Quantitative Modeling of the Effects of Temperature and Inoculum Density of *Fusarium oxysporum* f. sp. *ciceris* Races 0 and 5 on Development of Fusarium Wilt in Chickpea Cultivars

Juan A. Navas-Cortés, Blanca B. Landa, Miguel A. Méndez-Rodríguez, and Rafael M. Jiménez-Díaz

First and third authors: Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Apartado 4084, 14080 Córdoba, Spain; and second and fourth authors: Escuela Técnica Superior de Ingenieros Agrónomos y de Montes (ETSIAM), Universidad de Córdoba (UCO), Apartado 3048, 14080 Córdoba, Spain and IAS-CSIC.

Current address of M. A. Méndez-Rodríguez: Empresa Pública de Desarrollo Agrario y Pesquero, Consejería de Agricultura y Pesca, Junta de Andalucía, 14004 Córdoba, Spain.

Accepted for publication 30 October 2006.

ABSTRACT

Navas-Cortés, J. A., Landa, B. B., Méndez-Rodríguez, M. A., and Jiménez-Díaz, R. M. 2007. Quantitative modeling of the effects of temperature and inoculum density of *Fusarium oxysporum* f. sp. *ciceris* races 0 and 5 on development of Fusarium wilt in chickpea cultivars. *Phytopathology* 97:564-573.

Races 0 (*Foc-0*) and 5 (*Foc-5*) of *Fusarium oxysporum* f. sp. *ciceris* differ in virulence and induce yellowing or wilting syndrome, respectively, in chickpea. We modeled the combined effects of soil temperature and inoculum density of *Foc-0* and *Foc-5* on disease developed in chickpea cvs. P-2245 and PV-61 differing in susceptibility to those races, using quantitative nonlinear models. Disease development over time in the temperature range of 10 to 30°C and inoculum densities between 6 and 8,000 chlamydospores g⁻¹ of soil was described by the Weibull function. Four response variables (the reciprocal incubation period, the final disease intensity, the standardized area under the disease progress curve, and the intrinsic rate of disease development) characterized the

disease development. Response surface models that expressed the combined effect of inoculum density and temperature were developed by substituting the intrinsic rate of disease development in the Weibull or exponential functions with a beta function describing the relationship of response variables to temperature. The models estimated 22 to 26°C as the most favorable soil temperature for infection of cvs. P-2245 and PV-61 by *Foc-5*, and 24 to 28°C for infection of cv. P-2245 by *Foc-0*. At 10°C, no disease developed except in cv. P-2245 inoculated with *Foc-5*. At optimum soil temperature, maximum disease intensity developed with *Foc-5* and *Foc-0* at 6 and 50 chlamydospores g⁻¹ of soil respectively, in cv. P-2245, and with *Foc-5* at 1,000 chlamydospores g⁻¹ of soil in cv. PV-61. The models were used to construct risk threshold charts that can be used to estimate the potential risk of Fusarium wilt epidemics in a geographical area based on soil temperature, the race and inoculum density in soil, and the level of susceptibility of the chickpea cultivar.

Additional keywords: *Cicer arietinum*.

Fusarium wilt, caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *ciceris* (Padwick) Matuo & K. Sato, is the most important soilborne disease of chickpea (*Cicer arietinum* L.) throughout the world, particularly in the Indian subcontinent, the Mediterranean region, and California (15,17). Two pathotypes exhibiting differential symptoms, namely yellowing and wilting, exist in *F. oxysporum* f. sp. *ciceris* populations (39). The yellowing pathotype induces progressive foliar yellowing with vascular discoloration, while the wilting pathotype induces rapid and severe chlorosis, flaccidity, and vascular discoloration (39). In addition to these two pathotypes, there exist eight races (race 0, 1A, 1B/C, 2, 3, 4, 5, and 6) of *F. oxysporum* f. sp. *ciceris* that can be identified based on the disease reactions of a set of differential chickpea cultivars (14,18,20). Races 0 and 1B/C belong to the yellowing pathotype, whereas the remaining races form the wilting pathotype (20,23,24). The eight races also have a distinct geographic distribution. Races 2, 3, and 4 have been reported only in India (14), whereas races 0, 1B/C, 5, and 6 are found mainly in the Mediterranean region and California (12,18,23). Unlike the other races, race 1A is more widespread and has been reported in India, California, and the Mediterranean region (14,16,23). An intraspecific phylogeny of *F. oxysporum* f. sp. *ciceris* races in-

ferred from DNA fingerprinting with repetitive sequences indicated that each of the eight races forms a monophyletic lineage and that they have evolved in a simple stepwise pattern, with race 0 being hypothesized as ancestor of the wilting races (22).

Studies under controlled conditions indicated that Fusarium wilt of chickpea increases with decreasing soil matric potential, and that disease can develop severely at 25 and 30°C, but not at 15 and 20°C, with an inoculum density of *F. oxysporum* f. sp. *ciceris* at 500 and 1,000 propagules g⁻¹ of soil (3). Similarly, Sugha et al. (38) observed that an increase in inoculum density accelerated the development of chickpea wilt whereas low inoculum densities delayed the expression of wilt symptoms. However, in all of the above studies, the nature of the pathogen race was not known. In a recent study, the development of Fusarium wilt caused by race 5 was strongly affected by the incubation temperature (25). Thus, the rate of disease development was faster and final severity was higher at 25°C than at 20 and 30°C, and there was a significant incubation temperature-pathogen inoculum density interaction on disease development. Although the influence of inoculum density on disease development at 25°C was negligible, increasing inoculum densities significantly increased final disease severity at 20 and 30°C.

Differences in virulence among *F. oxysporum* f. sp. *ciceris* races bear significance in terms of disease development and management under field conditions. At equal inoculum densities, epidemics caused by race 5 develop earlier and more rapidly, and produce significantly greater yield loss, compared with those

Corresponding author: J. A. Navas-Cortés; E-mail address: aglnacoj@uco.es

doi:10.1094/PHYTO-97-5-0564

© 2007 The American Phytopathological Society

caused by race 0 (30,32). However, the effectiveness of wilt management practices, such as the use of resistant cultivars, choice of sowing dates (17,27,31), and biocontrol (26), is influenced by pathogen race (14,18,26,31).

Quantifying the relationships and effects of pathogen, plant, and environmental factors on disease development by means of quantitative models can help in the design and efficient use of management strategies for *Fusarium* wilt of chickpea and other *Fusarium* wilt diseases. However, such a quantitative modeling approach is mostly lacking for *Fusarium* wilt diseases. Therefore, the objectives of this study were to quantify the combined effects of biotic (a range of virulence, inoculum density, and cultivar susceptibility) and abiotic (soil temperature) factors on development of *Fusarium* wilt in chickpea. The data then were analyzed to develop models to assess combinations of temperature and inoculum density under which *Fusarium* wilt epidemics can be predicted in certain chickpea cultivars; in addition, risk thresholds for these predictions were established. Furthermore, how the virulence of races inducing yellowing and wilting influences these relationships also was deduced from the models.

MATERIALS AND METHODS

Fungal isolates and inoculum production. Monoconidial cultures of *F. oxysporum* f. sp. *ciceris* isolates Foc 7802 (race 0, *Foc*-0) and Foc 8012 (race 5, *Foc*-5) were obtained from infected chickpea in southern Spain and are well characterized (25,30). Selection of these races for the study was based on (i) their widespread distribution throughout the Mediterranean region, (ii) the large difference in virulence between them, and (iii) their belonging to different pathotypes (i.e., yellowing and wilting, for *Foc*-0 and *Foc*-5, respectively) (25,30). Isolates were stored in sterile soil tubes at 4°C. Active cultures of the isolates were obtained by placing aliquots of a soil culture onto a plate of fresh potato dextrose agar (PDA) (250 g of unpeeled potato, 20 g of agar, and 20 g of glucose liter⁻¹ of distilled water) and incubating for 5 days at 25°C with a 12-h photoperiod of fluorescent and near-UV light at 36 µE m⁻²s⁻¹.

Inoculum for the experiments consisted of chlamydospores produced in a sterile salt solution. Microconidia were produced in fresh potato dextrose broth in 250-ml flasks on an orbital shaker (Adolf Kühner AG, Birsfelden, Switzerland) adjusted at 140 rpm, and the same temperature and light conditions as PDA cultures for 7 days. Microconidia harvested by centrifugation (6,800 × g, 20 min at 4°C) were suspended to a final concentration of 1 × 10⁷ microconidia ml⁻¹ in 500 ml of a sterile salt solution ((NH₄)₂SO₄, 300 mg; KH₂PO₄, 120 mg; K₂HPO₄, 480 mg; and D-glucose, 1.8 g/liter of distilled water) (2; J. A. Navas-Cortés, M. A. Méndez-Rodríguez, and R. M. Jiménez-Díaz, *unpublished data*) in 1,000-ml flasks and incubated at 25°C in the dark for 4 weeks. The production of chlamydospores of *F. oxysporum* f. sp. *ciceris* in the salt solution was confirmed by microscopic observations. Chlamydospores were harvested by centrifugation (6,800 × g, 20 min at 4°C), washed free of nutrients, and suspended in deionized, sterile water. Chlamydospore suspension were used to infest 25 g of heat-sterilized talcum powder. The infested talcum then was dried at 37°C for 5 to 7 days and stored at 4°C until use. The amount of inoculum in the infested talcum powder was estimated by the number of CFU per gram of talcum. The number of CFU was determined by dilution plating on V8 juice-oxgall-pentachloronitrobenzene agar (VOPA) *Fusarium*-selective medium (4,25,30).

Chickpea plants and inoculation. ‘Kabuli’ (large, ram-head shaped, beige seed) chickpea cvs. P-2245 and PV-61 used in the Mediterranean region were used. Cultivar P-2245 is highly susceptible to both *Foc*-0 and *Foc*-5, whereas cv. PV-61 is moderately resistant to *Foc*-0 and susceptible to *Foc*-5 (19,20). Seed were surface disinfested in 2.5% NaOCl for 3 min and

germinated on autoclaved layers of filter paper in moist chambers at 25°C for 48 h. Germinated seed, selected for uniformity (length of radicle = 1 to 2 cm), were sown into 15-cm-diameter clay pots (four plants per pot) filled with an autoclaved (121°C, 1 h, twice, on two consecutive days) soil mixture (clay loam/peat, 2:1, vol/vol), artificially infested with *Foc*-0 or *Foc*-5.

Effect of incubation temperature and inoculum density of *F. oxysporum* f. sp. *ciceris*. Plants were incubated in soil tanks (Environmental Growth Chambers; Integrated Development & MFG/Chagrin, Fall, OH) placed inside a walk-in growth chamber adjusted to 25 ± 1°C and a 14-h photoperiod of fluorescent light at 360 µE m⁻²s⁻¹ for 60 days. Pots with soil and plant roots were set inside the soil tanks at constant temperature of 10, 15, 20, 25, and 30°C, with a maximum variation of ±1°C for all temperatures. Plants in a pot were watered daily and fertilized with 100 ml of a 0.1%, 20-5-32+micronutrients hydro-sol fertilizer (Haifa Chemicals, Ltd., Haifa, Israel) solution every week. Upon termination of experiments, isolations were made from stem segments of symptomless plants to determine the occurrence of vascular infections. Stem pieces of individual plants were cut into 5- to 10-mm-long segments, surface disinfested (0.2% NaOCl for 2 min), plated on VOPA, and incubated at 25°C and a 12-h photoperiod for 3 to 5 days (25,30).

The infested talcum powder was mixed in proportion with the autoclaved soil mixture to achieve an inoculum density of 0, 24, 50, 100, 200, 500, 1,000, 2,000, and 8,000 chlamydospores g⁻¹ of soil for cv. P-2245-*Foc*-0; 0, 6, 24, 50, 100, 500, 1,000, 2,000, and 5,000 for cv. P-2245-*Foc*-5; and 0, 6, 24, 50, 100, 1,000, and 5,000 for cv. PV-61-*Foc*-5. Soil in pots that served as controls or with pathogen inoculum densities <5,000 (*Foc*-5) or 8,000 (*Foc*-0) chlamydospores g⁻¹ of soil were amended with an amount of uninfested talcum powder equivalent to that of infested powder used for the highest pathogen inoculum density. The inoculum density of *Foc*-0 and *Foc*-5 in the infested soil mixture, estimated as CFU per gram of soil, was determined just after sowing by dilution plating as previously described (25,30). Estimated values of inoculum densities corresponded to those theoretically established and the number of CFU achieved for equal inoculum densities did not differ significantly among the experimental treatments. Separate experiments for each chickpea cultivar-pathogen race combination were performed using a completely randomized split-plot design, with temperature as the main plot factor and pathogen inoculum density level as the subplot factor. There were four replications (four pots with four plants each) of each treatment combination. In each separate experiment, the combination of chickpea cv. P-2245 and 1,000 chlamydospores g⁻¹ of soil of each of *F. oxysporum* f. sp. *ciceris* races 0 and 5 incubated at 25°C air and soil temperature also were included to test reproducibility of results across experiments. Each experiment was repeated once and preliminary analyses of variance of treatments suggested no significant differences (*P* > 0.05) among experiments. Therefore, pooled data from the experiments were analyzed.

Disease assessment and data analyses. The incidence (0 = plant showing no disease symptoms and 1 = plant showing disease symptoms) and severity of symptoms were assessed at 2- to 3-day intervals. Severity was assessed on a 0-to-4 rating scale based on the percentage of foliage with yellowing or wilting in acropetal progression (0 = 0%, 1 = 1 to 33%, 2 = 34 to 66%, 3 = 67 to 100%, and 4 = dead plant). Incidence and severity were used to calculate a disease intensity index (DII) for each pot (25,30) using the equation $DII = (\sum S_i \times N_i) / (N_t \times 4)$; where *S_i* is the symptoms severity, *N_i* is the number of plants with *S_i* symptoms severity, and *N_t* is the total number of plants. Thus, DII expresses the mean value of disease intensity at any given moment as a proportion of the maximum possible amount of disease.

Temporal analysis. Disease progress curves (DPCs) were obtained from the accumulated DII over time in days from the

date of sowing. The nonlinear form of the Weibull model was evaluated for goodness-of-fit to the set of DII progress data using nonlinear regression analysis. The Weibull model (equation 1) is a generalized simple model that includes both rate and shape parameters allowing it to model a wide variety of response curves (29,33). In the Weibull equation:

$$DII(t) = A_W(1 - \exp\{-[B_W(t - C_W)]^{D_W}\}) \quad (1)$$

where DII = disease intensity index, A_W = upper asymptote parameter (i.e., upper limit of DII), B_W = scale parameter (i.e., intrinsic rate of DII increase over time), C_W = location parameter (i.e., time lag before the onset of symptom development), D_W = shape parameter (i.e., location of inflection point), and t = time of disease assessment in days after inoculation (10,33). For analyses, non-zero points for DII in experimental units and the average DII values of the replicated blocks were used.

Relationship between disease development and DPC associated variables. To further examine disease development, four additional variables associated with DPC were examined. These included (i) the incubation period (IP), established as the time in days to initial symptoms, or its reciprocal ($IP_R = 1/IP$); (ii) the final disease intensity (DII_{final}); (iii) the standardized area under the DII progress curve (SAUDPC) calculated by trapezoidal integration method standardized by duration of disease development in days (6,27,31); and (iv) B_W .

Response surface models. To describe the effects of temperature (T) and inoculum density (ID) on the DPC-associated variables (IP_R , B_W , DII_{final} , and SAUDPC) for the different chickpea cultivar-pathogen race interactions, response surface models were fitted to data using nonlinear regression analysis following two steps. In the first step, two models were used to evaluate the effect of ID on DPC-associated variables. The Weibull model (equation 2) was used to represent the effect of ID on IP_R , DII_{final} , and SAUDPC:

$$Y_{ID} = f(ID) = A(1 - \exp\{-(r[\log(ID) - C])^D\}) \quad (2)$$

where Y is the response of IP_R , DII_{final} , or SAUDPC to ID, A is the upper limit of the response, r is the intrinsic rate of increase in the response, C is the minimum ID value for the response, and D is the portion of the ID range in which the response decelerate.

Similarly, the exponential model (equation 3) was used to represent the effect of ID on B_W :

$$Y_{ID} = f(ID) = E \exp[r \log(ID)] \quad (3)$$

where Y_{ID} is the response of B_W to ID, E is a constant, and r is the rate of the response increase.

The response of the four DPC-associated variables to T was described by the modified β function (equation 4) (13,26):

$$Y_T = f(T) = G \frac{[(T - T_{min})/(T_{opt} - T_{min})]^{H \times (T_{opt} - T_{min})/(T_{max} - T_{opt})}}{[(T_{max} - T)/(T_{max} - T_{opt})]^H} \quad (4)$$

In this equation, Y_T is the response of the DPC-associated variables to temperature, and T_{max} and T_{min} were fixed to 37 and 5°C, respectively, which are known maximum and minimum temperatures for growth of *F. oxysporum* f. sp. *ciceris* isolates (9,26). The shape parameter, H , determines the temperature range near the optimum temperature, T_{opt} , at which the response values are close to the maximum response G .

In the second step, the combined effect of ID and T on IP_R , DII_{final} , and SAUDPC were characterized by substituting r in equation 2 with a description of the response to temperature (equation 5)

$$Y_{T,ID} = f(T,ID) = A\{1 - \exp[-f(T)(\log(ID) - C)]^D\} \quad (5)$$

in which $f(T)$ is given by equation 4. Similarly, the combined effect of ID and T on B_W were characterized by substituting r in equation 3 with a description of the response to temperature (equation 6):

$$Y_{T,ID} = f(T,ID) = E \exp[f(T) \log(ID)] \quad (6)$$

in which $f(T)$ is given by equation 4.

All regression analyses were conducted using the Levenberg-Marquardt's nonlinear least-squares iterative procedure of SPSS Software (version 12.0; SPSS Inc., Chicago). All derivatives were calculated numerically. The coefficient of determination (R^2), mean square error, standard errors associated with the parameter estimates, confidence intervals of predicted values, and pattern of the standardized residuals plotted against either predicted values or the independent variable were used to evaluate the appropriateness of a model to describe the data (6).

Risk chart models. Curves derived from equations 5 and 6 for the range of temperature and inoculum density required to attain thresholds values of the disease response variables (IP , SAUDPC, and B_W) were used to obtain a chart for predicting risk of Fusarium wilt development for each chickpea cultivar-*F. oxysporum* f. sp. *ciceris* combination.

RESULTS

Treatment effects. Soil temperature, chickpea cultivar, and race and inoculum density of *F. oxysporum* f. sp. *ciceris* influenced the development of Fusarium wilt. Neither disease symptoms nor infection were observed in cv. PV-61 grown in soil infested with *Foc-0*, in spite of the broad range of inoculum densities and soil temperatures tested; similarly, no disease symptoms or infection were found at 10°C in cv. PV-61 grown in soil infested with *Foc-5* and cv. P-2245 grown in soil infested with *Foc-0*. For the remaining chickpea cultivar-pathogen race interactions, progression of DII over time varied with soil temperature and increased steadily with the increasing inoculum density (Table 1; Fig. 1). Overall, development of Fusarium wilt was optimum between 20 and 25°C for *Foc-5* and between 25 and 30°C for *Foc-0*, with an interaction occurring between soil temperature and pathogen inoculum density (Table 1; Fig. 1). The increase in DII over time was adequately described by the Weibull model ($R^2 > 0.85$) for all chickpea cultivar-*F. oxysporum* f. sp. *ciceris* race-temperature-inoculum density combinations (Fig. 1).

At the optimum soil temperature indicated above and highest inoculum density, symptoms in the compatible interactions started to develop at 15 to 18 days after inoculation. At 10°C, symptoms developed only in cv. P-2245 grown in soil infested with *Foc-5* at any inoculum density, and required at least 39 days of incubation. At 15 to 30°C of soil temperature, the IP varied with the cultivar-race interactions and ranged from 15 to 38 days in cv. P-2245-*Foc-5*, from 17 to 48 days in cv. P-2245-*Foc-0*, and from 18 to 40 days in cv. PV-61-*Foc-5*, with IP decreasing as the inoculum density increased. The IP for infection by *Foc-5* always was smaller in cv. P-2245 than in cv. PV-61 for similar inoculum densities and incubation temperatures. The difference between the IP in these two cultivars decreased with the increase of *Foc-5* inoculum density. Also, for a comparable range of inoculum densities of the two *F. oxysporum* f. sp. *ciceris* races, the greatest IP always occurred in cv. P-2245 grown in soil infested with the yellowing-inducing *Foc-0*, regardless of soil temperature (Table 1; Fig. 2).

At constant soil temperature, the time lag to symptom expression in the plants, indicated by the reciprocal of the incubation period (IP_R), increased with the increase in inoculum density according to the Weibull model. At constant inoculum density, the beta function described well the IP_R increase with temperature, but tended to underestimate the IP_R in some combinations (Fig. 2).

The interaction between chickpea cultivar and *F. oxysporum* f. sp. *ciceris* race strongly influenced Fusarium wilt severity (assessed both by DII_{final} and SAUDPC). In the cv. P-2245-*Foc-5* interaction, disease intensity reached asymptote (i.e., ≥ 0.9) at all

inoculum density–soil temperature combinations studied, except for plants incubated at 10°C (DII_{final} ranged from 0.3 to 0.8) or at 30°C at the lowest inoculum density (6 chlamydospores g⁻¹ of soil) (DII_{final} = 0.5) (Table 1). When this same cv. P-2245 was inoculated with the *Foc*-0 race, the values of DII_{final} ≥ 0.9 were attained at the lowest inoculum density tested (24 chlamydospores g⁻¹ of soil) only when plants were incubated at 20, 25, and 30°C. Conversely, at 15°C, the DII_{final} ranged from 0.5 to 0.8 for ID values >24 chlamydospores g⁻¹ of soil, but no disease devel-

oped in plants incubated at 10°C (Table 1; Fig. 1). In contrast, for the cv. PV-61–*Foc*-5 interaction, a DII_{final} ≥ 0.9 was attained only when inoculum density in soil was >100 and 1,000 chlamydospores g⁻¹ of soil at 20 and 25°C, respectively (Table 1; Fig. 1).

SAUDPC also was influenced by chickpea cultivar and *F. oxysporum* f. sp. *ciceris* race. At constant soil temperature, DII_{final} and SAUDPC increased with the increase in inoculum density, and this increase was well described by the Weibull model (Fig. 3). Similar patterns of variation were found for both

TABLE 1. Effect of soil temperature (ST) on development of *Fusarium* wilt in chickpea cvs. P-2245 and PV-61 sown in soil infested with different inoculum densities (IDs) of *Fusarium oxysporum* f. sp. *ciceris* races 0 and 5

ST, ID ^b	Elements of disease progress curves ^a											
	P-2245– <i>F. oxysporum</i> f. sp. <i>ciceris</i> race 5				P-2245– <i>F. oxysporum</i> f. sp. <i>ciceris</i> race 0				PV-61– <i>F. oxysporum</i> f. sp. <i>ciceris</i> race 5			
	IP ± SE	DII _{final} ± SE	SAUDPC ± SE	B _W ± SE	IP ± SE	DII _{final} ± SE	SAUDPC ± SE	B _W ± SE	IP ± SE	DII _{final} ± SE	SAUDPC ± SE	B _W ± SE
10												
6	44.9 ± 2.3	0.52 ± 0.16	0.35 ± 0.12	0.018 ± 0.001	na	na	na	na	...	0.00 ± 0.00	0.00 ± 0.00	...
24	51.6 ± 2.1	0.30 ± 0.10	0.16 ± 0.03	0.021 ± 0.001	...	0.00 ± 0.00	0.00 ± 0.00	...	...	0.00 ± 0.00	0.00 ± 0.00	...
50	44.5 ± 2.0	0.45 ± 0.06	0.28 ± 0.05	0.024 ± 0.001	...	0.00 ± 0.00	0.00 ± 0.00	...	...	0.00 ± 0.00	0.00 ± 0.00	...
100	48.9 ± 1.6	0.60 ± 0.17	0.35 ± 0.12	0.020 ± 0.001	...	0.00 ± 0.00	0.00 ± 0.00	...	...	0.00 ± 0.00	0.00 ± 0.00	...
200	na	na	na	na	...	0.00 ± 0.00	0.00 ± 0.00	...	na	na	na	na
500	40.8 ± 5.8	0.70 ± 0.14	0.37 ± 0.10	0.024 ± 0.001	...	0.00 ± 0.00	0.00 ± 0.00	...	na	na	na	na
1,000	41.5 ± 2.8	0.72 ± 0.12	0.41 ± 0.10	0.026 ± 0.001	...	0.00 ± 0.00	0.00 ± 0.00	...	...	0.00 ± 0.00	0.00 ± 0.00	...
2,000	41.7 ± 2.3	0.66 ± 0.11	0.42 ± 0.05	0.025 ± 0.002	...	0.00 ± 0.00	0.00 ± 0.00	...	na	na	na	na
5,000	38.7 ± 2.3	0.84 ± 0.06	0.54 ± 0.06	0.033 ± 0.001	na	na	na	na	...	0.00 ± 0.00	0.00 ± 0.00	...
8,000	na	na	na	na	...	0.00 ± 0.00	0.00 ± 0.00	...	na	na	na	na
15												
6	33.1 ± 1.2	0.98 ± 0.02	0.64 ± 0.07	0.045 ± 0.001	na	na	na	na	...	0.00 ± 0.00	0.00 ± 0.00	...
24	32.4 ± 0.9	1.00 ± 0.00	0.73 ± 0.02	0.054 ± 0.001	38.4 ± 2.2	0.50 ± 0.17	0.25 ± 0.09	0.020 ± 0.001	39.8 ± 6.3	0.20 ± 0.09	0.11 ± 0.03	0.008 ± 0.001
50	32.6 ± 0.9	1.00 ± 0.00	0.70 ± 0.01	0.053 ± 0.001	46.0 ± 1.1	0.47 ± 0.12	0.27 ± 0.05	0.021 ± 0.001	40.0 ± 4.7	0.20 ± 0.20	0.09 ± 0.09	0.013 ± 0.001
100	36.1 ± 1.4	0.98 ± 0.02	0.70 ± 0.06	0.047 ± 0.001	42.8 ± 2.4	0.52 ± 0.14	0.34 ± 0.22	0.019 ± 0.001	38.8 ± 3.1	0.27 ± 0.24	0.17 ± 0.15	0.039 ± 0.001
200	na	na	na	na	45.1 ± 1.4	0.65 ± 0.03	0.37 ± 0.08	0.032 ± 0.001	na	na	na	na
500	34.1 ± 1.3	0.99 ± 0.01	0.69 ± 0.06	0.044 ± 0.001	47.5 ± 2.2	0.69 ± 0.12	0.37 ± 0.15	0.019 ± 0.001	na	na	na	na
1,000	34.1 ± 0.8	0.99 ± 0.01	0.78 ± 0.02	0.064 ± 0.011	37.4 ± 1.4	0.78 ± 0.05	0.43 ± 0.02	0.031 ± 0.001	36.0 ± 2.9	0.29 ± 0.13	0.16 ± 0.06	0.031 ± 0.001
2,000	33.0 ± 0.9	1.00 ± 0.00	0.78 ± 0.02	0.060 ± 0.001	37.4 ± 2.5	0.84 ± 0.13	0.50 ± 0.21	0.023 ± 0.001	na	na	na	na
5,000	34.2 ± 1.1	1.00 ± 0.00	0.73 ± 0.02	0.067 ± 0.002	na	na	na	na	26.5 ± 4.6	0.41 ± 0.24	0.25 ± 0.15	0.029 ± 0.002
8,000	na	na	na	na	39.8 ± 2.3	0.76 ± 0.12	0.45 ± 0.22	0.054 ± 0.011	na	na	na	na
20												
6	21.3 ± 0.8	1.00 ± 0.00	0.85 ± 0.03	0.050 ± 0.002	na	na	na	na	...	0.00 ± 0.00	0.00 ± 0.00	...
24	17.4 ± 0.4	1.00 ± 0.00	0.90 ± 0.01	0.082 ± 0.001	30.3 ± 1.6	0.99 ± 0.00	0.64 ± 0.05	0.043 ± 0.006	23.2 ± 2.9	0.34 ± 0.03	0.23 ± 0.04	0.024 ± 0.001
50	17.4 ± 0.6	1.00 ± 0.00	0.89 ± 0.01	0.080 ± 0.004	27.4 ± 0.9	1.00 ± 0.00	0.82 ± 0.03	0.064 ± 0.001	25.5 ± 2.2	0.88 ± 0.06	0.55 ± 0.07	0.033 ± 0.001
100	17.5 ± 0.6	1.00 ± 0.00	0.89 ± 0.01	0.095 ± 0.034	27.8 ± 1.0	1.00 ± 0.00	0.76 ± 0.03	0.054 ± 0.001	22.9 ± 1.4	0.91 ± 0.04	0.55 ± 0.06	0.043 ± 0.001
200	na	na	na	na	23.6 ± 0.9	1.00 ± 0.00	0.84 ± 0.02	0.040 ± 0.001	na	na	na	na
500	15.4 ± 0.4	1.00 ± 0.00	0.91 ± 0.01	0.130 ± 0.001	24.5 ± 1.0	1.00 ± 0.00	0.84 ± 0.01	0.074 ± 0.001	na	na	na	na
1,000	16.1 ± 0.6	1.00 ± 0.00	0.88 ± 0.02	0.151 ± 0.001	24.5 ± 0.8	1.00 ± 0.00	0.85 ± 0.03	0.075 ± 0.001	20.3 ± 1.5	1.00 ± 0.00	0.75 ± 0.01	0.072 ± 0.001
2,000	16.4 ± 0.4	1.00 ± 0.00	0.91 ± 0.01	0.172 ± 0.002	22.9 ± 0.5	1.00 ± 0.00	0.88 ± 0.03	0.086 ± 0.002	na	na	na	na
5,000	15.1 ± 0.4	1.00 ± 0.00	0.90 ± 0.02	0.175 ± 0.001	na	na	na	na	18.4 ± 0.3	1.00 ± 0.00	0.92 ± 0.02	0.082 ± 0.003
8,000	na	na	na	na	21.9 ± 1.0	1.00 ± 0.00	0.80 ± 0.02	0.085 ± 0.001	na	na	na	na
25												
6	19.1 ± 0.7	1.00 ± 0.00	0.86 ± 0.01	0.064 ± 0.011	na	na	na	na	29.5 ± 1.0	0.15 ± 0.09	0.13 ± 0.07	0.030 ± 0.001
24	19.3 ± 1.1	1.00 ± 0.00	0.88 ± 0.02	0.103 ± 0.001	31.1 ± 1.2	0.98 ± 0.02	0.64 ± 0.05	0.041 ± 0.001	25.3 ± 1.3	0.83 ± 0.09	0.57 ± 0.11	0.051 ± 0.015
50	16.6 ± 0.5	1.00 ± 0.00	0.93 ± 0.01	0.166 ± 0.004	25.9 ± 1.9	1.00 ± 0.00	0.81 ± 0.08	0.075 ± 0.001	21.4 ± 1.1	0.83 ± 0.10	0.63 ± 0.06	0.062 ± 0.020
100	19.4 ± 1.2	1.00 ± 0.00	0.86 ± 0.04	0.105 ± 0.030	26.1 ± 0.7	1.00 ± 0.00	0.80 ± 0.03	0.062 ± 0.015	19.4 ± 0.9	0.84 ± 0.09	0.68 ± 0.11	0.093 ± 0.003
200	na	na	na	na	20.6 ± 0.6	1.00 ± 0.00	0.90 ± 0.02	0.098 ± 0.013	na	na	na	na
500	15.3 ± 0.4	1.00 ± 0.00	0.93 ± 0.01	0.134 ± 0.021	23.1 ± 1.0	1.00 ± 0.00	0.83 ± 0.03	0.083 ± 0.001	na	na	na	na
1,000	16.6 ± 0.6	1.00 ± 0.00	0.93 ± 0.01	0.142 ± 0.010	21.1 ± 0.7	1.00 ± 0.00	0.89 ± 0.03	0.118 ± 0.043	21.5 ± 0.6	1.00 ± 0.00	0.84 ± 0.04	0.079 ± 0.029
2,000	15.0 ± 0.3	1.00 ± 0.00	0.94 ± 0.01	0.248 ± 0.068	18.3 ± 0.4	1.00 ± 0.00	0.91 ± 0.01	0.140 ± 0.023	na	na	na	na
5,000	18.3 ± 1.3	1.00 ± 0.00	0.91 ± 0.03	0.273 ± 0.001	na	na	na	na	18.9 ± 0.3	1.00 ± 0.00	0.92 ± 0.01	0.131 ± 0.005
8,000	na	na	na	na	16.6 ± 0.4	1.00 ± 0.00	0.91 ± 0.02	0.160 ± 0.004	na	na	na	na
30												
6	37.8 ± 4.2	0.49 ± 0.16	0.25 ± 0.09	0.016 ± 0.001	na	na	na	na	31.5 ± 4.6	0.09 ± 0.06	0.07 ± 0.04	0.029 ± 0.001
24	27.0 ± 3.3	0.96 ± 0.04	0.60 ± 0.11	0.044 ± 0.004	24.5 ± 1.3	1.00 ± 0.00	0.76 ± 0.10	0.074 ± 0.001	32.3 ± 4.8	0.20 ± 0.09	0.12 ± 0.04	0.023 ± 0.001
50	17.5 ± 1.1	1.00 ± 0.00	0.85 ± 0.05	0.100 ± 0.011	23.4 ± 1.2	0.94 ± 0.06	0.78 ± 0.05	0.069 ± 0.008	22.8 ± 4.3	0.16 ± 0.06	0.13 ± 0.05	0.033 ± 0.001
100	20.3 ± 1.7	1.00 ± 0.00	0.76 ± 0.06	0.063 ± 0.001	21.8 ± 1.0	1.00 ± 0.00	0.83 ± 0.03	0.078 ± 0.001	21.6 ± 2.1	0.09 ± 0.06	0.07 ± 0.05	0.038 ± 0.003
200	na	na	na	na	17.3 ± 0.6	1.00 ± 0.00	0.88 ± 0.02	0.077 ± 0.010	na	na	na	na
500	15.1 ± 0.4	1.00 ± 0.00	0.90 ± 0.02	0.170 ± 0.006	22.6 ± 2.2	0.98 ± 0.02	0.73 ± 0.12	0.075 ± 0.002	na	na	na	na
1,000	15.9 ± 0.4	1.00 ± 0.00	0.91 ± 0.02	0.200 ± 0.001	18.5 ± 0.7	1.00 ± 0.00	0.89 ± 0.01	0.100 ± 0.001	20.2 ± 2.1	0.37 ± 0.08	0.26 ± 0.06	0.039 ± 0.001
2,000	15.1 ± 0.4	1.00 ± 0.00	0.95 ± 0.01	0.220 ± 0.003	18.1 ± 0.6	1.00 ± 0.00	0.92 ± 0.01	0.104 ± 0.001	na	na	na	na
5,000	15.0 ± 0.3	1.00 ± 0.00	0.92 ± 0.01	0.231 ± 0.002	na	na	na	na	17.8 ± 0.6	0.48 ± 0.12	0.37 ± 0.08	0.045 ± 0.001
8,000	na	na	na	na	16.8 ± 1.0	1.00 ± 0.00	0.88 ± 0.01	0.130 ± 0.002	na	na	na	na

^a IP = incubation period, SE = standard error, DII_{final} = disease intensity index (DII) determined at the final date of disease assessment, SAUDPC = area under disease intensity progress curve estimated by the trapezoidal integration method standardized by duration time in days, B_W = estimated values of the intrinsic rate parameter of the Weibull model adjusted to DII progress over time, na = not assayed, and ... = no disease developed.

^b ID measured in chlamydospores g⁻¹ of soil.

DII_{final} and SAUDPC; therefore, only the analysis for SAUDPC is shown and described below (Fig. 3). SAUDPC increased steadily in the cv. PV-61–*Foc*-5 interaction with the increase in the inoculum density at all soil temperatures for which disease developed. In contrast, in cv. P-2245 infected with either *Foc*-5 or *Foc*-0, such relationships were observed only for the lower and higher temperatures assayed at which disease developed. For the remaining soil temperatures in the study, the maximum values of SAUDPC were attained with an inoculum density as low as 50 chlamydospores g⁻¹ of soil, and higher levels of inoculum made SAUDPC increase asymptotically (Fig. 3). For a given soil temperature, major differences in SAUDPC across levels of inoculum density were due to chickpea cultivar. In cv. P-2245, differences in SAUDPC due to *F. oxysporum* f. sp. *ciceris* races decreased as soil temperature approached the optimum. Thus, the mean SAUDPC across all the inoculum densities tested in plants infected with *Foc*-0 was 50% lower than that in plants infected with *Foc*-5 at 15°C, but that difference decreased to only 10% at 20 and 25°C, and the values for the two races were nearly identical at 30°C (Table 1). Similarly, according to SAUDPC values, cv. P-2245 showed a highly susceptible reaction to *Foc*-5, and this susceptibility was greater than that shown by cv. PV-61 to the same race. However, that difference in susceptibility to *Foc*-5 between these two chickpea cultivars was strongly influenced by soil temperature. Thus, compared with cv. PV-61, the mean SAUDPC attained in cv. P-2245 across inoculum densities was 78

and 76% higher at the extreme temperatures of 15 and 30°C, respectively, and 33 and 19% higher at 20 and 25°C, respectively (Table 1).

At each level of inoculum density, the effect of soil temperature on SAUDPC was well described by the beta function (equation 4). The estimates of the shape parameter *H* decreased with the increase in the level of inoculum density, indicating that a wide range of temperature exists for which the SAUDPC remains near to a maximum value (Table 1). Overall, for a given inoculum density, the *H* parameter was highest for the cv. PV-61–*Foc*-5 interaction, resulting in narrow optimum curves, compared with that estimated for the cv. P-2245–*Foc*-5 and cv. P-2245–*Foc*-0 interactions (Table 1; Fig. 3).

The intrinsic rate of DII increase (*B_w*) also was influenced by both the incubation temperature and the pathogen inoculum density. In general, for each inoculum density–incubation temperature combination, *B_w* increased for cv. PV-61–*Foc*-5, cv. P-2245–*Foc*-0, and cv. P-2245–*Foc*-5, in that order; that is, according to the increasing degree of compatibility in the plant–pathogen interaction. For all the experimental combinations, *B_w* increased with the increase in pathogen inoculum density according to an exponential model (Table 1; Fig. 4). Differences between *B_w* for low and high inoculum densities and all chickpea cultivar–pathogen race combinations were highest at incubation temperatures >15°C (Fig. 4), with the highest values of the exponential model rate parameter (*r* in equation 3) being attained at

Fig. 1. Fusarium wilt disease progress in chickpea cvs. P-2245 and PV-61 grown in soil infested with different inoculum densities (IDs) (6 to 8,000 chlamydospores g⁻¹ soil) of *Fusarium oxysporum* f. sp. *ciceris* races 0 and 5 and incubated at 10, 15, 20, 25, and 30°C. Each point is the mean disease intensity index calculated with data from two repeated experiments, each comprising four pots and four plants per pot at 2- to 3-day intervals. Solid lines represent the predicted disease progress curve calculated by the Weibull function.

Fig. 2. Relationship between reciprocal of the incubation period (time to initial symptoms) of Fusarium wilt in chickpea cvs. PV-61 and P-2245 and the inoculum density of *Fusarium oxysporum* f. sp. *ciceris* races 0 (*Foc*-0) and 5 (*Foc*-5) (left panels) or soil temperature (right panels). Each point is the mean of data from two repeated experiments, each comprising four pots and four plants per pot. Solid line represents the predicted function calculated by the Weibull equation for inoculum density, or the beta equation for soil temperature.

20, 25, and 30°C. On the other hand, at constant inoculum density, B_W increased with temperature to an optimum (>20°C) and then decreased at the highest temperature tested, 30°C, this pattern of variation being well described by the beta function (Fig. 4).

Response surfaces describing the effect of inoculum density and temperature on *Fusarium* wilt development. The response surfaces fitted to IP_R , SAUDPC, and B_W data are shown in Figure 5. The response surface for the combined effects of soil temperature and inoculum density on IP_R indicated that 27°C was the optimum soil temperature for symptom expression in cv. P-2245 by *Foc*-0. This optimum temperature was significantly higher ($P < 0.05$) than 25°C, predicted as optimum for plants infected with *Foc*-5 (Table 2). In the response surface, the A (upper limit) parameter estimate increased and the H (shape) estimate decreased for cv. PV-61-*Foc*-5, cv. P-2245-*Foc*-5, and cv. P-2245-*Foc*-0 interactions, in that order (Table 2; Fig. 5). This indicated that the maximum IP_R (i.e., shorter IP) increased and the range of temperatures near the optimum was broader for a maximum response as virulence of the *Foc*-race or susceptibility of chickpea cultivar in the interaction increased (Table 2; Fig. 5). At the optimum soil temperature, the IP was shortest, with *Foc*-5 density of at least 100 and 1,000 chlamydo spores g^{-1} of soil in cvs. P-2245 and PV-61, respectively; or with *Foc*-0 at >2,000 chlamydo spores g^{-1} of soil in cv. P-2245 (Fig. 5).

The response surface for the combined effects of soil temperature and inoculum density on SAUDPC estimated that 26°C

was an optimum temperature for maximum SAUDPC in the cv. P-2245-*Foc*-0 interaction, but such a temperature was significantly ($P < 0.05$) lower in cvs. P-2245 (24°C) and PV-61 (23°C) infected by *Foc*-5 (Table 2, Fig. 5). The highest value (7.1) of the shape parameter, H , was estimated for the cv. PV-61-*Foc*-5 interaction compared with that in cv. P-2245 infected with either *Foc*-0 or *Foc*-5, for which the H parameter was estimated at 2.7 and 1.9, respectively. This indicates that, for the cv. PV-61-*Foc*-5 interaction, there was a narrower range of temperatures near the optimum for a maximum disease response in the plant to the pathogen. Thus, SAUDPC values for cv. P-2245 infected by the two races were nearly constant over a broad temperature range around the optimum temperature (Table 2; Fig. 5). At the optimum soil temperature, maximum SAUDPC values in cv. P-2245 developed with an inoculum density of *Foc*-5 at ≥ 100 chlamydo spores g^{-1} of soil and *Foc*-0 at 1,000 chlamydo spores g^{-1} of soil. However, for the moderately susceptible cv. PV-61, maximum SAUDPC values were obtained only with an inoculum density of *Foc*-5 of >1,500 chlamydo spores g^{-1} of soil (Fig. 5).

In the response surface of B_W to the combined effects of soil temperature and inoculum density, the optimum temperature (24 to 25°C) for maximum rate of disease development (B_W) was similar ($P \geq 0.05$) in cvs. P-2245 and PV-61 infected with the highly virulent *Foc*-5; however, the maximum rate for disease development in cv. P-2245 infected with the less virulent *Foc*-0 occurred at a significantly ($P < 0.05$) higher optimum temperature (27°C) (Table 2; Fig. 5). Also, a significantly higher ($P < 0.05$)

Fig. 3. Relationship between the standardized area under the disease intensity progress curve of *Fusarium* wilt in chickpea cvs. PV-61 and P-2245 and the inoculum density of *Fusarium oxysporum* f. sp. *ciceris* races 0 (*Foc*-0) and 5 (*Foc*-5) (left panels) or soil temperature (right panels). Each point is the mean of data from two repeated experiments, each comprising four pots and four plants per pot. Solid line represents the predicted function calculated by the Weibull equation for inoculum density, or the beta equation for soil temperature.

Fig. 4. Relationship between the Weibull intrinsic rate of *Fusarium* wilt increase parameter in chickpea cvs. PV-61 and P-2245 and inoculum density of *Fusarium oxysporum* f. sp. *ciceris* races 0 (*Foc*-0) and 5 (*Foc*-5) (left panels) or soil temperature (right panels). Each point is the mean of data from two repeated experiments, each comprising four pots and four plants per pot. Solid line represents the predicted function calculated by the Weibull equation for inoculum density, or the beta equation for soil temperature.

value of the H shape parameter was obtained for the cv. PV-61–*Foc*-5 interaction compared with the estimated values in cv. P-2245 infected with either *Foc*-0 or *Foc*-5. This indicates that B_W varies in a narrow temperature range around the optimum temperature for disease development in cv. PV-61 plants infected with *Foc*-5 (Table 2; Fig. 5). At the optimum soil temperature, the highest B_W values in cv. P-2245 occurred with *Foc*-0 at $\geq 1,000$ chlamydo spores g^{-1} of soil; however, *Foc*-5 densities of only 25 and 500 chlamydo spores g^{-1} of soil were needed to obtain maximum B_W values for cvs. P-2245 and PV-61, respectively (Fig. 5).

Risk threshold models. Risk threshold models indicated that severe Fusarium wilt in the moderately susceptible cv. PV-61 would occur only when soil temperature was within the range of 20 to 30°C and inoculum density at least 1,500 chlamydo spores g^{-1} of soil for the highly virulent *Foc*-5. In contrast, development of a similar amount of disease in the highly susceptible cv. P-2245 required *Foc*-5 at only 50 chlamydo spores g^{-1} of soil, or 500 chlamydo spores g^{-1} of soil for the less virulent *Foc*-0. Under conditions of 20 to 30°C and 500 chlamydo spores g^{-1} of soil in the most compatible cv. P-2245–*Foc*-5 interaction, symptoms were predicted to appear 17 days after inoculation, disease severity to attain a SAUDPC value >0.9 , and epidemics to develop at a B_W rate >0.12 DII per day, achieving maximum SAUDPC values 25 days after inoculation (Fig. 6).

Similarly, low disease was predicted to develop in highly susceptible cv. P-2245 when soil temperature was $<12^\circ\text{C}$ or $>30^\circ\text{C}$

and inoculum density of *Foc*-0 or *Foc*-5 was <2 chlamydo spores g^{-1} of soil and, for the less susceptible cv. PV-61, when *Foc*-5 was <10 chlamydo spores g^{-1} of soil. Within these soil temperature and inoculum density ranges, symptoms of Fusarium wilt are predicted to take at least 45 days to develop and further disease is predicted to proceed at a B_W rate >0.03 DII per day, to eventually reach a maximum SAUDPC value >0.05 by 60 days after inoculation (Fig. 6). Finally, if soil temperature is $<10^\circ\text{C}$, no disease is predicted to occur in cv. PV-61 grown in soil infested with *Foc*-5 or *Foc*-0, and in cv. P-2245 grown in soil infested with *Foc*-0, even at an inoculum density of *Foc*-5 or *Foc*-0 at 5,000 or 8,000 chlamydo spores g^{-1} of soil, respectively.

DISCUSSION

In this study, under controlled conditions, the combined effects of virulence and inoculum density of the race, susceptibility of the cultivar, and soil temperature on development of Fusarium wilt in chickpea caused by *F. oxysporum* f. sp. *ciceris* were described and quantified for the first time using nonlinear models. Results illustrated the complexity of soil temperature–race virulence–cultivar susceptibility interactions in the response of chickpea cultivars to *F. oxysporum* f. sp. *ciceris* races. In our study, the most favorable soil temperatures for development of Fusarium wilt over a range of 6 to 8,000 chlamydo spores g^{-1} of soil were between 20 and 25°C for infection of cvs. P 2245 and PV-61 by

Fig. 5. Surface response for Fusarium wilt progress curve elements (reciprocal of incubation period, standardized area under disease intensity index progress curve [SAUDPC], and Weibull intrinsic rate of Fusarium wilt increase) in chickpea cvs. PV-61 and P-2245 grown in soil infested with different inoculum densities (6 to 8,000 chlamydo spores g^{-1} soil) of *Fusarium oxysporum* f. sp. *ciceris* races 0 (*Foc*-0) and 5 (*Foc*-5) and incubated at 10, 15, 20, 25, and 30°C, as a function of both inoculum density (ID) and soil temperature.

Foc-5, and between 20 and 30°C for infection of cv. P-2245 by *Foc-0*, which is consistent with previous studies in the growth chamber (25,30) and in field (7,11,27,31,32).

Sparse information was available about temperatures that limit infection or disease development by *Fusarium* wilt pathogens. Compared with previous studies, our results differ mainly in the cardinal temperatures for disease development. Indeed, unlike some previous reports (3,7,11) of disease developing at 10°C, no disease developed at this temperature in our study, except for the cv. P-2245–*Foc-5* interaction, for which symptoms occurred over the entire range of soil temperature (10 to 30°C) and inoculum density assayed (6 to 5,000 chlamydo-spores g⁻¹ of soil). Moreover, in our study, infection by the pathogen was not impaired at 30°C for race 0, the less virulent of *F. oxysporum* f. sp. *ciceris* races, as reported previously (20,23,30). Temperatures >30°C were reported to reduce infection and *Fusarium* wilt development in chickpea (3,36). Differences in virulence of the pathogen races, or in susceptibility of the chickpea cultivars, may account for the discrepancy in the temperature optima for *Fusarium* wilt development. It is remarkable that optimum temperature for mycelial growth and conidia production of different *F. oxysporum* f. sp. *ciceris* races also is optimum for infection (8,9,26). Furthermore, it is worth noting that, in races 0 and 5 used in this study, both maximum mycelial growth and maximum disease intensity occurred at a temperature significantly higher for *Foc-0* compared with that for *Foc-5* (9).

The effect of soil temperature on *Fusarium* wilt development in chickpea has important implications in the development and use of resistant cultivars as well as in race pathotyping of *F. oxysporum*

f. sp. *ciceris* isolates (28). Joint studies in Israel and Spain showed that cvs. Ayala and PV-1 were moderately resistant and resistant, respectively, to *F. oxysporum* f. sp. *ciceris* race 1A at 24°C but highly susceptible at 27°C. A similar but less pronounced effect occurred for cv. Ayala infected with *F. oxysporum* f. sp. *ciceris* race 6 (28). In our study, susceptible reaction of chickpea cultivars to *F. oxysporum* f. sp. *ciceris* races 0 and 5 was modified only in chickpea cv. P-2245 at 10°C, for which symptoms developed in plants infected by the highly virulent *Foc-5* only. The other chickpea cultivar–*Foc* race interactions in the study were conducive to disease at all of the inoculum densities assayed; however, soil temperature had a strong influence on the rate of development and final disease severity.

Increasing understanding of the effect of soil temperature on development of *Fusarium* wilt in chickpea also has significance regarding cropping practices that may be used to manage this disease. Recent field studies in Israel indicated that the high resistant reaction of cv. Ayala to *Fusarium* wilt expressed when sown in mid- to late January was shifted to a moderately susceptible reaction under warmer temperatures when the sowing was delayed to late February or early March (28). Conversely, in southern Spain, advancing the sowing date from early spring to early winter delayed epidemic onset, reduced the rate of *Fusarium* wilt development, reduced the final amount of disease, and increase chickpea seed yield (27,31,32). The optimum temperature range of 20 to 30°C found in this study is close to temperatures prevalent in southern Spain in spring, when chickpea traditionally is grown and *Fusarium* wilt develops more severely (27,31,32,39).

TABLE 2. Nonlinear fit of response surfaces for the combined effects of inoculum density and soil temperature on the reciprocal incubation period (IP_R), standardized area under disease intensity index progress curve (SAUDPC), and the Weibull intrinsic rate of disease intensity index (*B_W*) increase for *Fusarium* wilt in chickpea cvs. P-2245 and PV-61 sown in soil infested with different inoculum densities of *Fusarium oxysporum* f. sp. *ciceris* races 0 and 5 (*Foc-0* and *Foc-5*, respectively) (equations 5 and 6)

Elements, cultivar/race ^c	Parameter estimates ^a							Statistics ^b		
	<i>A</i> ± SE	<i>C</i> ± SE	<i>D</i> ± SE	<i>E</i> ± SE	<i>G</i> ± SE	<i>T</i> _{opt} ± SE	<i>H</i> ± SE	MSE	<i>R</i> ²	Res.
IP_R										
PV-61/ <i>Foc-5</i>	0.0600	0.0001	11.8968	...	0.0890	25.1414	<u>2.4854</u>	0.000029	0.779	R
	0.0035	<0.0001	1.1397	...	0.0085	<0.0001	0.3449			
P-2245/ <i>Foc-5</i>	<u>0.0720</u>	<u>0.0024*</u>	10.6335*	...	0.0805*	25.2000	1.4244	0.000115	0.669	R
	<0.0001	<0.0001	0.6386	...	0.0048	<0.0001	0.2198			
P-2245/ <i>Foc-0</i>	0.0722	0.0000	8.5795	...	0.0565	26.7992*	1.2290	0.000034	0.792	R
	0.0102	0.0000	0.7575	...	0.0113	0.0050	0.1840			
SAUDPC										
PV-61/ <i>Foc-5</i>	<u>0.9995</u>	<u>0.5583</u>	0.8845	...	<u>0.9997</u>	23.3822	<u>7.1116</u>	0.003915	0.965	R
	0.0062	0.0644	0.0849	...	0.0029	0.1742	0.4960			
P-2245/ <i>Foc-5</i>	0.9221*	0.1660*	<u>18.4123*</u>	...	0.1197	24.0173	1.9288	0.011097	0.860	R
	0.0297	0.0348	1.5119	...	0.0098	1.1694	0.5308			
P-2245/ <i>Foc-0</i>	0.8868	0.0000	5.3190	...	0.3203*	26.1321*	2.6874*	0.005400	0.961	R
	0.0007	0.0000	0.2664	...	0.0161	0.1040	0.0130			
<i>B_W</i>										
PV-61/ <i>Foc-5</i>	...	...	...	0.3983	0.0284	24.2560	<u>3.8018</u>	0.000141	0.882	R
	...	...	...	0.0264	0.0021	0.1765	0.3945			
P-2245/ <i>Foc-5</i>	...	...	...	<u>0.4555*</u>	<u>0.0497*</u>	24.8000	1.7595	0.000798	0.856	R
	...	...	...	<0.0001	0.0024	0.4618	0.3089			
P-2245/ <i>Foc-0</i>	...	...	...	0.3568	0.0391	26.5002*	1.6812	0.000095	0.952	R
	...	...	...	0.0065	<0.0001	0.0178	0.0032			

^a For IP_R and SAUDPC, a response surface was determined from the original data set using the function $Y_{T,ID} = f(T, ID) = A(1 - \exp[-f(T)(\log[ID] - C)])^D$ (equation 5), in which *A* is the upper limit of the response, *r* is the intrinsic rate of increase in the response, *C* is the length of delay in the response, and *D* is the portion of the period of inoculum density (ID) in which the response decelerate. Similarly, for *B_W*, a response surface was determined using the function $Y_{T,ID} = f(T, ID) = E \exp(f(T) \log[ID])$ (equation 6), in which *E* is a constant. In both equations, the intrinsic rate of increase is expressed as a function of temperature given by $Y_T = f(T) = G([T - T_{min}]/[T_{opt} - T_{min}])^{(H \times [T_{opt} - T_{min}]/[T_{max} - T_{opt}])}([T_{max} - T]/[T_{max} - T_{opt}])^H$ (equation 4). In this equation, *Y_T* is the response of the disease progress curve element to temperature, *T_{max}* and *T_{min}* were fixed to 37 and 5°C, respectively (9,26). The shape parameter, *H*, determines the temperature range near the optimum temperature, *T_{opt}*, at which the response values are close to the maximum response *G*. SE = standard error.

^b MSE = final mean square error of residuals, *R*² = coefficient of determination, Res. = residuals: standardized residuals plotted against dependent variable values observed or predicted from nonlinear regression analysis. Patterns of residuals with a random scatter (R), after visual inspection of residual plots. For each parameter estimate, underlined parameter value in a column is significantly higher (*P* < 0.05) than the corresponding value at the other chickpea cultivar. A parameter value followed by an asterisk indicates the parameter value for a pathogen race in a column is significantly higher (*P* < 0.05) than the corresponding parameter value at the other pathogen race.

^c Disease progress curve elements: IP_R = time in days to initial symptoms, expressed as the reciprocal of the lengths of the incubation period; SAUDPC = estimated by the trapezoidal integration method standardized by duration time in days; and *B_W* = intrinsic rate of DII increase obtained by the estimates of parameters of the Weibull model fitted to DII progress data.

The effects of inoculum density and soil temperature on Fusarium wilt development followed Liebig's Law of the Minimum (1,5). Thus, at extreme temperatures, plants were either asymptomatic or developed moderate disease even when inoculum density was optimum for disease development. Similarly, at low inoculum density, no or little disease developed even at soil temperatures optimal for *F. oxysporum* f. sp. *ciceris* infection. In contrast, when conditions led to development of disease, limitations in a deficient factor were compensated by another factor, according to the compensation phenomenon as defined by Rotem (1,34). In our study, disease in moderately compatible cultivar–race interactions developed when soil temperature was favorable even though inoculum density was marginal.

The development of risk threshold charts may have application in predicting the potential threat for development of Fusarium wilt epidemics at levels of inoculum density and temperatures under field conditions prevalent in the Mediterranean region. The model can be used to identify locations and soils of high risk for Fusarium wilt development. Based on our results, it is predicted that severe Fusarium wilt will develop at 20 to 30°C and an inoculum density of *F. oxysporum* f. sp. *ciceris* races 5 and 0 of at least 6 and 100 chlamydospores g⁻¹ of soil, respectively. The efficient application of the risk models will require the identification of the races of *F. oxysporum* f. sp. *ciceris* that prevail in a geographical area as well as the level of resistance of local or commercial cultivars. Polymerase chain reaction-based techniques currently

Fig. 6. Charts for risk predictions for Fusarium wilt disease progress curve elements (reciprocal of incubation period, standardized area under disease intensity index progress curve [SAUDPC], and Weibull intrinsic rate of Fusarium wilt increase) in chickpea cvs. P-2245 and PV-61 grown in soil infested with *Fusarium oxysporum* f. sp. *ciceris* races 0 (Foc-0) and 5 (Foc-5) based on inoculum density and soil temperature. Isopaths are based on the response surfaces (equations 5 and 6, Fig. 5).

are available for the rapid identification of *F. oxysporum* f. sp. *ciceris* races prevailing in a soil, and cultivars resistant to some of the most extensively spread races have been developed (21, 23, 24,35,37), thus making practical the implementation of the risk model developed in this study.

ACKNOWLEDGMENTS

This work was supported by grants AGF97-1479 and AGL 2004-01231/AGR from Comisión Interministerial de Ciencia y Tecnología of Spain, HA 1997-0033 from Ministerio de Educación y Cultura, and Fundación Alonso Martín Escudero of Spain. B. B. Landa is a contract holder of the Ramón y Cajal program from Ministerio de Educación y Ciencia of Spain. We thank P. Castillo, W. E. Fry, T. C. Paulitz, and K. V. Subbarao for critically reading the manuscript prior to submission; and the senior editor and reviewers for their helpful comments and suggestions.

LITERATURE CITED

- Aust, H. J., Bashi, E., and Rotem, J. 1980. Flexibility of plant pathogens in exploiting ecological and biotic conditions in the development of epidemics. Pages 46-56 in: Comparative Epidemiology. J. Palti and J. Kranz, eds. Pudoc, Wageningen, The Netherlands.
- Barran, L. R., Schneider, E. F., and Seaman, W. L. 1977. Requirements for the rapid conversion of macroconidia of *Fusarium sulphureum* to chlamydospores. Can. J. Microbiol. 23:148-151.
- Bhatti, M. A., and Kraft, J. M. 1992. Effects of inoculum density and temperature on root rot and wilt of chickpea. Plant Dis. 76:50-54.
- Bouhot, D., and Rouxel, F. 1971. Technique sélective et quantitative d'analyse des *Fusarium oxysporum* et *Fusarium solani* dans le sol. Mode d'emploi. Ann. Phytopathol. 3:251-254.
- Browne, C. A. 1942. Leibig and the Law of the Minimum. In: Leibig and After Leibig. F. R. Moulton, ed. Publ. Am. Assoc. Adv. Sci. 16:71-82.
- Campbell, C. L., and Madden, L. V. 1990. Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York.
- Chauhan, S. K. 1963. Influence of different soil temperatures on the incidence of *Fusarium* wilt of gram (*Cicer arietinum* L.). Proc. Nat. Acad. Sci. India B33:552-554.
- Desai, S., Nene, Y. L., and Reddy, A. G. R. 1994. Races of *Fusarium oxysporum* causing wilt in chickpea: Growth variability. Indian J. Mycol. Plant Pathol. 24:120-127.
- Duro-Almazán, M. D. 2000. Influencia de la temperatura y el pH en el desarrollo in vitro de razas de *Fusarium oxysporum* f. sp. *ciceris*. MS thesis. E.T.S.I.A.M. University of Córdoba, Spain.
- Duthie, J. A. 1997. Models of the response of foliar parasites to the combined effects of temperature and duration of wetness. Phytopathology 87:1088-1095.
- Gupta, O., Kotasthane, S. R., and Khare, M. N. 1987. Factors influencing epidemiology of vascular wilt of chickpea. Proc. Natl. Acad. Sci. India 57:86-91.
- Halila, M. H., and Strange, R. N. 1996. Identification of the causal agent of wilt of chickpea in Tunisia as *Fusarium oxysporum* f. sp. *ciceri* race 0. Phytopathol. Mediterr. 35:67-74.
- Hau, B. 1988. Ein erweitertes analytisches modell für epidemien von pflanzenkrankheiten. Justus-Liebig-Universität, Habilitationsschrift, Giessen, Germany.
- Haware, M. P., and Nene, Y. L. 1982. Races of *Fusarium oxysporum* f. sp. *ciceri*. Plant Dis. 66:809-810.
- Haware, M. P., Jiménez-Díaz, R. M., Amin, K. S., Phillips, J. C., and Halila, H. 1990. Integrated management of wilt and root rots of chickpea. Pages 129-133 in: Proc. Second Int. Workshop Chickpea Improvement, ICRISAT, Patancheru, India.
- ICRISAT. 1989. Annual Report 1988. ICRISAT, Patancheru, India.
- Jalali, B. L., and Chand, H. 1992. Chickpea wilt. Pages 429-444 in: Plant Diseases of International Importance. Vol. 1. Diseases of Cereals and Pulses. U. S. Singh, A. N. Mukhopadhyay, J. Kumar, and H. S. Chaube, eds. Prentice Hall, Englewood Cliffs, NJ.
- Jiménez-Díaz, R. M., Alcalá-Jiménez, A. R., Hervás, A., and Trapero-Casas, J. L. 1993. Pathogenic variability and host resistance in the *Fusarium oxysporum* f. sp. *ciceris*/Cicer arietinum pathosystem. Pages 87-94 in: Proc. Eur. Semin. Fusarium Mycotoxins, Taxonomy, Pathogenicity and Host Resistance, 3rd. Hodowla Róslin Aklimatyzacja i Nasiennictwo. Plant Breeding and Acclimatization Institute, Radzików, Poland.
- Jiménez-Díaz, R. M., Singh, K. B., Trapero-Casas, A., and Trapero-Casas, J. L. 1991. Resistance in kabuli chickpeas to *Fusarium* wilt. Plant Dis. 75:914-918.
- Jiménez-Díaz, R. M., Trapero-Casas, A., and Cabrera de la Colina, J. 1989. Races of *Fusarium oxysporum* f. sp. *ciceri* infecting chickpeas in southern Spain. Pages 515-520 in: Vascular Wilt Diseases of Plants. NATO ASI Series, Vol. H28. E. C. Tjamos and C. H. Beckman, eds., Springer-Verlag, Berlin.
- Jiménez-Gasco, M. M., and Jiménez-Díaz, R. M. 2003. Development of a specific polymerase chain reaction-based assay for the identification of *Fusarium oxysporum* f. sp. *ciceris* and its pathogenic races 0, 1A, 5, and 6. Phytopathology 93:200-209.
- Jiménez-Gasco, M. M., Milgroom, M. G., and Jiménez-Díaz, R. M. 2004. Stepwise evolution of races in *Fusarium oxysporum* f. sp. *ciceris* inferred from fingerprinting with repetitive DNA sequences. Phytopathology 94:228-235.
- Jiménez-Gasco, M. M., Pérez-Artés, E., and Jiménez-Díaz, R. M. 2001. Identification of pathogenic races 0, 1B/C, 5, and 6 of *Fusarium oxysporum* f. sp. *ciceris* with random amplified polymorphic DNA (RAPD). Eur. J. Plant Pathol. 107:237-248.
- Kelly, A., Alcalá-Jiménez, A. R., Bainbridge, B. W., Heale, J.B., and Pérez-Artés, E., and Jiménez-Díaz, R. M. 1994. Use of genetic fingerprinting and random amplified polymorphic DNA to characterize pathotypes of *Fusarium oxysporum* f. sp. *ciceris* infecting chickpea. Phytopathology 84:1293-1298.
- Landa, B. B., Navas-Cortés, J. A., Hervás, A., and Jiménez-Díaz, R. M. 2001. Influence of temperature and inoculum density of *Fusarium oxysporum* f. sp. *ciceris* on suppression of *Fusarium* wilt of chickpea by rhizosphere bacteria. Phytopathology 91:807-816.
- Landa, B. B., Navas-Cortés, J. A., and Jiménez-Díaz, R. M. 2004. Influence of temperature on plant-rhizobacteria interactions related to biocontrol potential for suppression of *Fusarium* wilt of chickpea. Plant Pathol. 53:341-352.
- Landa, B. B., Navas-Cortés, J. A., and Jiménez-Díaz, R. M. 2004. Integrated management of *Fusarium* wilt of chickpea combining the use of choice of sowing date, host resistance, and biological control. Phytopathology 94:946-960.
- Landa, B. B., Navas-Cortés, J. A., Jiménez-Gasco, M. M., Katan, J., Retig, B., and Jiménez-Díaz, R. M. 2006. Temperature response of chickpea cultivars to races of *Fusarium oxysporum* f. sp. *ciceris*, the causal agent of *Fusarium* wilt. Plant Dis. 90:365-374.
- Madden, L. V., and Campbell, C. L. 1990. Nonlinear disease progress curves. Pages 181-229 in: Epidemics of Plant Diseases, Mathematical Analysis and Modeling, 2nd ed. Ecological Studies 13. J. Kranz, ed. Springer-Verlag, Berlin.
- Navas-Cortés, J. A., Alcalá-Jiménez, A. R., Hau, B., and Jiménez-Díaz, R. M. 2000. Influence of inoculum density of races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris* on development of *Fusarium* wilt in chickpea cultivars. Eur. J. Plant Pathol. 106:135-146.
- Navas-Cortés, J. A., Hau, B., and Jiménez-Díaz, R. M. 1998. Effect of sowing date, host cultivar, and race of *Fusarium oxysporum* f. sp. *ciceris* on development of *Fusarium* wilt of chickpea. Phytopathology 88:1338-1346.
- Navas-Cortés, J. A., Hau, B., and Jiménez-Díaz, R. M. 2000. Yield loss in chickpeas in relation to development of *Fusarium* wilt epidemics. Phytopathology 90:1269-1278.
- Pennypacker, S. P., Knoble, H. D., Antle, C. E., and Madden, L. V. 1980. A flexible model for studying plant disease progression. Phytopathology 70:232-235.
- Rotem, J. 1978. Climatic and weather influences on epidemics. Pages 317-337 in: Plant Disease: An Advanced Treatise, Vol. II. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York.
- Rubio, J., Hajj-Moussa, E., Kharrat, M., Moreno, M. T., Millán, T., and Gil, J. 2003. Two genes and linked RAPD markers involved in resistance to *Fusarium oxysporum* f. sp. *ciceris* race 0 in chickpea. Plant Breed. 122:188-191.
- Sattar, A., Ghani Arif, A., and Mohy-ud-Din, M. 1953. Effect of soil temperature and moisture on the incidence of gram wilt. Pak. J. Sci. Res. 5:16-21.
- Sharma, K. D., Chen, W., and Muehlbauer, F. J. 2005. Genetics of chickpea resistance to five races of *Fusarium* wilt and a concise set of race differentials for *Fusarium oxysporum* f. sp. *ciceris*. Plant Dis. 89:385-390.
- Sugha, S. K., Kapoor, S. K., and Singh, B. M. 1994. Factors influencing *Fusarium* wilt of chickpea (*Cicer arietinum* L.). Indian J. Mycol. Plant Pathol. 24:97-102.
- Trapero-Casas, A., and Jiménez-Díaz, R. M. 1985. Fungal wilt and root rot diseases of chickpea in southern Spain. Phytopathology 75:1146-1151.