Influence of Temperature, Wetness Period, Plant Age, and Inoculum Concentration on Infection and Development of Ascochyta Blight of Chickpea

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ABSTRACT

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The optimum temperature for infection and development of Ascochyta blight of chickpea, caused by *Ascochyta rabiei,* in 2-wk-old seedlings in controlled environment studies was about 20 C. At this temperature, 7.6 and 17 h of wetness were required for the pathogen to cause significant light and severe infection, respectively. Increasing wetness periods greater than 6 h during the infection period resulted in increased disease severity, regardless of the temperature. Some infection (9.6%) occurred even when plants did not receive a wetting period after inoculation. Dry periods (6–48 h) immediately after inoculation increased disease severity over plants receiving the same wetness period without drying, while the opposite effect occurred when dry periods >12 h were initiated after an initial wetting period of 6 h. Although disease development and symptom expression were most affected by temperature during infection, postinfection temperature also was influential. The lower and upper limits for infection and disease development were < 5 and about 30 C, respectively. Disease development was suppressed at 30 C in plants incubated at 20 C during the infection period. At a constant temperature of 20 C, the minimum incubation and latent periods were 4.5 and 5.5 days, respectively. Lower or higher temperatures increased the duration of these periods. Disease developed more slowly in 8-wk-old plants at the podding stage than in 2-wk-old seedlings, but final disease severity in both groups of plants was similar for most temperatures. Increasing inoculum concentration from 4×10^4 to 1×10^7 conidia per milliliter increased disease severity, the magnitude of which depended on the level of resistance of the chickpea cultivars to blight. A multiple regression model was derived empirically to describe disease severity 14 days after inoculation as a function of temperature and wetness duration. Other models were developed to describe the duration of wetness period for a specified level of disease, incubation period, latent period, and disease development as functions of temperature.

Additional keywords: Cicer arietinum, quantitative epidemiology.

Ascochyta blight, or *rabia* in Spanish, caused by *Ascochyta rabiei* (Pass.) Labr., is a devastating disease of chickpea (*Cicer arietinum* L.) in many regions of the world (21,27), including the Palouse region of eastern Washington and northern Idaho as well as Spain (14,22,29). The fungus attacks all aboveground parts of the plant at any stage of crop development, causing characteristic necrotic lesions that can lead to death of the plant (21). Pycnidia develop extensively in the blighted areas and produce conidia that are the inoculum for secondary infections. The disease appears initially in limited, isolated foci in the crop and spreads rapidly when suitable weather conditions prevail. The number of secondary infection cycles throughout the growing season determines the intensity of attacks (21).

Although much research has been done on certain aspects of Ascochyta blight and its control by various means (21,24,27), little information exists on the effects of different environmental

factors on the epidemiology of the disease. Although temperature and wind (17,21,26,27,29,33) influence disease development and spread, rainfall is likely the critical factor in most epidemics (16,17,21). Most of this work has been done under field conditions, and information on the precise effects of environmental variables on Ascochyta blight under controlled conditions is scarce and contradictory (4,31). This information is necessary to develop a forecasting system to implement an integrated approach to disease control.

The objectives of this study were to determine more precisely the influence of temperature and wetness period on infection and development of Ascochyta blight, to use this information to quantify the relationship between disease severity and environmental variables, and to standardize conditions and inoculum concentration for artificial inoculations with *A. rabiei*. A preliminary report has been given (30).

MATERIALS AND METHODS

Isolates and inoculum production. Four monoconidial isolates of *A. rabiei* from naturally infected chickpea plants (two from Spain, one from the International Center for Agricultural Research in the Dry Areas [ICARDA] in Aleppo, Syria, and one from Genesee, ID) were used in the first experiment to study the effect of inoculum concentration. The isolate from Genesee was used in all subsequent experiments.

Conidia of *A. rabiei* were produced on chickpea seed meal-dextrose agar medium (CDA) prepared by boiling 50 g of chickpea seed meal in 500 ml of distilled water for 30 min, straining through cheesecloth, and adding 20 g of dextrose, 20 g of agar, and distilled water to bring the total volume to 1 L. CDA plates were flooded with 10 ml of a conidial suspension (about 1×10^6 conidia per milliliter) from a 7-day-old culture of *A. rabiei*. After 5–10 min, the liquid was poured off and the plates were incubated at 21–25 C with a 12-h photoperiod of fluorescent and near-ultraviolet light at 85 μE m^{-2} s^{-1}. Conidial suspensions were prepared from 5- to 7-day-old CDA cultures by adding sterile distilled water, rubbing the culture surface gently with a bent glass rod, and filtering through four layers of cheesecloth. The suspensions were adjusted with sterile distilled water to desired concentrations using a hemacytometer. Tween 20 (one drop per 100 ml) was added to the inoculum suspensions as a wetting agent.

Plant production and inoculation. Four kabuli chickpea cultivars were used in the inoculum concentration experiment: Burpee (PI 458870) from the United States, Blanco Lechoso and Pedrosillano from Spain, and ILC 3279 from ICARDA in Syria. Cultivars Blanco Lechoso and Burpee are highly susceptible to *A. rabiei* under field conditions, Pedrosillano is moderately susceptible, and ILC 3279 is resistant to several races of the pathogen (21,29). In subsequent experiments, only Burpee was used.

Except for the plant age experiment, all inoculations were performed with 2-wk-old chickpea seedlings that had five to seven leaves. Seeds were germinated in trays filled with vermiculite and transplanted at about 10 days into 10-cm-diameter pots (three plants per pot) containing 0.6 L of a potting mixture (55% peat moss, 35% pumice, and 10% sand, v/v/v). To test the influence of plant age, 8-wk-old plants at the podding stage (one plant per 10-cm-diameter pot) also were inoculated. Plants were grown in the greenhouse at 18–26 C and fertilized every 2 wk with a water-soluble fertilizer (20-10-20, N-P-K). Before inoculation, plants were preconditioned for 1 h at the same temperature to which they were exposed after inoculation.

Plants were sprayed to incipient runoff (1 ml per seedling or 5 ml per plant at the podding stage) with the conidial suspension using an airbrush sprayer (Paasche Airbrush Co., Harwood Heights, IL) operated at 0.1 MPa. In all inoculations, conidia germination was higher than 90% as determined by spraying 2% water agar plates with the conidial suspension.

Incubation treatments differed among experiments. In general, plants were incubated during the wetting period in moist chambers that consisted of closed transparent plastic cages placed in metal trays filled with water to provide 100% relative humidity (RH). Plastic cages were sprayed with water before use, and after arrangement of the plants they were placed inside darkened controlled-environment chambers (Controlled Environments, Winnipeg, Canada) set at various constant temperatures with RH > 75%. After the wetness period, moistened plants were completely dried at room temperature (about 23 C) using a fan and a hair drier for approximately 30 min, and then they were transferred to the greenhouse at 18–26 C or to growth chambers set at various temperatures for symptom development. In the growth chambers, fluorescent lights provided a 14-h photoperiod (324 μE m^{-2} s^{-1}) and RH was 40–80%. Uninoculated checks were similarly handled, except they were sprayed with sterile distilled water plus Tween 20.

Effect of inoculum concentration on disease severity. Inoculum suspensions of 4×10^4, 2×10^5, 1×10^6, and 1×10^7 conidia per milliliter of the four isolates of *A. rabiei* were applied to sets of pots replicated three times, each pot containing three chickpea seedlings of the four cultivars mentioned above. Inoculated seedlings were placed in a mist chamber in a greenhouse at 19–25 C for 96 h. Disease severity was recorded at 7, 14, and 21 days after inoculation using a 0–9 rating scale as indicated in the disease assessment section. The experiment was repeated once and the pooled data from two experiments were analyzed by analysis of variance. Because of variance heterogeneity among cultivars, analyses were performed separately by cultivar. A suspension of 5×10^5 conidia per milliliter was used in all subsequent inoculations.

Effect of temperature during the wetness period and wetness duration on infection. Inoculated chickpea seedlings were placed inside moist chambers in darkened growth chambers set at 5, 10, 15, 20, 25, and 30 C and in the greenhouse at 18–26 C. In each incubator, plants were subjected to 3-, 6-, 12-, 24-, 48-, and 96-h wetness periods. After plants were removed from the moist chambers, they were dried for 30 min and then transferred to the greenhouse bench for symptom development. Besides the uninoculated check, four inoculated pots were dried immediately after inoculation and transferred to the greenhouse to serve as a 0-h wetness period treatment. Disease severity was recorded 7 and 14 days after inoculation using the 0–9 rating scale. Each combination of temperature and wetness duration was applied to four pots with three seedlings each; this experiment was conducted three times. A split-plot design was used in which experiments were blocks, temperatures were main plots, wetness periods were subplots, and pots were replications. Because analysis of variance did not show significant differences between experiments (blocks), regression analysis was performed on the pooled data from the three experiments.

Effect of temperature on infection and disease development. Inoculated chickpea seedlings were separated in three different groups of treatments to study the influence of temperature during or after the wetness period. In the first group, plants were inoculated and handled as described previously for the temperature-wetness duration experiment, except that they were only subjected to a 48-h wetting period.

In the second group, inoculated seedlings were incubated for 48 h in moist chambers placed in darkened growth chambers at 20 C, the optimum temperature recorded in the temperature-wetness duration experiment. After the wetness period, plants were dried and transferred to growth chambers at 5, 10, 15, 20, 25, and 30 C with a 14-h photoperiod for symptom development.

In the third group, inoculated seedlings were incubated in moist chambers in darkened growth chambers at 5, 10, 15, 20, 25, and 30 C for 48 h. After the wetness period, plants were removed from the moist chambers, dried, and returned to the same growth chambers with a 14-h photoperiod for symptom development.

Plants were evaluated daily for symptoms of Ascochyta blight and formation of pycnidia. For each plant, the number of days to first symptoms (incubation period) and to first pycnidia (latent period) was recorded. Plants also were evaluated weekly for disease severity using the 0–9 rating scale. Final assessments were made when disease severity reached the maximum value or started

to decline, which was approximately 21 days after inoculation in the first group or 35 days after inoculation in the second and third groups.

There were four replicated pots (three plants per pot) per treatment and the experiment was conducted twice. For comparative purposes, the standardized area under the disease progress curve (SAUDPC) was calculated in each replication by dividing the AUDPC value by the total time duration of disease development. Transformed data were analyzed by analysis of variance. In the third experiment, regression analyses also were performed on the pooled data of disease severity, incubation and latent periods, and SAUDPC values.

Effect of plant age and temperature during the wetness period on disease development. Two-week-old seedlings or 8-wk-old plants at the podding stage were inoculated and placed in moist chambers in darkened growth chambers at 5, 10, 15, 20, 25, and 30 C for 48 h. After the wetness period, plants were removed from the moist chambers, dried, and transferred to the greenhouse bench at 18–26 C for symptom development. Disease severity was recorded at 7, 14, and 21 days after inoculation using a 0–9 rating scale. There were four replicated pots (three seedlings or one adult plant per pot) per treatment and the experiment was repeated twice. Analysis of variance was performed on the pooled data from the three experiments separately for each assessment time and on the SAUDPC values.

Effect of interrupted wetness periods on infection and disease development. Inoculated seedlings were subjected to one of three wet-dry treatments: 1) a continuous wetness period of 6, 12, 24, or 48 h and the 0-h wetness check; 2) a 24-h wetness period after a 6-, 12-, 24-, or 48-h dry period initiated immediately after inoculation; and 3) a 24-h wetness period after a dry period of 6, 12, 24, or 48 h begun 6 h after inoculation. During the wetness period, plants were incubated in the moist chambers in a darkened growth chamber at 20 C with RH $>$ 75%. For the dry treatment, plants were incubated in a growth chamber at 20 C with a 14-h photoperiod and RH $<$ 50%. Dried plants were rewetted by placing them in the moist chambers. Temperature and RH were monitored continuously with a hygrothermograph. After the last wetness period, plants were dried and incubated in the dry growth chamber mentioned above for a postinoculation period of 7 days. Plants then were transferred to the greenhouse at 18–26 C for symptom development. Disease severity was recorded 14 days after inoculation using a 0–9 rating scale. Each treatment was applied to three replicated pots, each with three seedlings, and the experiment was repeated three times. Analysis of variance was performed on the pooled data from the four experiments, using wetness/dry treatments as a factor with 13 levels.

Disease assessment and data analyses. Each plant was assessed for disease severity at weekly intervals using a 0–9 rating scale. The scale considers percentage of affected leaves and stems in a way similar to the 0–11 scale of Horsfall-Barratt (HB) (9), but for Ascochyta blight, the 8, 9, and 10 values of the HB scale, corresponding to percentage values of 88–100%, were reduced to the value 8, corresponding to 95% disease severity. HB class value 11 became value 9 in the modified scale for Ascochyta blight. Class values were converted to percentage of foliage affected: 0, 2.5, 5, 10, 40, 60, 80, 95, and 100%, respectively. This transformation follows the Weber-Fechner law adapted in the HB scale (10), and it is very close to the average function (Fig. 1), which coincides with the Elanco formula recommended (3) to convert class values into percentage of disease.

Percentage values were used for all data analyses and also transformed into arcsin $\sqrt{Y/100}$ for analysis of variance. All experiments were repeated at least once, and similarity among experimental runs tested by preliminary analyses of variance using experimental runs as blocks allowed combining data for analyses of variance and linear regression. Data were analyzed using Statistix (NH Analytical Software, Roseville, MN). Treatment means were compared using Fisher's protected least significant difference (LSD) at $P = 0.05$. The best regression model was chosen from many combinations of terms based on the significance of the estimated parameters ($P \leq 0.05$), coefficients of determination (R^2), coefficients of determination adjusted for degrees of freedom (R_a^2), Mallow's Cp statistic, and pattern of residuals (3,6).

RESULTS

Effect of inoculum concentration on disease severity. Disease severity increased with increasing inoculum concentration from 4×10^4 to 1×10^7 conidia per milliliter, but the pattern of this increase depended on the chickpea cultivar (Fig. 2). There was no significant effect of isolates, so the data for isolates have been pooled. In the highly susceptible cultivars, Blanco Lechoso and Burpee, disease severity was greater than 50% for all inoculum concentrations, and there was little or no significant increase in disease severity with increases in inoculum concentration. Conversely, the resistant cultivar ILC 3279 was more resistant than other cultivars at all inoculum concentrations, with a maximum disease severity of 12.7% at the highest dose (10^7 conidia per milliliter). In the moderately susceptible cultivar Pedrosillano,

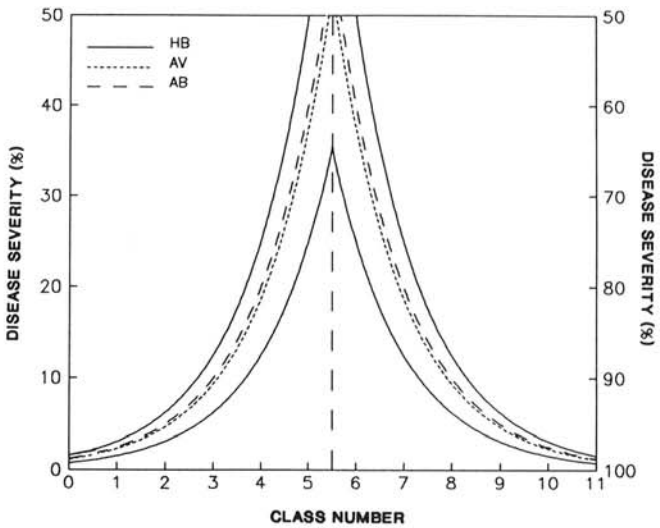

Fig. 1. Relationship between disease classes in the Horsfall-Barratt (HB) scale and percentage of disease. All curves fulfill the general function $Y = a \times 2^n$ ($n \leq 5.5$, $Y \leq 50\%$), $Y = 100 - a \times 2^{11-n}$ ($n > 5.5$, $Y > 50\%$). HB, Functions for limits of the class intervals in the HB scale (upper limit, $a = a_l = 1.5625$; lower limit, $a = 1/2\ a_l$). AV, Average function to convert class values into percentage of disease in the HB scale ($a = 3/4\ a_l = 1.172$). AB, Function used to convert class values into percentage of disease severity in the Ascochyta blight scale ($a = 1.25$).

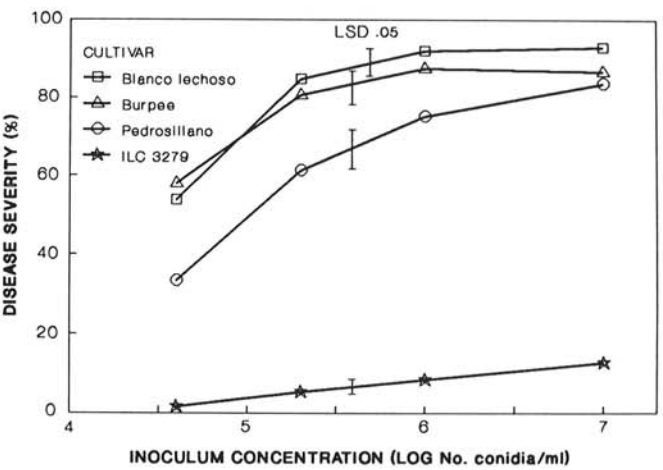

Fig. 2. Effect of inoculum concentration on infection of four chickpea cultivars by *Ascochyta rabiei*. Values represent the mean percent disease severity of 72 plants inoculated with each of four inoculum concentrations (4×10^4, 2×10^5, 1×10^6, and 1×10^7 conidia per milliliter).

disease severity depended greatly on inoculum dose, ranging from 32.9% at 4×10^4 conidia per milliliter to 83.5% at 1×10^7 conidia per milliliter (Fig. 2).

Effect of temperature and wetness duration on disease development. On the basis of analysis of variance, disease severity was significantly affected by temperature, wetness duration, and their interaction. In general, disease severity increased with increasing wetness duration for wetness periods > 6 h (Fig. 3). At wetness periods of 3 or 6 h, there was an average disease severity of 8.6% for all temperatures tested, which did not differ significantly from the 0-h control. Similarly, disease severity in plants incubated at 5, 10, and 30 C that received a 12-h wetness period did not differ significantly from the 0-h check. No significant differences in disease severity were observed between 48 and 96 h of wetness duration at 25 and 30 C. Disease severity increased with increasing temperatures to a maximum near 20 C and decreased dramatically between 25 and 30 C (Fig. 3). At 20 C, symptoms began to appear 96 h after inoculation with long wetting periods (48 or 96 h). However, symptom development was delayed with shorter wetting periods and at lower or higher temperatures. Symptoms initially appeared as white spots on the leaves and irregular, light green lesions on petioles and stems, which frequently girdled and broke the petioles and stems. Lesions with pycnidia turned brown 1–2 days later. Disease severity increased with time up to 14 days after inoculation at all temperatures and wetness periods tested. Plants not severely affected continued to grow actively in the greenhouse, and disease severity decreased with an increase in healthy tissue. For this reason, only disease severity data at 14 days after inoculation were analyzed.

The influence of temperature and wetness period on infection of chickpea seedlings by *A. rabiei* was described by the following equation:

$$Y = -38.05 + \ln W[23.69 - 1.99T + 0.24T^2 - 0.006T^3],$$

in which Y = percentage of foliage affected, T = temperature (C) during the wetness period, and ln W = natural logarithm of length of wetness period (h). For the regression analysis, the starting values were the shortest wetness periods at each temperature that significantly differed from the 0-h check (W_o = 6 for T = 15, 20, and 25 C, and W_o = 12 for T = 5, 10, and 30 C). Predictions cannot be extended below those values. The R^2 for this regression was 0.856, and R_a^2 was 0.855. All estimated parameters of the equation were significant ($P \leq 0.05$), and standardized residuals were randomly distributed over predicted Y, T, and ln W. The temperature of maximum disease severity predicted was 21.2 C, which was the optimum temperature for infection at all wetness durations. The wetness period of maximum disease severity predicted was 96 h, which was the highest wetness period used in these tests.

The curves derived from the equation for the effect of temperature on the wetness period required for light (Y = 25%) and severe (Y = 50%) infection, and the general equation describing them, are presented in Figure 4. The least time required for light or severe infection was 7.6 and 17 h, respectively, at the optimum predicted temperature (21.2 C).

In combinations of temperature and wetness duration in which disease severity was lower than 50–60%, stem lesions generally did not coalesce and it was possible to count them. The equation $Y = 10.5X$, where Y = percentage of foliage affected and X = number of stem lesions, described the relationship between disease severity and number of stem lesions with $R^2 = 0.882$ and $R_a^2 = 0.881$.

Effect of temperature on infection and disease development. Temperature during and after inoculation influenced infection and disease development. Infection occurred at all temperatures and incubation treatments tested, but symptom expression was affected most by the temperature during and after the wetness period. Characteristic lesions with pycnidia developed in all treatments at temperatures between 10 and 25 C, and at 5 and 30 C in plants exposed to these temperatures only during the wetness period (48 h). Plants exposed at 5 C during the entire experiment, or after wetting, developed blight lesions, but the lesions were lighter in color and had fewer pycnidia than those of plants

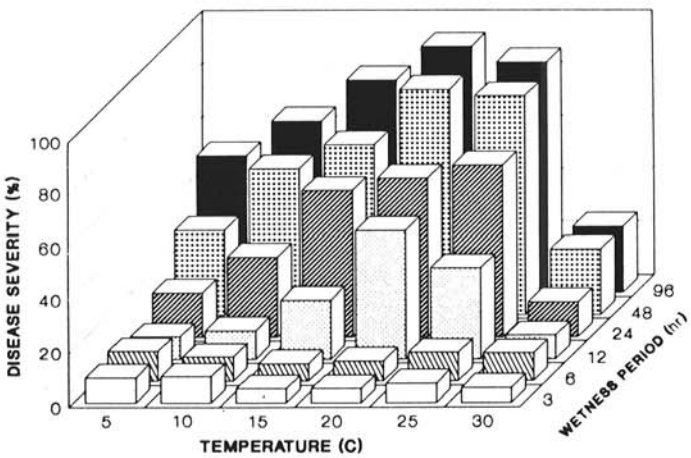

Fig. 3. Effect of temperature and wetness period duration on development of Ascochyta blight of chickpea. Each bar represents the mean percent disease severity of 48 plants inoculated with 5×10^5 conidia per milliliter of an isolate of *Ascochyta rabiei*. Equation 1 was adjusted to the data with $R^2 = 0.856$.

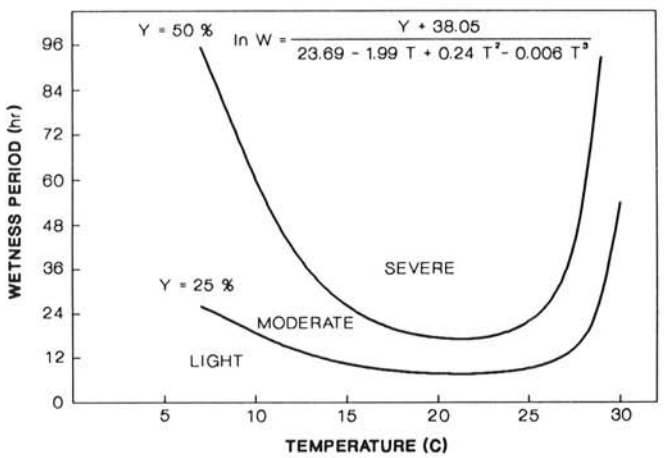

Fig. 4. Effect of temperature (T) on the predicted wetness period (W) required for light (Y = 25%) and severe (Y = 50%) infection of chickpea seedlings by *Ascochyta rabiei*.

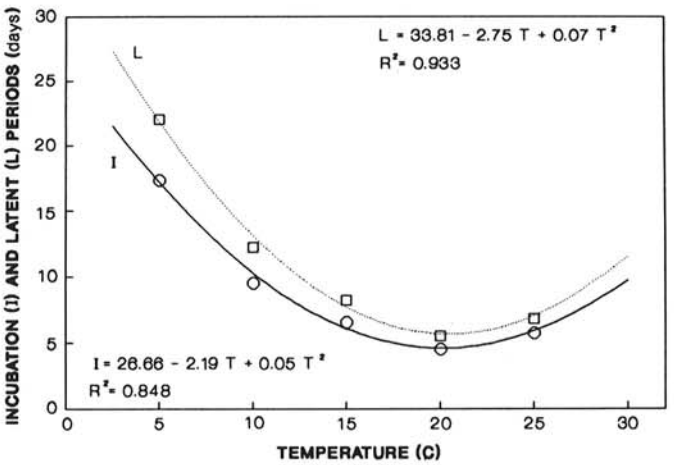

Fig. 5. Effect of constant temperature (T) on incubation period (I) and latent period (L) in chickpea seedlings inoculated with *Ascochyta rabiei*. ○ and □, observed means of 36 plants for incubation and latent periods, respectively.

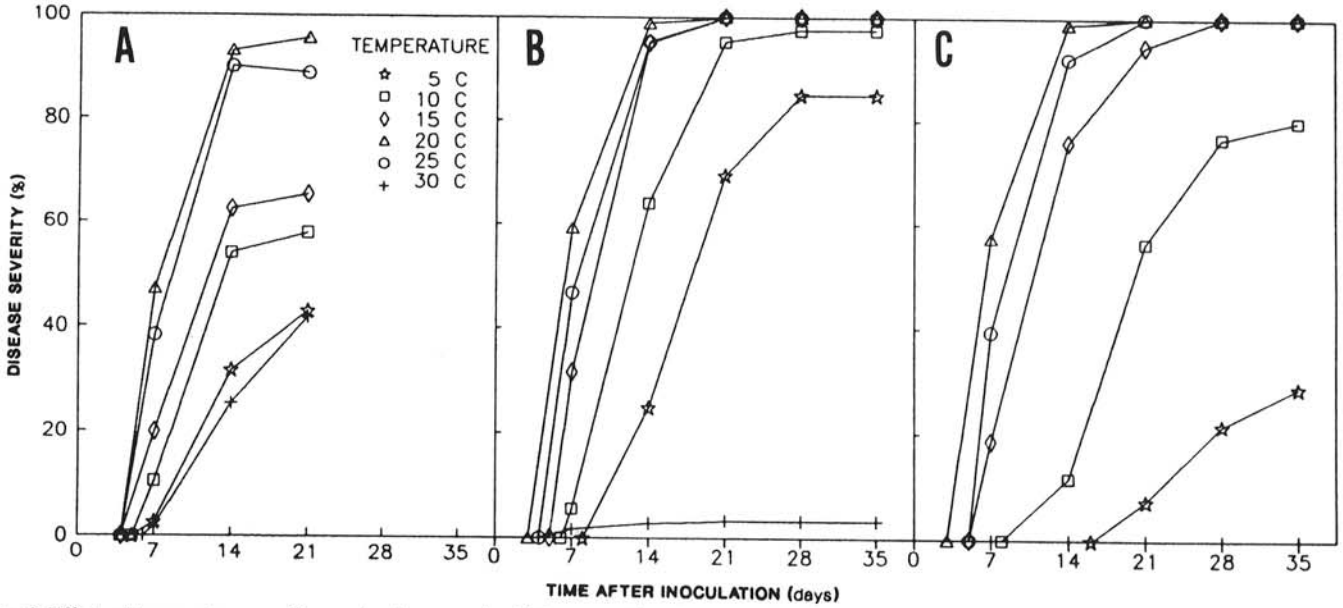

Fig. 6. Effect of temperature on disease development in chickpea seedlings inoculated with *Ascochyta rabiei.* After inoculation plants were subjected to a 48-h wetness period (infection period), dried, and transferred to growth chambers or to a greenhouse (postinfection period). **A,** Infection period at 5, 10, 15, 20, 25, and 30 C, and postinfection period in the greenhouse. **B,** Infection period at 20 C and postinfection periods at 5, 10, 15, 20, 25, and 30 C. **C,** Infection and postinfection periods at 5, 10, 15, 20, 25, and 30 C. For comparative purposes, the disease progress curves over time were summarized by the standardized area under the disease progress curve (SAUDPC) (Fig. 7).

exposed to other temperatures, except 30 C. At a constant 30 C, some infections occurred but visible lesions did not develop, and when 30 C was applied after a 48-h wetting period at 20 C, plants developed only a few small and irregular lesions without pycnidia.

Incubation and latent periods also were greatly influenced by temperature (Fig. 5). The shortest incubation and latent periods were 4.5 and 5.5 days, respectively, which occurred when temperature was 20 C during the entire experiment. At constant temperatures, the equations

$$I = 26.66 - 2.19T + 0.05T^2 \quad (R^2 = 0.848,\ R_a^2 = 0.840)$$

and

$$L = 33.81 - 2.75T + 0.06T^2 \quad (R^2 = 0.933,\ R_a^2 = 0.929),$$

where I = number of days to first symptoms, L = number of days to first pycnidia, and T = temperature (C), described the incubation or latent periods for infected chickpea plants. All estimated parameters of the equations were significant ($P < 0.05$), and there was a random distribution of standardized residuals. There was no significant difference ($P > 0.10$) in the regression results for the two experiments. The equations should not be used to make predictions near the maximum temperature (30 C). A temperature lower or higher than about 20 C also prolonged the incubation and latent periods in plants incubated in the greenhouse or in plants exposed to different temperatures after 48 h at 20 C (Fig. 6A, B). Disease progress over time also was greatly influenced by temperature during the infection and postinfection periods (Fig. 6). The SAUDPC was a good average of the whole process and was used for comparative purposes (Fig. 7). The maximum value of SAUDPC was 87.3% corresponding to plants incubated at a constant 20 C. Similar high values of SAUDPC also were obtained in plants placed at 20 C during the infection period and subsequently incubated at 25 or 15 C, or in plants incubated at 25 C during both periods (Fig. 7). Temperature during the infection period had a major influence on disease development, and plants exposed to 20 C during this period became severely diseased at all postinfection temperatures tested, except 30 C (Figs. 6, 7). Temperatures during the postinfection period also influenced disease severity, which increased as temperatures approached 20 C (Fig. 6). No disease or only a few atypical lesions developed on plants incubated continuously at 30 C. This temperature also suppressed disease

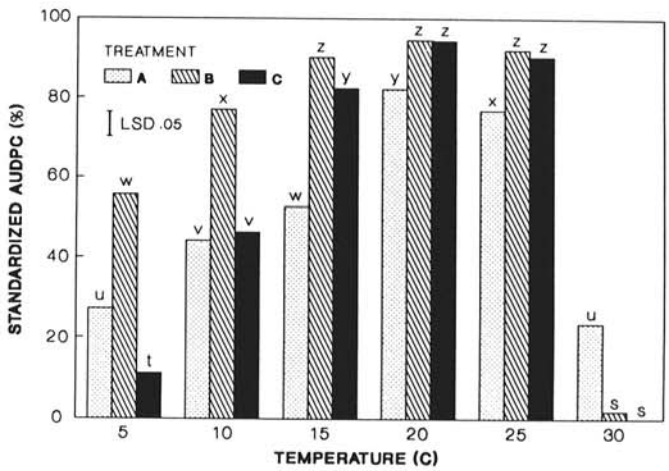

Fig. 7. Effect of temperature on disease development in chickpea seedlings inoculated with *Ascochyta rabiei.* After inoculation plants were given a 48-h wetness period (infection period), dried, and transferred to growth chambers or to a greenhouse (postinfection period). Treatment A, infection period at 5, 10, 15, 20, 25, and 30 C, and postinfection period in the greenhouse; treatment B, infection period at 20 C and postinfection period at 5, 10, 15, 20, 25, and 30 C; treatment C, infection and postinfection periods at 5, 10, 15, 20, 25, and 30 C. Bars represent the means of 24 plants for the standardized area under the disease progress curve (AUDPC). Bars with the same letter are not significantly different according to Fisher's protected LSD ($P = 0.05$).

development in plants incubated at 20 C during the infection period (Fig. 6).

At constant temperatures, the equations

$$Y_1 = 4.550 - 17.265T + 1.993T^2 - 0.049T^3 \quad (R^2 = 0.969)$$

and

$$Y_2 = 0.023T^2(30 - T) \quad (R^2 = 0.985),$$

where Y_1 = percentage of disease severity 14 days after inoculation, Y_2 = average of disease development over time as summarized by SAUDPC (percent), and T = temperature (C), described disease development at constant temperature (Fig. 8). In Y_1 and

Y_2, 21.8 and 20 C, respectively, were predicted as the optimum temperatures for disease severity and development (Fig. 8). Y_2 also predicted the cardinal temperatures for disease development at constant temperatures to be 0 and 30 C.

Effect of plant age and temperature during the wetness period on disease development. Disease developed more slowly in plants inoculated at the podding stage than in seedlings at all temperatures tested (Fig. 9). In adult plants, lesions and pycnidia appeared later and disease severity was significantly ($P = 0.05$) lower 7 and 14 days after inoculation at all temperatures except 15 C. After 21 days, differences in disease severity between adult plants and seedlings were not significantly different at 10, 20, and 25 C. However, at 15 C disease severity was significantly higher in adult plants than in seedlings. In general, the pattern of variation in disease severity with temperature in inoculated adult plants and seedlings was similar and comparable to that described in previous experiments under similar conditions.

Effect of interrupted wetness periods on disease development. Inoculated chickpea plants receiving a continuous wet period of 0, 6, 12, 24, or 48 h after inoculation showed an increase in disease severity as the length of the wetness period increased (Table 1). The inclusion of a variable dry period immediately or 6 h after inoculation greatly influenced disease severity. For the same duration of wetness (24 h), there was a different effect depending on when the wet period was interrupted and on the length of the dry period (Table 1).

When the dry period began immediately after inoculation, disease severity increased significantly (86.1 to 62.1% for the 6- to 48-h dry periods, respectively, compared with 51.1% for the same wetness period [24 h] without interruption). The increase in disease severity occurred regardless of the duration of the dry period, although disease severity was significantly lower with the 48-h dry period (Table 1). When the dry period began 6 h after inoculation, the highest disease severity was 55.3%, which corresponded to the shortest dry period (6 h). It did not differ significantly from 51.1%, the disease severity corresponding to the 24-h period of continuous wetting after inoculation. However, when the duration of the dry period was increased from 6 to 48 h, there was a significant reduction in disease severity from 55.3 to 19.3% (Table 1).

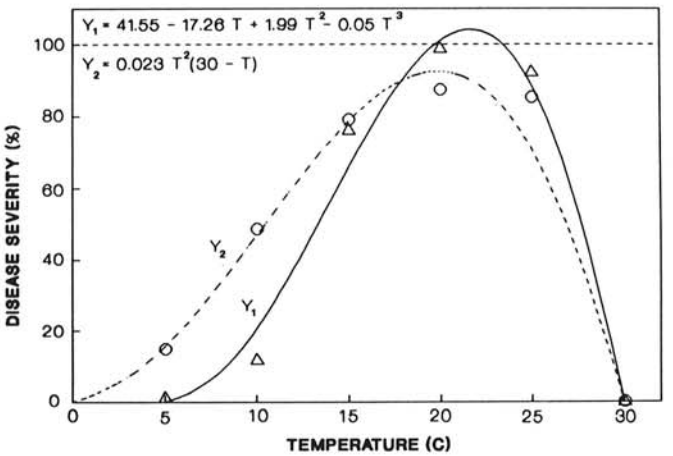

Fig. 8. Effect of different constant temperatures (T) on disease development in chickpea seedlings inoculated with *Ascochyta rabiei*. Y_1, Percentage of disease severity at 14 days after inoculation. Y_2, Standardized area under the disease progress curve (SAUDPC). △, observed means of disease severity at 14 days after inoculation; ○, observed means of the SAUDPC.

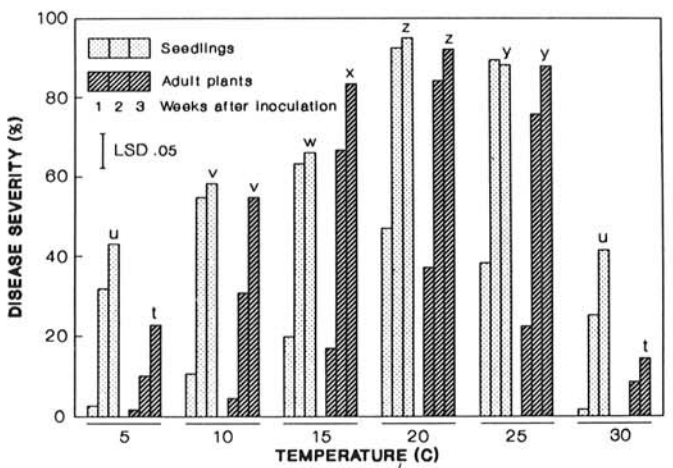

Fig. 9. Effects of plant age and temperature on infection and disease development in chickpea plants inoculated with *Ascochyta rabiei*. Two-week-old seedlings and 8-wk-old plants at the podding stage were inoculated, incubated for 48 h of wetness at different temperatures, dried, and transferred to a greenhouse at 18–26 C. Bars are means of three experiments with four replicated pots, each with three seedlings or one adult plant. Bars corresponding to 21 days after inoculation with the same letter are not significantly different according to Fisher's protected LSD ($P = 0.05$).

DISCUSSION

Temperature and wetness period are significant environmental factors influencing infection and disease development in chickpea by *A. rabiei*. Our results indicate that the effect of the length of the wetness period depends on the temperature, with an optimum at about 20 C. At temperatures lower or higher than 20 C, longer periods of wetness were required for significant infection. The regression model fitted to the data predicts an optimum temperature of 21.2 C and a minimum wetness period of 7.6 h for light and significant infection ($Y = 25\%$). The curve of predicted minimal wetness duration for significant infection shows a flat region over the range 15–25 C, predicting wetness periods of 7.6–10.3 h in this range.

These results agree with those reported by Weltzien and Kaack (31), who indicated a minimum wetness period of 6 h and a minimum temperature of 6 C for significant (> 1%) infection. In our study, a 6-h wetness period was the longest wetness duration

TABLE 1. Effect of interrupted wetness period on disease severity in chickpea inoculated with *Ascochyta rabiei*[a]

Postinoculation period (h)[b]			Disease severity (%)[c]
Wet	Dry	Wet	
0	...	...	7.3
6	...	...	3.5
12	...	...	22.2
24	...	...	51.1
48	...	...	81.1
...	6	24	86.1
...	12	24	85.6
...	24	24	81.9
...	48	24	62.1
6	6	18	55.3
6	12	18	42.8
6	24	18	36.0
6	48	18	19.3
LSD ($P = 0.05$)[d]			10.0

[a] Two-week-old seedlings of chickpea cultivar Burpee were inoculated with 5×10^5 conidia per milliliter of an isolate of *A. rabiei*, incubated at constant 20 C for the first week, and then in the greenhouse at 18–26 C.
[b] For the first or second wet periods, plants were incubated immediately after inoculation or after the dry period, respectively, in a saturated atmosphere in a growth chamber. After the respective wet period, plants were dried and incubated in a growth chamber with less than 50% relative humidity.
[c] Disease severity was assessed at 14 days after inoculation using a 0–9 rating scale and values were converted into percentage of foliage affected. Data are the average of four experiments with three replicated pots, each with three plants.
[d] LSD = Fisher's protected least significant difference.

tested that did not result in significant infection at any temperature. However, because some infection (9.6%, 0.8 stem lesions per plant) occurred even on plants subjected to a 0-h wetness period, it was not possible to establish an absolute threshold for infection, and arbitrary limits for light-moderate-severe infections were chosen from the data analyses (Fig. 4). The predicted minimal wetness period for light significant infection was not irrespective of the temperature as quoted by Weltzien and Kaack for the interval 9–24 C (31), and the minimum temperature for infection was lower than 5 C. Unlike our results, Chauhan and Sinha (4) reported a minimum wetting period of 60 h for light disease development at the optimum temperature (20 C). They found that wetness durations of 24–48 h were not adequate for disease development and that a duration of 144 h was found to be most suitable for disease development and sporulation (4). Differences in inoculum production or cultivar susceptibility may account for the different results.

In addition to temperature and wetness period, infection may depend on other factors, such as the chickpea cultivar or fungal isolate. In chickpea, Hafiz (7) reported that the minimum wetness period for 100% infection by *A. rabiei* was 24 h in a susceptible chickpea line and 96 h in two resistant lines. Similar results have been reported recently by ICARDA (11), indicating a change in disease reaction in moderately susceptible and resistant cultivars with increased wetness periods. The magnitude of this effect depended on the race of *A. rabiei* (11).

A few infections occurred in inoculated plants that did not receive a wetness period. Similarly, dry periods of up to 48 h that began immediately or 6 h after inoculation did not adversely affect infection, except in the latter case when the dry period was longer than 12 h. Even drying up to 48 h after inoculation had no adverse effect on infection. This result is not common (12,25), although it has been observed for other diseases (19,23,28,32). Conidia of *A. rabiei* are reported to survive adverse environmental conditions for relatively long periods of time (26,33). These facts may have important epidemiological consequences that need to be considered under field conditions. The occurrence of some infections in the absence of a wetness period would be related to the infection reported after 1 h at 100% RH (21) or to the insignificant (< 1%) infection mentioned by Weltzien and Kaack (31).

Temperature after the infection period affected disease development and symptom expression. At constant temperatures, the optimum temperature for disease development was about 20 C as predicted by the regression equations for disease severity 14 days after inoculation, the standardized AUDPC, and the minimum incubation and latent periods. Disease developed more slowly and severity was less at temperatures higher or lower than 20 C. Disease severity was higher when the temperature during the infection and postinfection periods approached 20 C. However, the temperature during infection had a greater effect on disease severity than the postinfection temperature. The lower and upper limits for disease development were < 5 C and about 30 C, respectively, similar to in vitro effects of temperature on growth of the fungus. The optimum temperature for mycelial growth, pycnidial formation, and spore germination of different isolates of *A. rabiei* is about 20 C, with lower and upper limits around 0–5 C and 30–32 C, respectively (1,13,16,18,21,33). The optimum temperature for infection also is about 20 C (4,16,17,21, 31,33), but little information is available on temperatures that limit infection. Temperatures below 6–10 C and above 30 C have been reported to limit infection and disease development (16,17,21,31,33). Chauhan and Sinha (4) reported no infection of chickpea by *A. rabiei* at 10 or 30 C.

Incubation and latent periods also were significantly affected by temperature. The values observed at constant temperatures or predicted by the regression models were similar to those reported from field and laboratory studies (2,4,16,17,21,33).

It is often difficult to compare field studies on the effects of temperature and wetness period on disease development with those conducted under controlled conditions (growth chambers) where only one infection cycle occurs. However, under field conditions it is possible to reach a 100% disease severity after only one infection cycle when healthy chickpea plants are exposed to abundant inoculum for short periods of time (A. Trapero-Casas and W. J. Kaiser, *unpublished*). This situation may account for the explosive nature of the disease in wet weather at temperatures ranging from 15 to 25 C (17,21,26,33). In our study, 100% disease severity required 85–166 h at these temperatures. Continuous, long wet periods are not common in Mediterranean or semiarid climates where chickpeas are traditionally planted in the spring. However, the pathogen may compensate for this weakness (25) by tolerating intermittent desiccation periods during the infection process, as suggested by our data on the effect of interrupted wetness periods.

The results of our studies on the effect of temperature and wetness periods on disease development may help to predict the risk of blight in different regions or under different crop management practices and to develop a disease forecasting system for the proper timing of foliar fungicidal sprays. Ketelaer et al (15) found that a monthly average temperature of at least 8 C and a monthly rainfall of at least 40 mm was needed before an epidemic of Ascochyta blight would occur. These predictions of risk could be improved and applied to more restricted areas if the quantitative relationships between disease and environmental variables were better understood.

Plant age has been considered a factor affecting disease expression and susceptibility of chickpea to *A. rabiei* (5,7,21,22,26,27). Sattar (26) reported that susceptibility in chickpea plants increased with age. In contrast, in our study 2-wk-old seedlings or 8-wk-old plants at the podding stage showed a similar disease severity when maximum disease severity was used for comparison, and maximum disease developed 1 wk later in adult plants (21 days after inoculation). This pattern occurred regardless of the temperature with some exceptions. A similar effect was reported by Hafiz at 20 C (7).

Inoculum concentration is a significant factor influencing disease severity, and its effect depends on susceptibility of the chickpea cultivar. Besides its possible application in predictive systems, results on the effect of inoculum doses are of great importance in screening chickpea germplasm for disease resistance to Ascochyta blight (5,11,20,21). In this study, a range of 2×10^5 to 1×10^6 conidia per milliliter was considered appropriate for reproducing the disease reaction in the same cultivar with favorable weather conditions in the field, particularly in the moderately susceptible cultivar Pedrosillano. Changes in disease susceptibility with inoculum dose have been reported (5). For that reason, an inoculum concentration of 5×10^5 conidia per milliliter was used in all other experiments. Inoculum doses in this range are being used by other researchers in artificial inoculations under field and greenhouse conditions (5,11). Lower doses of inoculum have been used successfully, but under different controlled conditions (21).

The rating scale for disease severity is another factor to be considered in screening for disease resistance. The scale used in this study has been adapted from the HB rating scale by reducing the higher class values, which were considered not relevant to the objectives of this study. The transformation used (Fig. 1) converts rating values into integer percentage values, making it easy to use the scale and the transformation. Despite some limitations and recent criticism, the HB scale is a valuable tool (3), and the modified scale employed in this study could be used in assessing disease severity in single plants as well as disease incidence or intensity in field plots.

All of the regression models used in this study were developed with an empirical approach, that is, the form of the model was determined by the collected data (3). Curves describing the relationship of temperature and minimal wetness duration, disease severity at 14 days, incubation period, and latent period have the typical asymmetrical U-shape or inverted U-shape associated with this type of relationship (8). Similarly, SAUDPC values had the best adjustment to a beta model, which has biological significance (3,8). However, in our study, minimum and maximum temperatures were not preassigned values but estimated by linear

regression analysis. The better fit of SAUDPC values to a model with biological significance than those of disease severity may be because SAUDPC values are an average of the disease development over time, while disease severity at 14 days after inoculation is only a single observation in the process of disease development, which was still not complete at some temperatures by this time.

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