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MATHEMATICAL MODELS BASED ON DIFFERENT THERMAL AND MOISTURE REGIMES FOR DEVELOPMENT OF *ASCOCHYTA* BLIGHT OF CHICKPEAS

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Abstract

Two separate models have been developed to describe the mathematical relationship of temperature and leaf wetness durations with Ascochyta blight development on two chickpea cultivars under controlled conditions. Plants of Balkasar-2000 (moderately resistant cultivar) and AUG-424 (highly susceptible cultivar) of our field trials were artificially inoculated with *Ascochyta rabiei* isolate ID-1 prepared @$5x10^5$ conidia ml^{-1} and subjected to various controlled environments to determine the impact of temperature and leaf wetness durations on disease establishment. Disease severity (%) was significantly affected by temperature, wetness durations and their interactions and depended on the level of resistance of the cultivar. It increased with increasing wetness duration (6-96h) at all the temperatures tested (10-25°C). At least 18h of leaf wetness were required for significant disease establishment (50%) at the optimum temperature of 20°C in AUG-424 and for Balkasar-2000, this value was 96 h. Quadratic trend in disease severity was found in relation to temperature and linear trend was recorded with regard to leaf wetness periods in both the cultivars. This study gives a systematic evaluation of host-pathogen interactions in controlled conditions. This approach of finding out quantitative relationship of disease with these most important variables may serve as a criterion for selection of cultivar for a specific area.

Key words: *Ascochyta rabiei*, Controlled conditions, Regression equations, Wetness periods.

Introduction

Chickpea (*Cicer arietinum* L.) is the second most important cool season food legume crop in the world after dry peas (Robertson *et al*., 1995). It ranks first among pulses in Indo-Pak subcontinent and is a major source of protein in many parts of the world, particularly in Pakistan. The crop is subjected to a wide range of photoperiods and hydrothermal regimes that may affect crop yield but large fluctuations in its productivity commonly coincides with the severity of attacks of Ascochyta blight, caused by *Ascochyta rabiei* (Pass) Labrousse (teleomorph: *Didymella rabiei* (Kovacheviski) v. Arx.) (Jhorar *et al.*, 1997). This disease is a major limiting factor in low average national yield and is a constant threat to chickpea crop and industry in Pakistan (Iqbal *et al.*, 2003). The disease was reported first time from Attock, Pakistan in 1911(Butler, 1918) and then onward appeared occasionally in epiphytotic forms in many parts of the world with more frequency in Pakistan. Records of epidemics have been reviewed by Malik & Bashir, 1984 and Iqbal *et al.*, 2003). It is a multiple cycle disease and its development depends upon the inoculum's concentration in the field, temperature, plant age and genetic make-up of the cultivars (Trapero-Casas & Kaiser, 1992a; Chongo & Gossen, 2001; Riaz *et al.*, 2006). Under favorable conditions rain-splashed conidia are important in spreading the disease. This can also spread quickly among fields because of dispersal of airborne ascospores produced on over wintered plant residue (Trapero-Casas & Kaiser, 1992b; Armstrong *et al*., 2001).

It has been observed that blight is most prevalent at latitude 29°-45°N and causes tremendous losses to the chickpea crop whenever the environmental conditions favor at flowering and pod formation stages (Kaiser, 1994). Outside this zone tropical and temperate climates limit the disease. These conditions clearly indicate different temperature and humidity requirements for onset of the disease. In a detailed study, Weltzien & Kaack (1984) found that blight development was favored by temperatures of 9-24°C and wetness periods of 10 h or more under controlled conditions. Trapero-Casas & Kaiser (1992a) noted severe infection by *A. rabiei* in chickpea at an optimum temperature of 20°C and 17 h of leaf wetness. Jhorar *et al.* (1998) reported that sporulation and germ tube penetration increased linearly with increasing wetness periods in case of *D. rabiei* when recorded 42 h after inoculation. However, effect of range of wetness periods at different thermal regimes on disease development in artificial conditions has been less widely investigated and this lack of information may limit the development and application of forecasting system. This aspect of disease demanded further work, regressing these two important variables with the disease severity describing their quantitative relationship with it. Therefore, objectives of the present study were to determine more precisely the effects of different thermal regimes for different leaf wetness durations on the establishment of the disease on two genetically different cultivars under controlled conditions and then to use this information for the mathematical models. This study may be helpful in terms that this approach can be used by agriculture extension agents in recommending different blight resistant chickpea cultivars for different agro-ecological zones and has implications in application of disease forecasting system for adoption of appropriate measures preventing spread of the disease.

Materials and Methods

Seed of the cultivars: The most resistant and the most susceptible cultivars of our epidemiological field trial, Balkasar-2000 and AUG-424 respectively, were selected for these trials. Balkasar-2000 (with a cream colored seed testa) was developed by Barani Agricultural Research Institute, Chakwal, Pakistan in 2000, is a high yielding partially resistant cultivar to Ascochyta blight, recommended for arid chickpea growing areas of Pakistan. AUG-424 is a well known susceptible cultivar developed by Agriculture University, Faisalabad, Pakistan (AUG stands for Agriculture University Gram) in 1980 and not recommended for cultivation anymore and is used as spreader for blight trials. Their levels of resistance/ susceptibility were further confirmed by activity of defense related GST enzyme in a study undertaken by Riaz *et al*., 2013. Seeds of these cultivars were obtained

from Pulses Program, National Agricultural Research Centre (NARC), Islamabad, Pakistan.

Isolation, maintenance and preparation of fungal culture: The isolate of *A. rabiei* ID-1 used in this study was collected from chickpea trial area of Pulses Program, National Agricultural Research Centre (NARC), Islamabad, Pakistan during a detailed survey of chickpea growing areas. This survey was conducted in the month of March during the crop season of 2011-12 when the crop was at the flowering to pod forming stage.

Diseased stems/pods were cut into 2-3 mm^2 pieces, surface sterilized with domestic bleach solution (0.1 % available chlorine) for 2 minutes and washed three times with sterile distilled water (SDW). The infected stem/pod pieces were placed on potato dextrose agar (PDA). Fungal growth was observed near lesions after 7 days of incubation at 20°C. The isolate was single spored and preserved on PDA slants at –30°C for further studies. Single spored culture was then multiplied on PDA plates and harvested after 12 days of incubation at 20°C and suspension was adjusted each time @ 5x 10^5 conidia per ml of water using Neubauer hemocytometer.

Growth chamber experiments: Four seeds of Balkasar-2000 and AUG-424 were sown in each pot. The pots were placed in growth chambers set at different temperatures *i.e.*, 10, 15, 20 and 25°C and two-week old plants were subjected to 6-, 12-, 18-, 24-, 48-, 72- and 96-h wetness periods at each temperature by putting transparent polyethylene bags (to serve as moist chamber) after artificial spray-inoculation till runoff to provide 100% relative humidity (RH) with spore suspension of the isolate prepared @ 5x10^5 spores per ml. Non-inoculated plants served as control. After plants were removed from the chamber each time, they were dried for 30 minutes and then arranged on a green house bench with four replicates for symptom development at 18-26°C. Each experiment was conducted thrice.

Data recording, analysis and development of mathematical models: Disease severity was rated after 14 days of inoculation (10 days after 96 hours of incubation) using the scale of Gowen *et al.*, 1989. 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), pooled data from three experiments was subjected to multiple regression analysis using statistical software Minitab 13.1 (Minitab Inc. 2003). The data was plotted graphically for each cultivar to determine relationship between environmental variables and severity of Ascochyta blight. Based upon data or in other words level of resistance/susceptibility, separate model was developed for each cultivar.

Results

On the basis of analysis of variance, disease severity of both cultivars were significantly affected ($p \leq 0.05$) by temperature, wetness duration and their interaction. In general, disease severity increased with increasing wetness durations and depended on their level of resistance (Figs. 1&2). At wetness periods of 72 and 96-h at 10°C, there was no significant difference ($p \leq 0.05$) in disease severity of both Balkasar–2000 and AUG-424, however, significant effect on disease was observed for duration of 48 and 72-h at this temperature in case of AUG-424. At 15°C, gradual increase in disease severity was noticed in Balkasar-2000 with increase in wetness period but there was a rapid increase in disease severity in AUG-424 after 48-h wetness period at this temperature. In general, at this temperature, disease severity was more on both varieties as compared to 10°C at corresponding wetness durations.

Most of the foliage of AUG-424 became dead after 48-h and all plants died completely at 20°C after 72 and 96-h of wetness and no significant difference was noticed in disease at this temperature for both wetness periods. Balkasar-2000 also showed more disease at 20°C at all wetness periods. At 25°C, a drastic negative trend of disease severity was noticed on both the cultivars. Although data in all the experiments was taken 14 days after inoculation but symptoms began to appear after 96 h after inoculation with wetting periods of 48h or longer at 20°C. However, symptoms 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. The lesions with conidia in typical concentric rings 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 which were not severely affected continued to grow actively in the green house and disease severity decreased with an increase in healthy tissue. The speed of active growth was however faster in Balkasar-2000 than AUG-424.

Overall a quadratic trend in disease severity was found in relation to temperature, being maximum at 20°C and linear trend was recorded with regard to wetness periods in both the cultivars (Figs. 1&2). Based upon the data, two separate models for disease severity were developed for Balkasar–2000 and AUG-424 in equation (1) and (2) respectively which are as follows:

$$DS = -48.7+8.24\ T -0.234\ T^2+0.200\ W+0.0021WT-0.0016\ WT^2 \quad \text{----- (1)}$$

$R^2 = 85.4\ \%$

P values for all predictors given in the equation viz. constant, T, W, T^2 ,WT and WT^2 are 0.005, 0.001, 0.050, 0.001, 0.050 and 0.040, respectively.

$$DS = -32.8 + 6.39T - 0.170T^2+ 0.404W +0.0444\ WT –0.00123WT^2 \quad \text{- (2)}$$

$R^2 = 93.0\ \%$

where DS= disease severity (%), T =temperature (°C) during the wetness period, W= length of wetness periods (hours) and R^2 =coefficient of variation. Similarly, P values for all predictors i.e. constant, T, W, T^2, WT and WT^2 in equation (2) are 0.050, 0.040, 0.050, 0.050, 0.040 and 0.050 respectively.

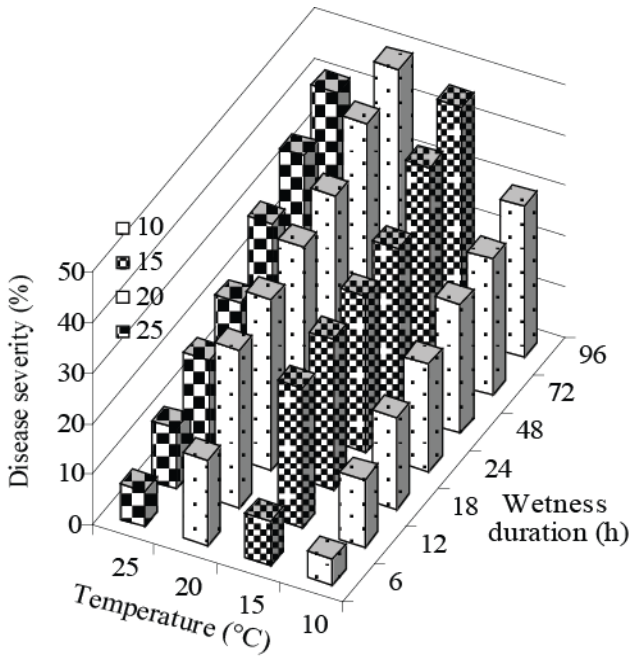

Fig. 1. Effect of temperature and wetness period on disease development on 2-weeks old Balkasar-2000 seedlings inoculated with spore suspension of an isolate ID-1 of *Ascochyta rabiei* (5x 10^5 conidia per ml).

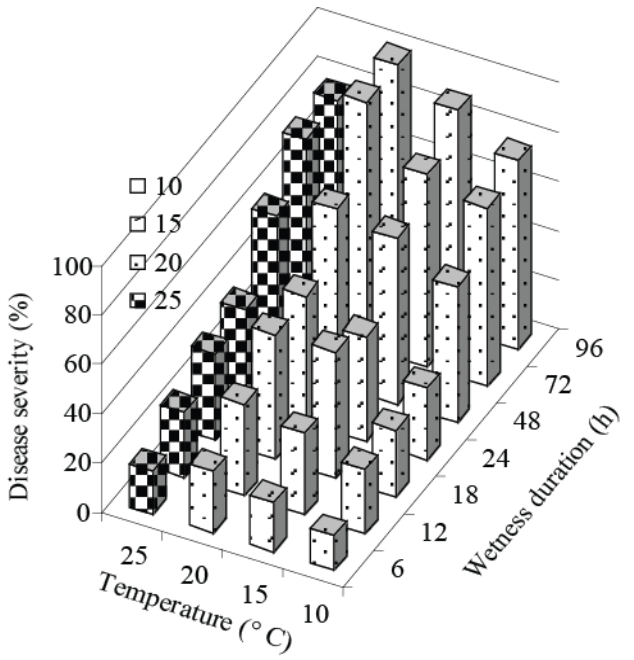

Fig. 2. Effect of temperature and wetness period on disease development on 2-weeks old AUG-424 seedlings inoculated with spore suspension of an isolate ID-1 of *Ascochyta rabiei* (5x 10^5 conidia per ml).

Discussion

Regression coefficients value of each variable represents the strength of the effect of this variable on output function i.e., disease severity and a positive sign implies that the effect is positive, whereas a minus sign implies that effect is negative. It was evident from high values of coefficients of T (temperature) in both the equations that temperature was more important factor and effects of length of wetness periods depended on the temperature with an optimum at 20°C. At temperatures, lower or higher than 20°C, longer periods of wetness were required for significant infection. These results confirm some of the studies reported by Nene and Reddy (1987) and Trapero-Casas & Kaiser (1992a) and contradict those of Chauhan & Sinha (1973) who reported that there was no infection at 10°C and 30°C and a minimum 60h wetness period was required for blight disease development at the optimum temperature of 20°C. These latter researchers also found a wetness period of at least 144 h conducive for disease development. Non-significant difference in disease severity of both the cultivars at 10°C for 72h and 96h indicate that maximum physiological changes would have taken place at former wetting period. In the present studies, it was noticed that in addition to temperature and wetness periods, the chickpea cultivar affects disease development. Iqbal (2002) reported 24 h as minimum wetness period required to produce 100% disease in a susceptible cultivar whereas the same level of disease in the resistant cultivar needed a 96h wetness period.

Considering the values of R^2 and P for all predictors and randomness and normality of residuals, above mentioned equations fulfill the criteria of judgment of any model to determine the quantitative relationship between the parameters and the disease. Our results further suggest that development of a separate model is necessary for cultivars falling in every disease reaction category (resistant, tolerant and susceptible) and this approach can be employed to know the accurate level of resistance /susceptibility before recommending a cultivar for cultivation in specific environmental conditions.

These models are consistent with the concept that the duration of leaf wetness determines the amount of spore germination and host penetration, whereas temperature determines the rapidity and extent of infection (Lalancette *et al*., 1988; Evans *et al.*, 1992). At least 18 h of leaf wetness were required for significant disease establishment (50%) at the optimum temperature of 20°C in AUG-424 (Fig. 2) and for Balkasar-2000, this value was 96 h. These results are consistent with the controlled environment studies of Trapero-Casas & Kaiser (1992a) who found that 17h of leaf wetness was necessary for significant levels of infection of disease at 20°C in a susceptible cultivar. It is likely that when temperature becomes limiting, extended leaf wetness periods have potential for compensation.

The results of the present study highlight the importance of temperature, leaf wetness durations and genetic resistance of the cultivar in relation to epidemiology of Ascochyta blight. These findings are in line with those reported by Riaz *et al*. (2013) in which they determined that any one factor out of temperature and relative humidity alone cannot help in wheat leaf rust development It was concluded that temperature setting of 20°C and wetness period of 72 h or more should be treated as equivalent conditions for disease establishment for moderately resistant cultivars like Balkasar-2000 under controlled conditions. Although the models developed here, apply to controlled environmental conditions used in the study, relative differences between sets of environmental factors could be used to develop daily or weekly humid thermal index of environmental favorability for disease increase in the field.

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