European Journal of Plant Pathology **106:** 135–146, 2000.

Influence of inoculum density of races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris* on development of Fusarium wilt in chickpea cultivars

Juan A. Navas-Cortés[1,**], Antonio R. Alcalá-Jiménez[2], Bernhard Hau[1] and Rafael M. Jiménez-Díaz[2,*]
[1]*Institut für Pflanzenkrankheiten und Pflanzenschutz, Universität Hannover, Herrenhäuser Str. 2, 30419 Hannover, Germany;* [2]*Instituto de Agricultura Sostenible, Consejo Superior de Investigaciones Científicas, and Departamento de Agronomía, Universidad de Córdoba, Apartado 4084, 14080 Córdoba, Spain;* [*]*Author for correspondence (Fax: +34 957 499252; E-mail: ag1jidir@lucano.uco.es);*
[**]*Present address: Instituto de Agricultura Sostenible, Consejo Superior de Investigaciones Científicas, Apartado 4084, 14080 Córdoba, Spain*

Accepted 8 November 1999

Key words: Cicer arietinum, quantitative epidemiology, disease modelling

Abstract

Artificial inoculation experiments were carried out at 25 °C to determine the effects of inoculum density of *Fusarium oxysporum* f.sp. *ciceris* races 0 (*Foc*-0) and 5 (*Foc*-5) and susceptibility of chickpea cultivars P-2245 and PV-61 on development of Fusarium wilt. *Foc*-5 proved much more virulent than *Foc*-0. Increasing the inoculum density of *F. oxysporum* f.sp. *ciceris* caused an exponential reduction in disease incubation period and a monomolecular increase of disease incidence and the area under the disease intensity progress curve. The extent of these effects was highest in the most conducive 'P-2245'/*Foc*-5 combination and decreased in the less susceptible 'PV-61' and for the less virulent *Foc*-0, in that order. For 'P-2245'/*Foc*-5, the highest disease intensity was attained with 6 chlamydospores g^{-1} of soil, the lowest inoculum density in the study. One thousand chlamydospores g^{-1} of soil of the same race were needed to attain a comparable disease intensity in 'PV-61'. Twenty thousand chlamydospores g^{-1} of soil of *Foc*-0 were required for maximum disease intensity in 'P-2245'.

The disease intensity curves were adequately described by the Gompertz model. Using this model, a response surface for disease intensity was developed, in which the model parameters are expressed as a function of both time from inoculation and inoculum density. This response surface confirmed that the final amount of disease intensity increases in a monomolecular relationship with increasing inoculum density and showed that the relative rate of disease progress increases exponentially with increasing inoculum density of the pathogen.

Introduction

Chickpea (*Cicer arietinum* L.) is an important, high protein food crop in many areas of the world (Saxena, 1990). Fusarium wilt, caused by *Fusarium oxysporum* Schlechtend.: Fr.f.sp. *ciceris* (Padwick) Matuo & K. Sato, is the most important soilborne disease of chickpea throughout the world particularly in the Indian subcontinent, the Mediterranean basin, and California (Haware, 1990; Nene and Reddy, 1987). Attacks by this pathogen can destroy the crop completely (Halila and Strange, 1996; Haware and Nene, 1980), or cause annual yield losses which amount to 10% in India (Singh and Dahiya, 1973) and Spain (Trapero-Casas and Jiménez-Díaz, 1985) and 40% in Tunisia (Bouslama, 1980). Disease symptoms in a highly susceptible cultivar develop within 25 days after sowing, but can occur up to podding stage. Early wilting causes more loss than late wilting, but seeds from late-wilted plants are lighter, rougher, and duller

than those from healthy plants (Haware and Nene, 1980).

Fusarium wilt of chickpea is best managed by the use of resistant cultivars (Jalali and Chand, 1992; Nene and Reddy, 1987), the effectiveness of which is complicated by the occurrence of pathogen races. Haware and Nene (1982) first identified races 1–4 in India. Three additional races, 0, 5, and 6, were later identified in southern Spain (Jiménez-Díaz et al., 1989b). Race 0 induces a progressive foliar yellowing compared to the severe leaf chlorosis, flaccidity, and early wilt induced by races 1–6 (Jiménez-Díaz et al., 1989b). Both syndromes arise as a result of vascular infections (Trapero-Casas and Jiménez-Díaz, 1985). Race 0 occurs in California, Spain and Tunisia; races 1 and 6 were found in California, Morocco and Spain and race 5 has been reported from California and Spain (Halila and Strange, 1996; Jiménez-Díaz et al., 1989b; R.M. Jiménez-Díaz, unpublished).

The annual variation in severity of chickpea root diseases has been attributed to differences in temperature and inoculum density in the soil (Gupta et al., 1987; Westerlund et al., 1974). Field observations on disease development in Andalucia, southern Spain, suggested that Fusarium wilt incidence and severity were enhanced by warm, dry soils occurring in crops sown in spring time (Trapero-Casas and Jiménez-Díaz, 1985). Field experiments in microplots artificially infested with races 0 and 5 of *F. oxysporum* f.sp. *ciceris* at Córdoba, southern Spain, also indicated that advancing chickpea sowing from early spring to early winter slowed down the development of Fusarium wilt epidemics, delayed the epidemic onset, and minimized the final amount of disease (Navas-Cortés et al., 1998). However, the net effect of this disease management practice was also influenced by the susceptibility of the chickpea cultivar and the nature and inoculum density of the *F. oxysporum* f.sp. *ciceris* race.

In laboratory studies, Bhatti and Kraft (1992) reported that the incidence of wilt symptoms increased linearly with increasing inoculum density of the pathogen, the most severe wilt reaction occurring at an inoculum density of 5000 propagules g^{-1} of soil (Bhatti and Kraft, 1992). Similarly, Sugha et al. (1994) observed that an increase in inoculum load accelerated development of chickpea wilt, while lower concentrations delayed the expression of wilt symptoms. However, the nature of the pathogen race, which might influence the disease-incidence–inoculum-density relationship, was not considered in these studies.

A better understanding of the influence of inoculum density on disease, as related to pathogen race, may lead to better prediction of disease development and to the development of more efficient disease management strategies. The present study was undertaken to determine the effects of cultivar susceptibility and inoculum density of races of *F. oxysporum* f.sp. *ciceris* inducing yellowing or wilting in chickpea, on development of Fusarium wilt.

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 stored in sterile soil tubes at 4 °C. Active cultures were obtained from aliquots of a soil culture placed on potato dextrose agar (PDA) and incubated at 25 °C and a 12-h photoperiod of fluorescent and near-UV light at 36 $\mu E\, m^{-2}\, s^{-1}$.

Inoculum consisted of chlamydospores produced in dry chickpea stem debris artificially colonized by the fungus. Stem debris was collected from healthy plants of the chickpea cultivar Blanco Lechoso in a disease-free field near Córdoba and stored in sacks in a dry place at 4 °C until used. Chopped, autoclaved (121 °C, 30 min, twice), chickpea straw was placed in a sterile nutrient solution (5 mM KNO_3 + 0.5% glucose) (Harris and Ferris, 1991) in 225-ml flasks at a rate of 10 g/30 ml solution and infested with 1 ml of a microconidial suspension. Microconidia were obtained from *F. oxysporum* f.sp. *ciceris* cultures on PDA incubated at 25 °C and a 12-h photoperiod of fluorescent and near-UV light at 36 $\mu E\, m^{-2}\, s^{-1}$ for 10 days. The chopped, infested straw was incubated for 2 weeks, transferred aseptically into sterile paper bags and air-dried for 1 month. Pieces of chickpea straw extensively colonized by the fungus were ground to a very fine powder in a rotating mill (Cyclotec 1093 Sample Mill Tecator, Tecator AB, Höganäs, Sweden), sieved through a 0.5 mm pore sieve, and the powder (averaging 60–100 μm in diameter) used to infest an autoclaved soil mixture at the appropriate proportion. The occurrence of chlamydospores of the fungus in the infested straw powder was confirmed by microscopic observations. Inoculum density of *F. oxysporum* f.sp. *ciceris* in the infested powder was determined by dilution plating on V8 juice–oxgall-PCNB agar (VOPA)

Fusarium-selective medium (Bouhot and Rouxel, 1971). Each of three 0.1-g samples of the infested straw powder was placed in a vessel containing 100 ml of sterile 0.1% water agar and stirred in a blender for 1 min. One ml of this suspension was spread on to each of four plates of VOPA. The plates were incubated at 25 °C and a 12-h photoperiod of fluorescent and near-UV light at 36 μE m^{-2} s^{-1} for 3–7 days, and the number of *F. oxysporum* colonies that grew on the selective medium counted. The number of colonies of *F. oxysporum* formed per gram of sampled powder was used to calculate the density of chlamydospores in the infested powder assuming that each colony had developed from a single chlamydospore.

Chickpea plants and inoculation

Chickpea cultivars P-2245 and PV-61 were used. 'P-2245' and 'PV-61' are 'kabuli' (large, ram-head shaped, beige seeds) chickpeas representative of those used in the Mediterranean region. 'P-2245' is highly susceptible to *Foc*-0 and *Foc*-5, while 'PV-61' is moderately resistant to *Foc*-0 and susceptible to *Foc*-5 (Jiménez-Díaz et al., 1989a). Seeds 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 seeds, selected for uniformity (length of radicle = 1–2 cm), were sown in 15 cm clay pots (four plants per pot) filled with an autoclaved (121 °C, 1 h, twice) soil mixture (clay loam/sand/peat, 1 : 1 : 1, vol/vol/vol), artificially infested with *Foc*-0 or *Foc*-5 at the appropriate inoculum density.

Inoculum density of F. oxysporum *f.sp.* ciceris

The infested powder of chickpea straw was incorporated into the autoclaved soil mixture at the appropriate proportion to achieve an inoculum density of 0, 50, 100, 200, 1000, 2000, 4000, 10,000 and 20,000 chlamydospores g^{-1} of soil of *Foc*-0, and 0, 6, 12, 24, 50, 100, 500, 1000, 2000, 8000 and 25,000 chlamydospores g^{-1} of soil of *Foc*-5. Soil in control pots received an amount of non-infested straw powder similar to that of infested powder used for the corresponding inoculum density. The inoculum density of *Foc*-0 and *Foc*-5 in the infested soil mixture was determined before use by plating on VOPA medium. Plants were grown in a growth chamber adjusted to 25 ± 1 °C, 60–90% relative humidity, and a 14-h photoperiod of fluorescent light at 360 μE m^{-2} s^{-1} for 48 days. Plants were watered as needed and fertilized weekly with 100 ml of Hoagland's nutrient solution (Hoagland and Arnon, 1950).

Disease assessment and data analyses

Three experiments were carried out, each one included cultivars P-2245 and PV-61 as well as *Foc*-0 and *Foc*-5. The inoculum density of the pathogen varied among experiments. However, an inoculum density of 2000 chlamydospores g^{-1} of soil of *Foc*-0 and of 50 chlamydospores g^{-1} of soil for *Foc*-5 was used in all experiments, to test the reproducibility of results. All experiments were conducted following a factorial treatment design arranged in four randomized complete blocks, each treatment consisting of three pots and four plants per pot. Analysis of variance of common treatments did not show significant differences ($P \geq 0.05$) among experiments. Further analysis was performed on the pooled data from the three experiments.

Disease reactions were assessed by the incidence and severity of symptoms. Each plant was assessed for symptom severity at 2-day intervals using a 0–4 rating scale according to percentage of foliage with yellowing or necrosis in acropetal progression (0 = 0%, 1 = 1–33%, 2 = 34–66%, 3 = 67–100%, and 4 = dead plant). Upon termination of experiments, the pathogen was isolated from stem segments of symptomless plants to determine the occurrence of vascular infections. Stem pieces were cut into 5- to 10 mm-long pieces, surface-disinfested in 0.2% NaOCl for 2 min, plated on VOPA, and incubated at 25 °C and a 12-h photoperiod of fluorescent and near-UV light at 36 μE m^{-2} s^{-1} for 3–5 days. Incidence of foliar symptoms, I (on a 0–1 scale) and severity data, S (categorized from 0 to 4) were used to calculate a disease intensity index (*DII*) by the following equation: $DII = (\sum S_i N_i) 4^{-1} N_t$, where S_i = symptom severity; N_i = number of plants with S_i symptom severity; and N_t = total number of plants. Thus, *DII* expresses the mean value of disease intensity at any given moment as a proportion of the maximum possible disease. The accumulated *DII* over time in days from the date of inoculation was used to obtain curves of disease progress. The nonlinear form of the Richards and Gompertz models was evaluated for goodness-of-fit to the entire set of *DII* progress data using nonlinear regression analysis. For analyses, non-zero points for

DII in experimental units and the average *DII* values of the four replicated blocks were used.

A standardized area under the *DII* progress curve (SAUDPC) was calculated by trapezoidal integration method standardized by the duration of disease development in days (Campbell and Madden, 1990). To describe the effects of inoculum density (*ID*) on the SAUDPC, the monomolecular model SAUDPC $(ID) = K_U \{1.0 - B \exp[-r_m \log(ID)]\}$ was fitted to data where K_U is the asymptote for the maximum SAUDPC, B is a constant, and r_m is the relative rate of SAUDPC increase over *ID*. Similarly, to describe the effects of *ID* on the disease incidence (DI), the monomolecular model DI $(ID) = 1.0 - B \exp[-r_m \log(ID)]$ was fitted to the data; B is a constant, and r_m is the relative rate of DI increase over *ID*.

An incubation period (*IP*) for disease development was established as the number of days taken for *DII* to become > 0. To describe the effects of inoculum density on the *IP*, the expanded negative exponential model $IP(ID) = C \exp[-r_I \log(ID)] + K_I$ was fitted to data, where *IP* is the incubation period in days, C is a constant, r_I is a rate of *IP* decrease, *ID* is the inoculum density in soil, and K_I is the asymptote for the minimum *IP*.

Regression analyses were conducted by the least-squares programme for nonlinear models of the Statistical Analysis System 6.08 (SAS Institute Inc., Cary, NC). Coefficient of determination (R^2), the mean square error, the asymptotic standard error associated with the estimated parameter, and the 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. The standard errors of the parameters obtained from regression analyses were used to compare the effects of chickpea genotypes as well as *F. oxysporum* f.sp. *ciceris* races on disease development (Campbell and Madden, 1990).

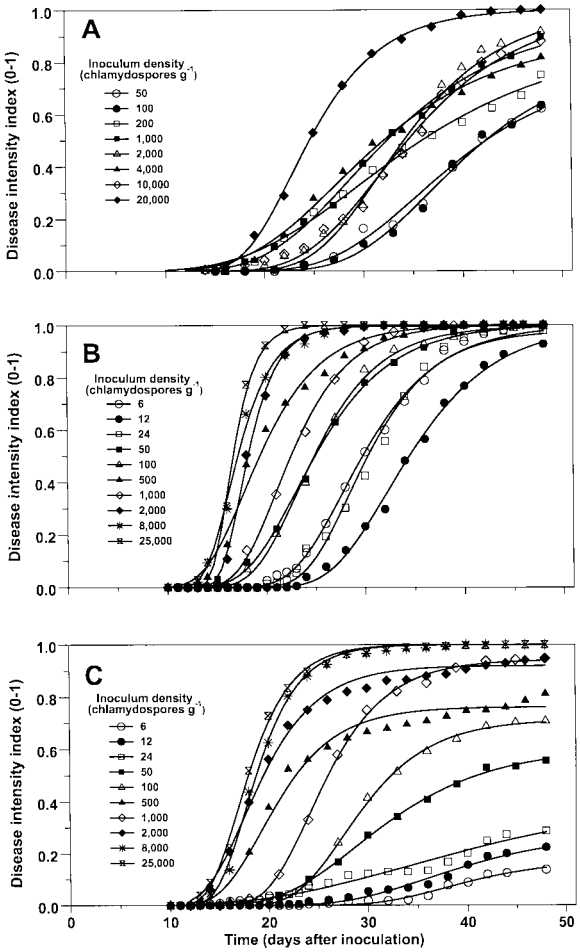

Figure 1. Progress of Fusarium wilt disease in two chickpea cultivars grown in soil infested with different inoculum densities of *F. oxysporum* f.sp. *ciceris* races 0 and 5. Each point is the mean *DII* calculated with data from four randomized blocks each with three pots and four plants per pot at 2-day intervals. The solid line represents the predicted disease progress curve calculated by the Gompertz function. (A) Cultivar P-2245 grown in soil infested with *F. oxysporum* f.sp. *ciceris* race 0. (B) Cultivar P-2245 grown in soil infested with *F. oxysporum* f.sp. *ciceris* race 5. (C) Cultivar PV-61 grown in soil infested with *F. oxysporum* f.sp. *ciceris* race 5.

Results

Both the inoculum density and race of *F. oxysporum* f.sp. *ciceris* significantly influenced the quantitative development of Fusarium wilt in the two chickpea cultivars. No disease developed in 'PV-61' grown in soil infested with *Foc*-0, in spite of the high inoculum densities used. For the remaining chickpea cultivar $\times$ pathogen race combinations, disease intensity (*DII*) for both the wilt ('P-2245'/*Foc*-5, 'PV-61'/*Foc*-5) and yellowing ('P-2245'/*Foc*-0) syndromes increased with the increase in inoculum density in soil (Figure 1).

The incubation period (*IP*) of the disease decreased exponentially with the increase in inoculum density of both *Foc*-0 and *Foc*-5 in soil (Figure 2A) irrespective of the cultivar used, the shortest *IP* occurring for the most conducive interaction, i.e. cultivar P-2245 grown in soil infested with the wilt-inducing *Foc*-5. The *IP*

in cultivar P-2245 inoculated with *Foc*-5 was always shorter than that in cultivar PV-61, and the difference between the *IP* in the two cultivars infected by *Foc*-5 decreased with the increase in inoculum density. Conversely, for disease reaction within a comparable range of inoculum density of either *F. oxysporum* f.sp. *ciceris* race, the highest *IP* always occurred in cultivar P-2245 grown in soil infested with the yellowing-inducing *Foc*-0, irrespective of inoculum density in soil (Figure 2A). Both the K_I and r_I parameters of the *IP–ID* negative exponential model were significantly ($P < 0.05$) influenced by the chickpea cultivar and *F. oxysporum* f.sp. *ciceris* race. The highest values of both the maximum *IP* (K_I asymptote) and the rate of *IP* decrease occurred in cultivar P-2245 grown in soil infested with *Foc*-0 (Figure 2A).

Disease incidence and SAUDPC were also influenced both by the chickpea cultivar and *F. oxysporum* f.sp. *ciceris* race. Disease incidence showed a monomolecular-type of increase with the increase in inoculum density in soil, except for the 'P-2245'/*Foc*-5 combination for which a disease incidence higher than 97% occurred at any inoculum density. Disease incidence in 'PV-61' grown in soil infested with *Foc*-5 ranged from 25% to 100%, as compared to that in 'P-2245' grown in soil infested with *Foc*-0 which ranged from 85% to 100% (Figure 2B). Also, SAUDPC values increased with the increase in inoculum density in soil according to the monomolecular model (Figure 2C). The estimated asymptotic value of SAUDPC (K_U parameter) was highest in the most conducive interaction, i.e. 'P-2245'/*Foc*-5, and it decreased in the disease reaction of the less susceptible 'PV-61' to the same race, as well as in that of the highly susceptible 'P-2245' to *Foc*-0, in that order. The relative rate of SAUDPC progress with increasing inoculum density was significantly higher ($P < 0.05$) in cultivar PV-61 inoculated with *Foc*-5 than in any other cultivar–race combination. Conversely, SAUDPC in cultivar P-2245 progressed with increasing inoculum density at a rate which was not significantly different ($P \geq 0.05$) between the two races of the pathogen (Figure 2C).

In order to analyze Fusarium wilt development over time as influenced by inoculum density of *F. oxysporum* f.sp. *ciceris*, the nonlinear form of both the Richards and Gompertz models was fitted to the observed *DII* progress curves. The Richards model offered an improved fit to disease progression data compared to the Gompertz model, which lacked the shape parameter. However, for most of the *DII* progress curves studied, the estimated value of the shape parameter

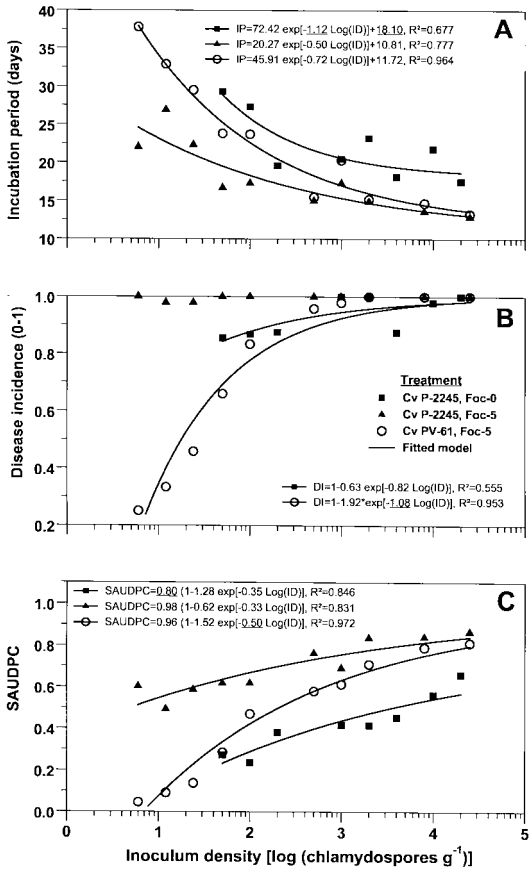

Figure 2. Relationship between *ID* of *F. oxysporum* f.sp. *ciceris* races 0 (*Foc*-0) and 5 (*Foc*-5) and the following parameters of Fusarium wilt development in chickpea cultivars P-2245 and PV-61. (A) *IP*, calculated as the number of days until *DII* became > 0. (B) *DI*. (C) SAUDPC. Each point is the mean of data from four randomized blocks each with three pots and four plants per pot. The solid line represents the predicted function calculated by the expanded negative exponential model (A) and the monomolecular model (B and C). Underlined parameter values are significantly different ($P < 0.05$).

in Richards function was not significantly different from 1. Since the Richards model, as a generalized form of growth curve, becomes closer to the Gompertz model as the shape parameter approaches 1, for further analyses the Gompertz function $DII(t) = K_G \exp[-B \exp(-r_G t)]$ was used where *DII* is the disease intensity index, K_G is the asymptote parameter, B is a constant of integration, r_G is the relative rate of disease increase, and t is the number of days after inoculation. Appropriate descriptions of *DII* increase over time were obtained with the Gompertz model (Tables 1–3). *DII* progress curves for the different

Table 1. Nonlinear fit of the Gompertz function to the increase of Fusarium wilt disease intensity over time in chickpea cultivar P-2245 inoculated with *F. oxysporum* f.sp.*ciceris* race 0

Inoculum density (chl. g^{-1})	Parameter estimates[a]			Statistics[b]		Residual plots[c]
	$K_G \pm$ SE	$B \pm$ SE	$r_G \pm$ SE	MSE	R^2	
50	0.7748 ± 0.0328	60.87 ± 16.29	0.1162 ± 0.0079	0.000630	0.991	R
100	0.8167 ± 0.0308	96.26 ± 6.55	0.1245 ± 0.0001	0.000508	0.993	R
200	0.8800 ± 0.0001	12.99 ± 1.69	0.0871 ± 0.0042	0.000755	0.990	R?
1000	0.9600 ± 0.0001	27.20 ± 0.71	0.1149 ± 0.0001	0.000443	0.997	R
2000	1.0000 ± 0.0000	107.93 ± 17.96	0.1478 ± 0.0057	0.001115	0.992	R?
4000	0.9000 ± 0.0001	19.90 ± 2.71	0.1096 ± 0.0042	0.000581	0.994	R?
10,000	1.0000 ± 0.0000	58.97 ± 1.81	0.1286 ± 0.0001	0.000794	0.993	R?
20,000	1.0000 ± 0.0000	98.99 ± 2.44	0.2015 ± 0.0001	0.000174	0.999	R

[a] K_G = Asymptotic value of *DII*; B = constant of integration; r_G = rate parameter; SE = asymptotic standard error for the parameter estimates.
[b] R^2 = Coefficient of determination; MSE = final mean square error.
[c] Standardized residuals plotted against *DII* values observed or predicted from nonlinear regression analysis. Patterns of residuals with a random scatter (R) or scarcely non-random scatter (R?), after visual inspection of residual plots.

Table 2. Nonlinear fit of the Gompertz function to the increase of Fusarium wilt disease intensity over time in chickpea cultivar P-2245 inoculated with *F. oxysporum* f.sp.*ciceris* race 5

Inoculum density (chl. g^{-1})	Parameter estimates[a]			Statistics[b]		Residual plots[c]
	$K_G \pm$ SE	$B \pm$ SE	$r_G \pm$ SE	MSE	R^2	
6	0.9974 ± 0.0001	212.30 ± 28.51	0.1924 ± 0.0042	0.000365	0.998	R
12	1.0000 ± 0.0000	254.59 ± 5.10	0.1714 ± 0.0001	0.000260	0.998	R
24	0.9792 ± 0.0001	517.34 ± 24.31	0.2193 ± 0.0001	0.001072	0.994	R?
50	1.0000 ± 0.0001	85.44 ± 0.22	0.1929 ± 0.0012	0.000313	0.998	R
100	0.9962 ± 0.0073	175.90 ± 0.03	0.2227 ± 0.0001	0.000361	0.998	R
500	1.0000 ± 0.0000	69.80 ± 3.54	0.2382 ± 0.0001	0.001262	0.993	NR
1000	1.0000 ± 0.0000	204.51 ± 6.93	0.2525 ± 0.0001	0.000343	0.998	R?
2000	1.0000 ± 0.0000	4602.60 ± 124.35	0.4839 ± 0.0001	0.000172	0.999	R
8000	1.0000 ± 0.0000	722.77 ± 22.81	0.4055 ± 0.0001	0.000284	0.998	R
25,000	1.0000 ± 0.0000	23087.01 ± 106.5	0.6249 ± 0.0001	0.000461	0.997	R

[a,b,c] See Table 1.
NR – Non-random scatter.

chickpea cultivars–*F. oxysporum* f.sp. *ciceris* race combinations, together with the fitted Gompertz model are illustrated in Figure 1. For this model, the cumulative amount of disease at the inflection point is given by $DII(t_{ip}) = K_G$ 0.37 and the time taken to reach it (t_{ip}) is given by $t_{ip} = \ln(B) r_G^{-1}$ (Madden and Campbell, 1990).

A response surface for *DII* progression as a function of both time from inoculation and inoculum density was developed with a two-stage method (Hau et al., 1985). Using the Gompertz function in the first step, the time component was separately analyzed for each inoculum density data subset (Tables 1–3). In the second step, the influence of inoculum density on the estimated values of the three Gompertz parameters, the asymptotic *DII* (K_G), the constant of integration (B), and the rate parameter (r_G) were analyzed.

The estimated values of K_G increase asymptotically with *ID* approaching the maximum possible value of 1.0. The relationship between K_G and *ID* can be well described by the monomolecular model $K_G(ID) = 1 - c_1 \exp[-c_2 \log(ID)]$ (Figure 3A). The estimated values for the rate parameter, r_G, showed an exponential increase with increasing inoculum densities in soil, so that r_G can be modelled by the function $r_G(ID) = c3 + c_4 \exp[c_5 \log(ID)]$ (Figure 3B). As expected, no relationship was found between the estimated values for the third parameter, B (Figure 3C), and therefore B

Table 3. Nonlinear fit of the Gompertz function to the increase of Fusarium wilt disease intensity over time in chickpea cultivar PV-61 inoculated with *F. oxysporum* f.sp.*ciceris* race 5

Inoculum density (chl. g^{-1})	Parameter estimates[a]			Statistics[b]		Residual plots[c]
	$K_G \pm$ SE	$B \pm$ SE	$r_G \pm$ SE	MSE	R^2	
6	0.1756 ± 0.0051	372.24 ± 79.70	0.1580 ± 0.0025	0.000169	0.914	R
12	0.2742 ± 0.0007	92.09 ± 7.60	0.1266 ± 0.0011	0.000105	0.981	NR
24	0.4101 ± 0.0082	10.77 ± 0.53	0.0700 ± 0.0001	0.000252	0.972	NR
50	0.6027 ± 0.0060	46.97 ± 1.26	0.1345 ± 0.0001	0.000073	0.999	R
100	0.7143 ± 0.0001	291.13 ± 7.55	0.2068 ± 0.0001	0.000123	0.999	R
500	0.7621 ± 0.0001	98.47 ± 40.36	0.2395 ± 0.0196	0.001414	0.987	R?
1000	0.9431 ± 0.0001	266.96 ± 6.83	0.2321 ± 0.0001	0.000190	0.999	R
2000	0.9200 ± 0.0001	76.42 ± 17.07	0.2476 ± 0.0116	0.000713	0.995	NR
8000	1.0000 ± 0.0000	386.10 ± 9.49	0.3368 ± 0.0001	0.000205	0.999	R
25,000	1.0000 ± 0.0000	285.19 ± 7.70	0.3348 ± 0.0001	0.000252	0.998	R

[a,b,c]See Table 1.
NR – Non-random scatter.

Table 4. Nonlinear fit of response surfaces as a function of time and *ID* for the increase of a Fusarium wilt *DII* in chickpea cultivars P-2245 and PV-61 inoculated with races 0 and 5 of *F. oxysporum* f.sp. *ciceris* (*Foc*)

Parameter estimates[a]						Statistics[b]		Residual plots[c]
$K_G(ID)$		$B(ID)$	$r_G(ID)$			MSE	R^2	
$c_1 \pm$ SE	$c_2 \pm$ SE	$c_6 \pm$ SE	$c_3 \pm$ SE	$c_4 \pm$ SE	$c_5 \pm$ SE			
Cultivar P-2245 / *Foc* race 0								
9.5651	1.8886	33.9566	2.92E-06	2.1794	0.1096	0.007149	0.935	R
12.2987	0.7251	3.0490	2.59E-07	0.0077	0.0039			
Cultivar P-2245 / *Foc* race 5								
1.0000	1.0000	189.4743	0.1477	0.2027	0.0000	0.007061	0.961	R
0.0000	0.0000	38.9258	0.0066	0.0048	0.0000			
Cultivar PV-61 / *Foc* race 5								
2.1123	0.9790	67.9609	0.0971	0.2368	0.0024	0.004417	0.971	R
0.1368	0.0567	18.2366	0.0046	0.0142	0.0127			

[a]A response surface was determined from the original data set using the function $DII(t, ID) = K_G(ID) \exp\{-B(ID) \times \exp[-r_G(ID)t]\}$. The parameters c_1–c_6 of this function were simultaneously estimated for each chickpea cultivar and *F. oxysporum* f.sp. *ciceris* race combination The relationship between K_G and *ID* was well described by the monomolecular model $K_G(ID) = 1 - c_1 \exp[-c_2 \log(ID)]$. The estimated values for the rate parameter, r_G, was modelled as a function of inoculum density in soil according to $r_G(ID) = c_3 + c_4 \exp[c_5 \log(ID)]$. No relationship was found between the estimated values for the third parameter, *B*, and *ID*, therefore *B* can be regarded as a constant, i.e. $B(ID) = c_6$. SE = asymptotic standard error for the parameter estimates.
[b]R^2 = Coefficient of determination; MSE = final mean square error.
[c]Standardized residuals plotted against *DII* values observed or predicted from nonlinear regression analysis. Patterns of residuals with a random scatter (R), after visual inspection of residual plots.

can be regarded as a constant, i.e. $B(ID) = c_6$. Based on results from the two-stage method, a response surface could be determined directly from the original data set (Hau et al., 1985) using the function $DII(t, ID) = K_G(ID) \exp\{-B(ID) \exp[-r_G(ID)t]\}$. Parameters ($c_1$–$c_6$) in that function were simultaneously estimated for each chickpea cultivar–*F. oxysporum* f.sp. *ciceris* race combination (Table 4, Figure 4).

The threshold of *ID* for disease to develop was influenced both by the nature of the *F. oxysporum* f.sp. *ciceris* race and susceptibility of the chickpea cultivar (Figures 1 and 4). In the cultivar–race combination most conducive for disease development ('P-2245'/*Foc*-5), the maximum final *DII* value was attained even at a very low initial inoculum density, i.e. 6–50 chlamydospores g^{-1} of soil (Figures 1B, 3A and

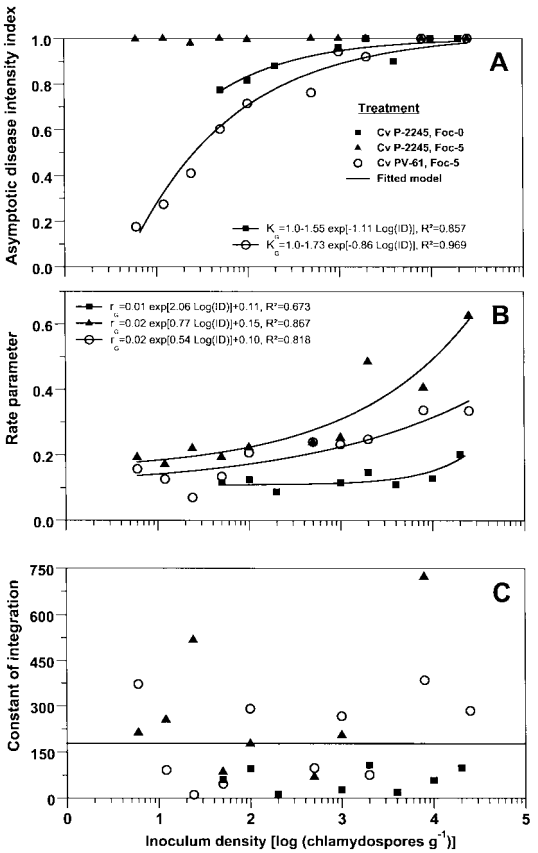

Figure 3. Relationship between *ID* of *F. oxysporum* f.sp. *ciceris* races 0 (*Foc*-0) and 5 (*Foc*-5) and the estimated parameter values of the Gompertz model adjusted to Fusarium wilt developed in chickpea cultivar P-2245 and PV-61. (A) The asymptotic *DII* (K_G). (B) The rate parameter (r_G). (C) The constant of integration (*B*). The solid line represents the predicted function calculated by the monomolecular model (A) and the expanded negative exponential model (B). Parameter *B* was regarded as a constant (C).

4B). On the contrary, when the moderately susceptible cultivar PV-61 was grown in soil infested with the same wilt-inducing *Foc*-5, the maximum disease intensity occurred only with an inoculum density of at least 1000–2000 chlamydospores g^{-1} of soil (Figures 1C, 3A and 4C). Conversely, when the highly susceptible 'P-2245' was inoculated with the yellowing-inducing *Foc*-0, the maximum disease intensity was attained with an inoculum density of 20,000 chlamydospores g^{-1} in soil, only (Figures 1A and 4A).

When data from all chickpea cultivar–*F. oxysporum* f.sp. *ciceris* race experimental combinations were pooled together, the estimated values for the relative rate of *DII* increase, r_G, and SAUDPC decreased exponentially and linearly, respectively, with the increase

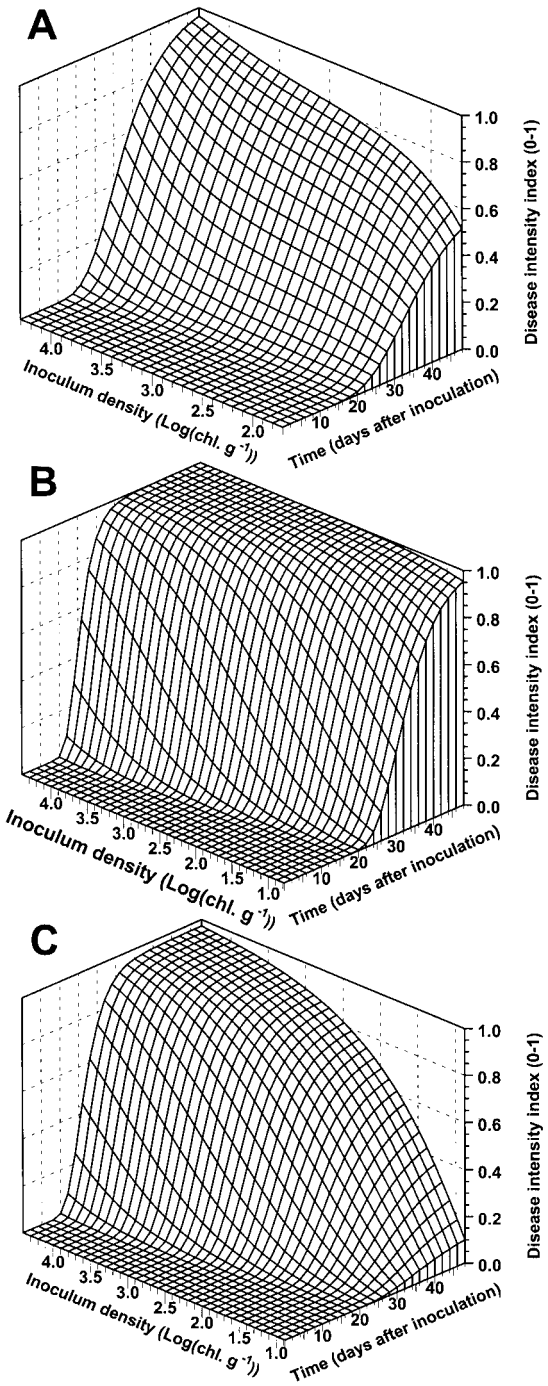

Figure 4. Surface response for *DII* of Fusarium wilt in chickpea cultivars caused by races of *F. oxysporum* f.sp. *ciceris* as a function of both time from inoculation and *ID*.(A) Cultivar P-2245 grown in soil infested with *F. oxysporum* f.sp. *ciceris* race 0. (B) Cultivar P-2245 grown in soil infested with *F. oxysporum* f.sp. *ciceris* race 5. (C) Cultivar PV-61 grown in soil infested with *F. oxysporum* f.sp. *ciceris* race 5.

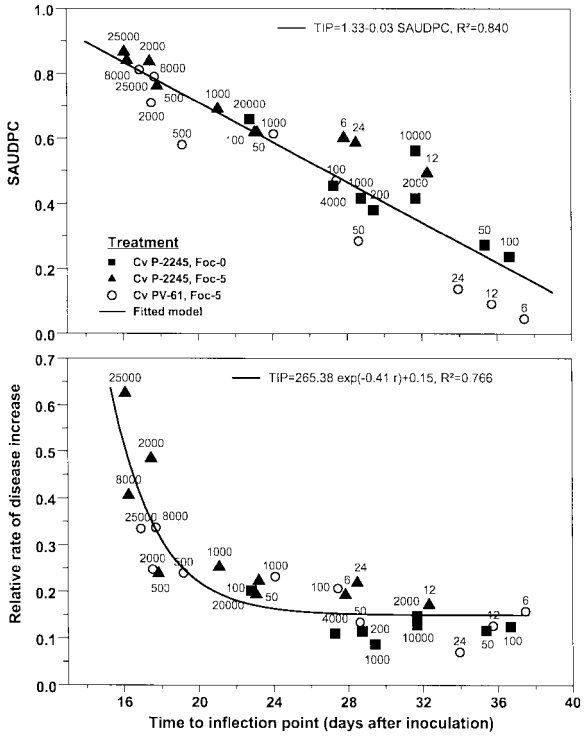

Figure 5. Relationship between time from inoculation to reach the inflection point (TIP) and the following parameters for disease intensity progress curves in chickpea cultivars P-2245 and PV-61 inoculated with different inoculum densities of *F. oxysporum* f. sp. *ciceris* race 0 (*Foc*-0) and 5 (*Foc*-5). (A) The estimated relative rate parameter of Gompertz model for *DII* increase over time (r_G). (B) SAUDPC. The solid line represents the predicted function calculated by the linear model (A) and the expanded negative exponential model (B). Numbers displayed near each data point indicate the inoculum density as number of chlamydospores g^{-1} in soil.

in the time period needed for the cumulative *DII* to reach the inflection point of the adjusted Gompertz function (Figure 5). That is, disease reactions with the most delayed onset had the lowest SAUDPC, and therefore the least disease intensity. Disease reactions with earlier onset and higher disease intensity developed on cultivar P-2245 grown in soil infested with higher inoculum densities of the wilt-inducing *Foc*-5. Conversely, disease reactions which were most delayed and with the least disease intensity were those which developed in either of the chickpea cultivars inoculated with the lowest inoculum density of *Foc*-5, or in 'P-2245' inoculated with low or intermediate inoculum densities of the yellowing-inducing *Foc*-0. Both for 'PV-61' inoculated with *Foc*-5 and 'P-2245' inoculated with *Foc*-0, the relative rate of *DII* progress and the final *DI* increased steadily with inoculum within the higher range of inoculum densities in the study (i.e. 1000 chlamydospores g^{-1} of soil or more for *Foc*-5, and 4000–10,000 chlamydospores g^{-1} of soil for *Foc*-0 (Figure 5).

Discussion

The role of initial inoculum density of soilborne fungi in disease development has been well documented in many pathosystems. Generally, for Fusarium wilt diseases, increasing the amount of initial inoculum enhances disease severity and reduces the time required for maximum disease development (Ben-Yephet et al., 1996; Bhatti and Kraft, 1992; Harris and Ferris, 1991; ICRISAT, 1989; Martyn and McLaughlin, 1983; Sugha et al., 1994; Zote et al., 1996). However, the influence of virulence in the pathogen race and susceptibility in the host cultivar on that relationship has not been addressed in many cases.

In this study, development of severe disease in chickpea cultivar P-2245 required much less inoculum density of the wilt-inducing race *Foc*-5 compared to that of the yellowing-inducing race *Foc*-0 (Figures 1 and 2). This clearly indicates that *Foc*-5 was much more virulent than *Foc*-0 to susceptible chickpea, irrespective of the disease syndrome induced in the plant. This difference in virulence between *Foc*-0 and *Foc*-5 could be associated with the genetic nature of these races, as indicated by the grouping of the isolates into two distinct clusters when subjected to random amplified polymorphic DNA analyses (Kelly et al., 1994). Differences in several of the stages during the plant–pathogen interaction might account for the difference in virulence between *Foc*-0 and *Foc*-5 observed in our study. One of these would be the faster and more extensive colonization of xylem vessels in 'P-2245' root and stem bases by *Foc*-5 compared to *Foc*-0, as revealed in histological studies (Jiménez-Díaz et al., 1989a).

Increasing the inoculum density of *F. oxysporum* f.sp. *ciceris* influenced development of Fusarium wilt in chickpea through an exponential reduction of the time for disease onset (decreasing *IP*) and a monomolecular increase of the overall amount of disease (increasing disease incidence and SAUDPC) (Figure 2). However, the extent of these effects was significantly influenced both by virulence of the pathogen race and susceptibility of the chickpea cultivar. Thus, effects on *IP* and SAUDPC were highest in the most conducive cultivar–race combination

('P-2245'/*Foc*-5), and decreased in the less susceptible cultivar, PV-61, and for the less virulent *Foc*-0 race, in that sequence (Figures 1 and 2).

Sugha et al. (1994) reported that an increase in inoculum load of *F. oxysporum* f.sp. *ciceris* accelerated the progress of Fusarium in chickpea and that lower inoculum concentrations delayed the expression of wilt symptoms. Similarly, Bhatti and Kraft (1992) showed that severity of Fusarium wilt of chickpea increased with increasing inoculum density of the pathogen, with severe disease developing at 25 and 30 °C at 500 and 1000 propagules g^{-1} of soil. However, the nature of the *F. oxysporum* f.sp. *ciceris* race used was not specified in these studies. The present results showed that a threshold of inoculum density of *F. oxysporum* f.sp. *ciceris* depended on the virulence of the pathogen race and susceptibility of the chickpea cultivar. Thus, for the most disease-conducive 'P-2245'/*Foc*-5 combination, the highest amount of disease was attained with 6 chlamydospores g^{-1} of soil which was the lowest inoculum density of *Foc*-5 used. Conversely, 1000 chlamydospores g^{-1} of soil of the same race were needed to attain a comparable amount of disease in the less susceptible cultivar PV-61. Likewise, some 20,000 chlamydospores g^{-1} of soil of the less virulent *Foc*-0 were required to attain the highest amount of disease in the highly susceptible 'P-2245' (Figures 1 and 4).

The influence of cultivar susceptibility on the relationship between inoculum density of *F. oxysporum* f.sp. *ciceris* and development of Fusarium wilt in chickpea was also found in greenhouse experiments with *F. oxysporum* f.sp. *ciceris* race 1 and chickpea cultivars JG-62 and K-850 in India (ICRISAT, 1989). While disease incidence in the susceptible 'JG-62' increased from 30% at an inoculum density of 33 propagules g^{-1} of soil up to 100% with 483 propagules g^{-1} of soil or higher, in the partially resistant 'K-850' a minimum of 483 propagules g^{-1} of soil were required to produce 10% disease incidence, and 43% disease incidence was attained with 3283 propagules g^{-1} of soil, the highest inoculum density of the pathogen used.

Results from the present study allowed the development of a response surface function for disease development over time as influenced by inoculum density (Figure 4). Such a surface is based on the Gompertz model, in which the model parameters are expressed as a function of the pathogen inoculum density. This response surface confirmed that in the chickpea/*F. oxysporum* f.sp. *ciceris* pathosystem the final amount of disease increases in a monomolecular relationship with increasing inoculum density, except in the most susceptible cultivar for which a low inoculum density of the most virulent race saturates the system (Figure 3A). Similarly, the response surface indicated an exponential increase in the relative rate of disease progress with increasing inoculum density of the pathogen, except for the least conducive 'P-2245'/*Foc*-0 combination. This relationship suggests a synergistic interaction within *Foc*-5 propagules at high inoculum densities for the rate of disease development over time, the effect of such an interaction being more pronounced in the most susceptible cultivar (Figure 3B).

Our results have practical implications for strategies of Fusarium wilt management in chickpeas, including biological control, development and use of resistant cultivars, and cultural practices. Protection from disease conferred by seed treatment with a conidial suspension of non-pathogenic *F. oxysporum* can be annulled by high inoculum densities of *Foc*-5 (Hervás et al., 1995; 1997). Similarly, high inoculum densities of the pathogen can overcome a valuable partial resistance (delayed wilting) (ICRISAT, 1989), which is controlled by homozygous recessive alleles at either of two loci or by a dominant allele at a third locus (Upadhyaya et al., 1983). Such a partial resistance is expressed under field conditions (Jiménez-Díaz et al., 1991), and may be of interest because yield loss in late wilting genotypes is expected to be lower than in early wilting genotypes (Haware and Nene, 1980). Furthermore, late wilting genotypes have important implications in breeding for resistance to *F. oxysporum* f.sp. *ciceris*, as complete resistance can be obtained from crosses involving only late wilting parents (Singh et al., 1987). Thus, while screening chickpea genotypes for partial resistance to *F. oxysporum* f.sp. *ciceris* an appropriate inoculum density of the pathogen must be used in order to avoid that level of resistance being undetected (Figures 1C and 3A) (ICRISAT, 1989). The importance of pathogen inoculum density in Fusarium wilt resistance screening has been pointed out in several pathosystems (Alou et al., 1974; Martyn and McLaughlin, 1983).

Fusarium wilt of chickpea can be managed by choice of sowing date (Jalali and Chand, 1992; Navas-Cortés et al., 1998). Under the Mediterranean environment prevailing in southern Spain, changing the chickpea sowing date from early spring to early winter significantly delayed epidemic onset, slowed down epidemic development, and reduced the final amount of disease (Navas-Cortés et al., 1998), and consequently

the loss of seed yield caused by Fusarium wilt (J.A. Navas-Cortés and R.M. Jiménez-Díaz, unpublished). However, as shown by results from this research, the benefits provided by such a cultural practice for Fusarium wilt management can be overridden if a cultivar too susceptible to the disease is used, or if soils where a highly virulent race of the pathogen prevails are present, or both (Navas-Cortés et al., 1998).

Acknowledgements

Research support was provided by a Postdoctoral fellowship for J.A. Navas-Cortés from Consejo Superior de Investigaciones Científicas, Spain, and by grants AGR97-1479 and HA 1997-0033 from Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT) and Ministerio de Educación y Cultura, respectively.

References

Alou H, Katan J and Kedar N (1974) Factors affecting penetrance of resistance to *Fusarium oxysporum* f.sp. *lycopersici* in tomatoes. Phytopathology 64: 455–461

Ben-Yephet Y, Reuven M, Zviebil A and Shtienberg D (1996) Effects of initial inoculum and cultivar resistance on incidence of Fusarium wilt and population densities of *Fusarium oxysporum* f.sp. *dianthi* on carnation and in soil. Phytopathology 86: 751–756

Bhatti MA and Kraft JM (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

Bouslama M (1980) Chickpea improvement in Tunisia. In: Proc Int Workshop Chickpea Improv (pp 277–280) ICRISAT, Hyderabad, India

Campbell CL and Madden LV (1990) Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York

Gupta O, Kotasthane SR and Khare MN (1987) Factors influencing epidemiology of vascular wilt of chickpea. Proc Natl Acad Sci India 57: 86–91

Halila MH and Strange RN (1996) Identification of the causal agent of wilt of chickpea in Tunisia as *Fusarium oxysporum* f.sp. *ciceri* race 0. Phytopath Medit 35: 67–74

Harris AR and Ferris H (1991) Interactions between *Fusarium oxysporum* f. sp. *tracheiphilum* and *Meloidogyne* spp. in *Vigna unguiculata*. I. Effects of different inoculum densities on Fusarium wilt. Plant Pathol 40: 445–456

Hau B, Eisensmith SP and Kranz J (1985) Construction of temporal models: II. Simulation of aerial epidemics. In: Gilligan CA (ed) Advances in Plant Pathology. Vol 3 (pp 31–65) Academic Press, London

Haware MP (1990) Fusarium wilt and other important diseases of chickpea in the Mediterranean area. Options Méditerranéennes. Série Séminaires 9: 63–166

Haware MP and Nene YL (1980) Influence of wilt at different stages on the yield loss in chickpea. Trop Grain Legume Bull 19: 38–40

Haware MP and Nene YL (1982) Races of *Fusarium oxysporum* f.sp. *ciceri*. Plant Dis 66: 809–810

Hervás A, Landa B and Jiménez-Díaz RM (1997) Influence of chickpea genotype and *Bacillus* sp. on protection from Fusarium wilt by seed treatment with nonpathogenic *Fusarium oxysporum*. Eur J Plant Pathol 103: 631–642

Hervás A, Trapero-Casas JL and Jiménez-Díaz RM (1995) Induced resistance against Fusarium wilt of chickpea by nonpathogenic races of *Fusarium oxysporum* f.sp. *ciceris* and nonpathogenic isolates of *F. oxysporum*. Plant Dis 79: 1110–1116

Hoagland DR and Arnon DI (1950) The water culture method for growing plants without soil. Calif Agr Exp Stn Circ 347: 32 pp

ICRISAT (1989) Annual Report 1988. ICRISAT, Patancheru, India

Jalali BL and Chand H (1992) Chickpea wilt. In: Singh US, Mukhopadhayay AN, Kumar J and Chaube HS (eds) Plant Diseases of International Importance, Vol I. Diseases of Cereals and Pulses (pp 429–444) Prentice Hall, Englewood Cliffs

Jiménez-Díaz RM, Basallote-Ureba MJ and Rapoport H (1989a) Colonization and pathogenesis in chickpea infected by races of *Fusarium oxysporum* f.sp. *ciceri*. In: Tjamos EC and Beckman CH (eds) Vascular Wilt Diseases of Plants, NATO ASI Series, Vol H28 (pp 113–121) Springer-Verlag, Berlin

Jiménez-Díaz RM, Singh KB, Trapero-Casas A and Trapero-Casas JL (1991) Resistance in kabuli chickpea to Fusarium wilt. Plant Dis 75: 914–918

Jiménez-Díaz RM, Trapero-Casas A and Cabrera de la Colina J (1989b) Races of *Fusarium oxysporum* f.sp. *ciceri* infecting chickpeas in southern Spain. In: Tjamos EC and Beckman CH (eds) Vascular Wilt Diseases of Plants, NATO ASI Series, Vol H28 (pp 515–520) Springer-Verlag, Berlin

Kelly A, Alcalá-Jiménez AR, Bainbridge BW, Heale JB, Pérez-Artés E and Jiménez-Díaz RM (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

Madden LV and Campbell CL (1990) Nonlinear disease progress curves. In: Kranz J (ed) Epidemics of Plant Diseases, Mathematical Analysis and Modeling, 2nd edn, Ecological Studies 13 (pp 181–229) Springer-Verlag, Berlin, Germany

Martyn RD and McLaughlin RJ (1983) Effect of inoculum concentration on the apparent resistance of watermelons to *Fusarium oxysporum* f.sp. *niveum*. Plant Dis 67: 493–495

Navas-Cortés JA, Hau B and Jiménez-Díaz RM (1998) Effect of sowing date, host cultivar, and race of *Fusarium oxysporum* f.sp. *ciceris* on development of Fusarium wilt of chickpea. Phytopathology 87: 1338–1346

Nene YL and Reddy MV (1987) Chickpea diseases and their control. In: Saxena MC and Singh KB (eds) The Chickpea (pp 233–270) CAB International, Oxon, UK

Saxena MC (1990) Problems and potential of chickpea production in the nineties. In: Chickpea in the Nineties: Proceedings of the Second International Workshop on Chickpea Improvement (pp 13–27) ICRISAT, Patancheru, India

Singh KB and Dahiya BS (1973) Breeding for wilt resistance in chickpea. In: Symposium on Wilt Problem and Breeding for Wilt Resistance in Bengal Gram (pp 13–14) Indian Research Institute, New Delhi, India

Singh H, Kumar J, Smithson JB and Haware MP (1987) Complementation between genes for resistance to race 1 of *Fusarium oxysporum* f.sp. *ciceri* in chickpea. Plant Pathol 36:539-543

Sugha SK, Kapoor SK and Singh BM (1994) Factors influencing Fusarium wilt of chickpea (*Cicer arietinum* L.). Indian J Mycol Pl Pathol 24: 97–102

Trapero-Casas A and Jiménez-Díaz RM (1985) Fungal wilt and root rot diseases of chickpea in southern Spain. Phytopathology 75: 1146–1151

Upadhyaya HD, Smithson JB, Haware MP and Kumar J (1983) Resistance to wilt in chickpea. II. Further evidence for two genes for resistance to race 1. Euphytica 32: 749–755

Westerlund FV Jr, Campbell RN and Kimble KA (1974) Fungal root rots and wilt of chickpea in California. Phytopathology 64: 432–436

Zote KK, Haware MP, Jayanthi S and Narayana Rao J (1996) Effects of inoculum density of *Fusarium oxysporum* f.sp. *ciceri* race 1 and 2 on chickpea wilt. Phytopath Medit 35: 43–47