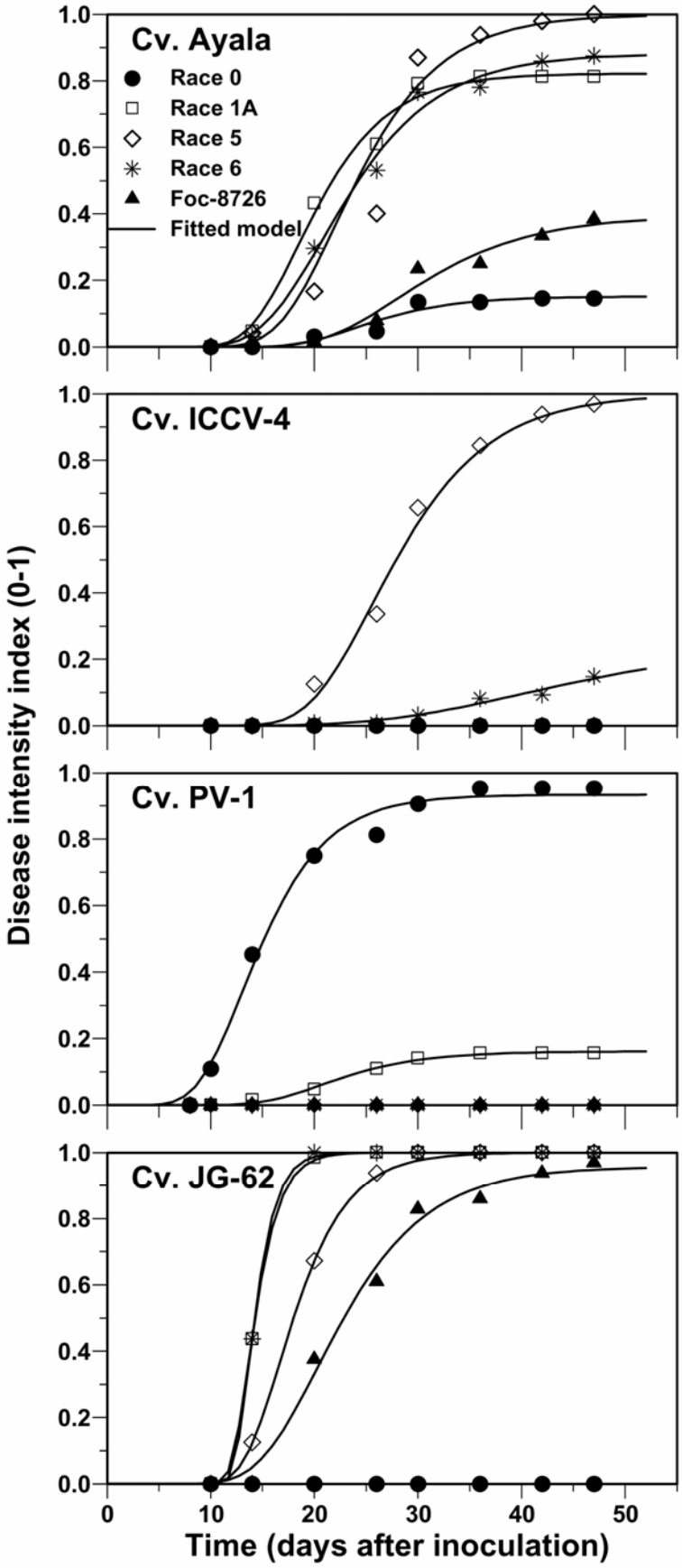

Fig. 2. Progress of Fusarium wilt on the chickpea cultivars Ayala, ICCV-4, PV-1, and JG-62 grown at 25 ± 2°C in soil infested with race 0 (isolate Foc-7802), race 1A (isolate Foc-7989), race 5 (isolate Foc-8012), race 6 (isolate Foc-9620), and isolate Foc-8726 (unknown race) of *Fusarium oxysporum* f. sp. *ciceris*. Each data point is the mean disease intensity index calculated from four pots with four plants per pot. The solid line represents the predicted disease progress curve calculated by the Gompertz function.

the yellowing isolate of race 0 was not influenced by temperature, disease reactions to the three wilting isolates were significantly ($P < 0.05$) influenced by temperature, but the nature of this effect depended on the pathogen isolate. Thus, although Ayala showed complete resistance to isolate Foc-8726 at 27°C (no disease developed and no vascular infection was detected), some disease developed at 24°C (DII_{final} = 0.09), and 43.8% of the plants were infected (Fig. 3, Table 3). On the contrary, Ayala showed a moderately susceptible disease reaction to race 1A and 6 at 24°C, but it was significantly ($P < 0.035$) more susceptible to these two isolates at 27°C (Fig. 3, Table 3).

DISCUSSION

Abiotic factors, including soil moisture and temperature, can significantly influence development of Fusarium wilt of chickpeas (3,9,31,32,40). In particular, temperature may modify plant–pathogen interactions by affecting metabolic processes and development of the plants, as well as pathogen growth and virulence (26,28). Understanding the precise influence of temperature on Fusarium wilt of chickpea is important for management of the disease, e.g., through adjustment of sowing date and the use of host resistance (27,31,32). In this study, we show that temperature may have several important effects on interactions between *F. oxysporum* f. sp. *ciceris* and chickpea. Temperature affected the severity and rate of development of wilt symptoms, as well as the expression of resistance or susceptibility of certain chickpea genotypes to races of the pathogen. Consequently, lack of consideration of the effects of temperature on Fusarium wilt may lead to wrong race and virulence (i.e., the amount of disease caused in a host genotype) characterization of isolates of *F. oxysporum* f. sp. *ciceris.*

Several studies have demonstrated the critical role of temperature on development of Fusarium wilt of chickpea. Chickpea wilt is favored at a temperature range of 20 to 30°C (26,40), with the optimum temperature for disease development between 24.5 and 28.5°C (3,26,40). In previous research (26), we showed that disease caused by *F. oxysporum* f. sp. *ciceris* race 5 on the chickpea cultivar PV-61 can develop over a range of temperatures from 20 to 30°C, and a range of inoculum densities of the pathogen (25 to 1,000 chlamydospores per gram of soil). However, disease severity and the rate of increase in severity were strongly influenced by temperature, with significantly greater disease development at 25°C compared with that at 20 and 30°C (26). Nevertheless, the final expression of susceptibility of PV-61 to *F. oxysporum* f. sp. *ciceris* race 5 was not modified at any of the temperature–inoculum density combinations evaluated in that study.

Conversely, in the present study we demonstrated that temperature had a significant influence on the resistance response of certain chickpea cultivars to races of *F. oxysporum* f. sp. *ciceris*. Under greenhouse conditions, the cultivar Ayala was moderately resistant to *F. oxysporum* f. sp. *ciceris* at a daily temperature regime of 24/21°C, but became highly susceptible at a temperature regime of 27/25°C. Further inoculation experiments conducted in growth chambers adjusted to 24°C versus 27°C confirmed these results and demonstrated that the chickpea cultivars Ayala and PV-1 were moderately resistant and resistant to race 1A of *F. oxysporum* f. sp. *ciceris* at 24°C, respectively. However, both cultivars became highly susceptible to this race of the pathogen at 27°C. A similar but less pronounced effect was found for Ayala inoculated with *F. oxysporum* f. sp. *ciceris* race 6. Interestingly, the disease reaction of the cultivar JG-62 to races 1A and 6 of the fungus was not influenced by temperature. JG-62 is highly susceptible to all races of *F. oxysporum* f. sp. *ciceris* except race 0, the least virulent of the known races, to which JG-62 shows a complete resistance phenotype (12,15, 16,19). Thus, it appears that the effect of temperature on the resistance phenotype is cultivar dependent. Moreover, less disease developed on JG-62 plants inoculated with a variant of *F. oxysporum* f. sp. *ciceris* race 5 (isolate Foc-8726) at 27°C compared with 24°C, which suggests that the effect of temperature on the resistance phenotype may also depend on the race of *F. oxysporum* f. sp. *ciceris*.

Table 2. Reaction of chickpea cultivars to races and isolates of *Fusarium oxysporum* f. sp. *ciceris* in experiments under controlled conditions at 25°C

Cultivar	Race/isolate	Disease assessment (mean ± SE)[a]			$r \pm SE$[b]	Infected plants (%)[c]	Cultivar reaction[d]
		IP	DII_{final}	SAUDPC			
Ayala	0 / Foc-7802	24.0 ± 2.5	0.15 ± 0.04	0.10 ± 0.02	0.17 ± 0.08	46.7	R
	1A / Foc-7989	18.5 ± 1.5	0.81 ± 0.09	0.62 ± 0.07	0.21 ± 0.03	100.0	S
	5 / Foc-8012	17.0 ± 1.7	1.00	0.60 ± 0.02	0.20 ± 0.05	100.0	S
	U[e] / Foc-8726	27.0 ± 3.3	0.48 ± 0.28	0.20 ± 0.06	0.14 ± 0.05	46.7	M
	6 / Foc-9620	21.0 ± 3.3	0.88 ± 0.05	0.65 ± 0.05	0.19 ± 0.02	100.0	S
ICCV-4	0 / Foc-7802	…[f]	0.00	0.00	…	0.0	R
	1A / Foc-7989	…	0.00	0.00	…	0.0	R
	5 / Foc-8012	21.5 ± 1.5	0.97 ± 0.02	0.58 ± 0.07	0.16 ± 0.02	100.0	S
	U / Foc-8726	…	0.00	0.00	…	0.0	R
	6 / Foc-9620	33.5 ± 5.3	0.15 ± 0.07	0.08 ± 0.04	0.07 ± 0.04	12.5	R
PV-1	0 / Foc-7802	22.5 ± 2.5	0.95 ± 0.05	0.62 ± 0.05	0.23 ± 0.03	100.0	S
	1A / Foc-7989	23.0 ± 3.0	0.16 ± 0.09	0.10 ± 0.06	0.18 ± 0.02	12.5	R
	5 / Foc-8012	…	0.00	0.00	…	0.0	R
	U / Foc-8726	…	0.00	0.00	…	0.0	R
	6 / Foc-9620	…	0.00	0.00	…	0.0	R
JG-62	0 / Foc-7802	…	0.00	0.00	…	0.0	R
	1A / Foc-7989	14.0 ± 0.0	1.00	0.87 ± 0.02	0.60 ± 0.03	100.0	S
	5 / Foc-8012	17.0 ± 3.0	1.00	0.82 ± 0.02	0.28 ± 0.01	100.0	S
	U / Foc-8726	20.0 ± 0.0	0.97 ± 0.03	0.67 ± 0.10	0.17 ± 0.03	100.0	S
	6 / Foc-9620	14.0 ± 0.0	1.00	0.87 ± 0.02	0.69 ± 0.08	100.0	S

[a] A disease intensity index (DII) was calculated based on the incidence and severity of Fusarium wilt symptoms recorded at 2- to 3-days intervals. IP = incubation period, estimated as the number of days to reach a DII of 0.05; DII_{final} = disease intensity index at the final date of disease assessment; SAUDPC = area under disease intensity progress curve estimated by the trapezoidal integration method and standardized for the epidemic duration. Data are the means ± standard error (SE) of four replicated pots, each with four plants.

[b] r = rate parameter of the Gompertz model.

[c] After 7 weeks, isolations were made from stem segments of individual plants showing a severity score ≤2 (34 to 66% of foliage with yellowing or necrosis) to determine the incidence of plants with vascular infection.

[d] Average disease reactions of $DII_{final} < 0.25$ and > 0.75 were considered resistant (R) and susceptible (S), respectively. Intermediate DII_{final} scores (≥0.25 and ≤0.75) were considered moderately susceptible reactions.

[e] U = Unknown race.

[f] No disease developed in this combination.

Temperature-sensitive host resistance has been reported for a range of different plant pathogen interactions, e.g., wheat (*Triticum aestivum*) leaf and stripe rusts (2,29,34), powdery mildew (8), and Stagonospora blotch (24); leaf rust of flax (*Linum usitatissimum*) (13), downy mildew of lettuce (*Lactuca sativa*) (23); root-knot nematode (*Meloidogyne* spp.) on tomato (*Lycopersicon esculentum*) and common bean (*Phaseolus vulgaris*) (1,33,41); and *Tomato mosaic virus* in *Gomphrena globosa* (7). However, few studies have focused on the effect of temperature on resistance against Fusarium wilt diseases. Harling et al. (11) found that carnation (*Dianthus caryophyllus*) cultivars scarcely were colonized by *F. oxysporum* f. sp. *dianthi* and remained symptomless at 14 to 15°C, but the cultivars could be differentiated into resistant, partially resistant, and susceptible types at 22°C, depending on the severity of symptoms and the extent of fungal colonization of the plants. However, all cultivars became infected and showed wilt symptoms at 26°C. Similarly, the resistance response of the banana (*Musa* spp.) cultivar Cavendish to Fusarium wilt was affected by temperature, but a differential effect was observed depending on the race of *F. oxysporum* f. sp. *cubense* (5). Foliar symptoms and rhizome discoloration of Cavendish plants caused by race 1 of the pathogen were more severe at 20 than at 28°C, whereas *F. oxysporum* f. sp. *cubense* race 4 induced similar levels of disease at both temperatures (5). The results for chickpea cultivars Ayala and PV-1 in the greenhouse and growth chamber experiments of this study showed similar pathogen race, host cultivar, and temperature interactions.

The underlying mechanism of the effect of temperature on increased susceptibility or resistance of plants to diseases is not clear. Several studies have described cellular and biochemical changes induced by high temperatures in plants, which suggested that high temperatures (22 to 30°C, depending on the study) could alter products of avirulence or resistance genes, their interactions, later steps in the resistance response, or the ability of the pathogen to overcome certain types of host resistance (23,33). Also, various studies have identified resistance genes that are temperature sensitive. Thus, resistance (assessed by the absence of sporulation) in the *Bremia lactucae*/lettuce pathosystem involving resistance genes *Dm6*, *Dm7, Dm11, Dm15,* and

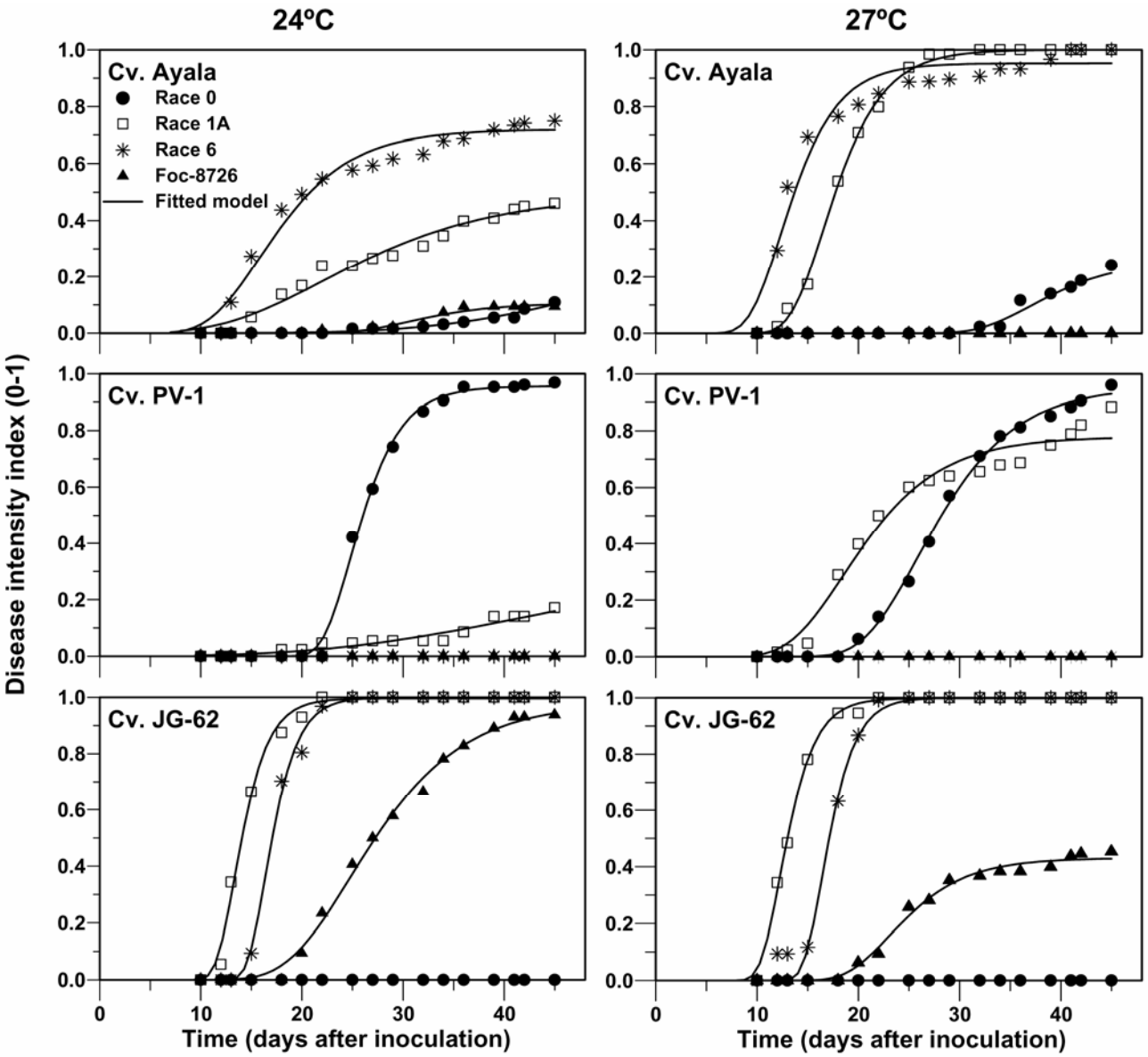

Fig. 3. Effect of temperature on progression of Fusarium wilt disease on the chickpea cultivars Ayala, PV-1, and JG-62 grown in soil infested with race 0 (isolate Foc-7802), race 1A (isolate Foc-7989), race 6 (isolate Foc-9620), and isolate Foc-8726 (unknown race) of *Fusarium oxysporum* f. sp. *ciceris*. Each data point is the mean disease intensity index calculated from four pots and four plants per pot. The solid line represents the predicted disease progress curve calculated by the Gompertz function.

Dm16 and the matching *Avr* genes became less effective at low temperatures (5 to 10°C), while interactions involving eight other pairs of *Dm*/*Avr* genes were insensitive to temperature (23). Recessive resistance to *Meloidogyne incognita* conferred by *me3* was expressed at 26°C, but not at 28°C. However, resistance to *M. javanica* or *M. incognita* conferred by the dominant genes *Me1* or *Me2*, respectively, at 28°C is not observed at 30°C (33). Interestingly, these genes are completely dominant at 26°C, but show an allelic dosage response of incomplete dominance at 28°C. Similarly, the wheat stem rust resistance gene *Sr6* was effective and dominant at 18°C but was not operative at 25°C (29).

Resistance to Fusarium wilt in chickpea has been shown to be race-specific and governed by major resistance genes (38,44,46). Resistance to races 1A and 2 is controlled by at least three independent genes, of which two confer resistance in the recessive form and a third is operative as a dominant allele (25,46). Resistance to race 4 is recessive and monogenic in the cultivar WR-315, but is recessive and digenic in the cultivar Surutato-77 (44,45). Resistance to race 0, the least virulent of the eight races of *F. oxysporum* f. sp. *ciceris,* is conferred by one gene in the cultivar JG-62 and by another gene in the cultivar CA-2139 (36). Also, a recessive gene conferring resistance to race 0, *foc*-0, was identified in line ICC-4958. This line also carries *foc*-5, a single recessive gene that confers resistance against race 5, but *foc*-0 and *foc*-5 are not linked (42). This complexity in chickpea resistance genes to different races of *F. oxysporum* f. sp. *ciceris* may help explain the results of this study, in which the differential responses of resistance phenotypes to temperature depended on the race of *F. oxysporum* f. sp. *ciceris* and the chickpea cultivar.

The influence of temperature on the resistance of chickpea cultivars to Fusarium wilt has important implications on the efficient use of choice of sowing dates and chickpea genotypes in infested soils with known races of the pathogen for which the cultivars may show a temperature-sensitive reaction. Field experiments conducted in microplots artificially infested with races 0 and 5 of *F. oxysporum* f. sp. *ciceris* at Córdoba in southern Spain demonstrated that advancing chickpea sowing from early spring (March) to early winter (December) (i.e., from warm to cooler temperatures) suppressed Fusarium wilt by delaying disease onset and slowing the development of epidemics. However, the usefulness of this effect depended on the level of disease resistance in chickpea cultivars (27,31,32). Similarly, experiments in this study conducted under field conditions in Israel indicated that delaying chickpea sowing from late January (traditional chickpea sowing time in Israel) to late February or early March resulted in the resistant cultivar Ayala being partially affected by Fusarium wilt at the late sowings, but not at the early sowings, in one of the two locations evaluated. Thus, an earlier sowing may ensure escape from Fusarium wilt in some locations. Results from this research confirmed that the choice of sowing date influences development of Fusarium wilt, as reported previously (27,31,32), but may also modify the resistance response of chickpea cultivars depending on the cultivar and

Table 3. Effect of temperature on development of Fusarium wilt on chickpea cultivars inoculated with different races and isolates of *Fusarium oxysporum* f. sp. *ciceris* in experiments under controlled conditions

Cultivar	Race/isolate	T(°C)	Disease assessment (mean ± SE)[a]			Wilks' lambda (*P*)[b]	*r* ± SE[c]	Infected plants (%)[d]	Cultivar reaction[e]
			IP	DII_{final}	SAUDPC				
Ayala	0 / Foc-7802	24	36.8 ± 5.0	0.11 ± 0.07	0.09 ± 0.01	0.498 (0.644)	0.22 ± 0.02	25.0	R
		27	34.0 ± 1.2	0.24 ± 0.08	0.14 ± 0.05		0.20 ± 0.04	25.0	R
	1A / Foc-7989	24	15.7 ± 2.6	0.46 ± 0.22	0.29 ± 0.09	0.039 (0.003)	0.10 ± 0.02	87.5	M
		27	13.4 ± 2.1	1.00	0.81 ± 0.03		0.30 ± 0.01*	100.0	S
	U[f] / Foc-8726	24	29.0 ± 5.1	0.09 ± 0.11	0.07 ± 0.09	…	0.20 ± 0.05*	43.8	R
		27	…[g]	0.00	0.00		…	0.0	R
	6 / Foc-9620	24	12.9 ± 0.8	0.75 ± 0.20	0.57 ± 0.11	0.140 (0.035)	0.20 ± 0.03	100.0	S
		27	10.5 ± 0.5	1.00	0.82 ± 0.04		0.32 ± 0.03*	100.0	S
PV-1	0 / Foc-7802	24	22.4 ± 0.2	0.97 ± 0.04	0.77 ± 0.11	0.376 (0.229)	0.36 ± 0.03*	100.0	S
		27	17.4 ± 4.8	0.96 ± 0.06	0.66 ± 0.07		0.19 ± 0.01	100.0	S
	1A / Foc-7989	24	30.5 ± 8.9	0.17 ± 0.14	0.07 ± 0.06	0.069 (0.030)	0.05 ± 0.03	31.3	R
		27	16.4 ± 4.6	0.88 ± 0.13	0.58 ± 0.20		0.19 ± 0.03*	100.0	S
	U / Foc-8726	24	…	0.00	0.00	…	…	18.8	R
		27	…	0.00			…	0.0	R
	6 / Foc-9620	24	…	0.00	0.00	…	…	43.8	R
		27	…	0.00	0.00		…	31.3	R
JG-62	0 / Foc-7802	24	…	0.00	0.00	…	…	0.0	R
		27	…	0.00	0.00		…	0.0	R
	1A / Foc-7989	24	11.9 ± 1.1	1.00	0.90 ± 0.01	0.533 (0.210)	0.54 ± 0.04	100.0	S
		27	10.6 ± 0.7	1.00	0.90 ± 0.04		0.52 ± 0.03	100.0	S
	U / Foc-8726	24	21.7 ± 3.5	0.94 ± 0.08	0.65 ± 0.15	0.064 (0.008)	0.16 ± 0.01	100.0	S
		27	21.9 ± 2.4	0.45 ± 0.12	0.34 ± 0.10		0.24 ± 0.02*	93.8	M
	6 / Foc-9620	24	14.4 ± 1.0	1.00	0.89 ± 0.02	0.944 (0.866)	0.56 ± 0.03	100.0	S
		27	14.0 ± 2.5	1.00	0.89 ± 0.03		0.54 ± 0.01	100.0	S

[a] A disease intensity index (DII) was calculated based on the incidence and severity of Fusarium wilt symptoms recorded at 2- to 3-days intervals. IP = incubation period, estimated as the number of days to reach a DII of 0.05; DII_{final} = disease intensity index at the final date of disease assessment; SAUDPC = area under disease intensity progress curve estimated by the trapezoidal integration method and standardized for the epidemic duration. Data are the means ± standard error (SE) of four replicated pots, each with four plants.

[b] Wilks' lambda statistic and associated probability (*P*) for the hypothesis of no overall temperature effects after multivariate analysis of variance, for each cultivar–*F. oxysporum* f. sp. *ciceris* isolate combination.

[c] *r* = rate parameter of the Gompertz model. The estimated values and confidence intervals (*P* = 0.05) for the estimated rate of DII increase over time (*r*) were used to compare DII progress curves of different treatments statistically. * Indicates significant differences between inoculated plants incubated at 24°C versus 27°C.

[d] After 7 weeks, isolations were made from stem segments of individual plants showing a severity score ≤2 (34 to 66% of foliage with yellowing or necrosis) to determine the incidence of plants with vascular infection.

[e] Average disease reactions of DII_{final} < 0.25 and > 0.75 were considered resistant (R) and susceptible (S), respectively. Intermediate DII_{final} scores (≥0.25 and ≤0.75) were considered moderately susceptible reactions.

[f] U = Unknown race.

[g] No disease developed in this combination.

the prevalent race of the pathogen in the soil.

In summary, we report for the first time that temperature can affect the relative resistance response of certain chickpea cultivars to races of *F. oxysporum* f. sp. *ciceris* under growth chamber, greenhouse, and field conditions. This demonstrates the importance of temperature in identifying resistant genotypes and races of the pathogen, as well as choosing sowing dates and using resistant chickpea genotypes for the management of Fusarium wilt in different growing areas.

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