Relation of Plant Water Potential at Flowering to Subsequent Cottonseed Infection by *Aspergillus flavus*

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ABSTRACT

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The involucral nectaries of cotton flowers in field plots in Arizona were tagged and inoculated with spores of *Aspergillus flavus* on the day of anthesis. To determine the degree of plant water stress, water potentials were measured in the plots between 1200 and 1230 hr on the tagging day. Tagged bolls were harvested at maturity and the seeds assessed for *A. flavus*. Seed infection levels were affected significantly by water potentials on the day of anthesis and inoculation, with the highest infection levels generally occurring in seed from plants with water potentials between −1.6 and −1.9 MPa.

Additional key words: aflatoxin, fungal ecology, *Gossypium*.

Aspergillus flavus Lk. ex Fr. and *A. parasiticus* Speare produce aflatoxins—potent, naturally formed carcinogens—in the seeds of several major crop plants, including corn (*Zea mays* L.), peanuts (*Arachis hypogaea* L.), and cotton (*Gossypium hirsutum* L.)(3). Field infection by these fungi and formation of the toxins in the seeds occur predominantly when the plants are grown at high temperatures or under water-stressed conditions (5,6,12–14).

In cotton, aflatoxin is a chronic problem in hot, dry, low-elevation areas in Arizona and California (1,2). Even within these areas, aflatoxin levels vary greatly from year to year and field to field (18), indicating that relatively small changes in environmental or physiological factors may influence fungal entry and toxin production.

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A. flavus may enter cotton plants in a variety of ways (10,21). In the absence of obvious injury to surface plant tissues, early-season entry has been demonstrated to occur through natural openings such as cotyledonary leaf scars and involucral (bracteal) nectaries (9,10). Although inoculations at these points resulted in significant increases in the number of contaminated seeds, not all of the inoculated plants contained *A. flavus* in the seeds. Factors other than presence of the inoculum apparently influence fungal entry and establishment in the seeds.

The purpose of this study was to determine whether the degree of plant water stress is a factor in infection of cottonseed by *A. flavus*.

MATERIALS AND METHODS

Experiments were conducted in 1984 and 1985 in a commercial cotton-growing area near Gila Bend, AZ. The 30-ha fields were planted with *G. hirsutum* 'Deltapine 120' during both years and furrow irrigated at 4- to 10-day intervals. Four and six randomly selected plots were established within a field in 1984 and 1985,

respectively. The plots were separated from one another by at least 30 m. Each plot consisted of seven (1984) or six (1985) 4-m sections of row. Flowers on plants in a given section within a plot were inoculated once during the study. Between 0700 and 1000 hr on a given date, 10 white flowers in one 4-m section per plot were tagged and conidia of *A. flavus* isolate SRRC 1000A were dusted onto the involucral nectaries with an artist's brush as described previously (10) to assure that the flower was exposed to the fungus. Cotton flowers undergo anthesis in a single day, and the flower is white only on that day. Between 1200 and 1230 hr on the inoculation day, the method of Radin et al (16) was used to harvest three leaves from plants in the freshly inoculated section of each plot and to determine the xylem water potentials (water potential, ψ) with a pressure chamber. Temperatures were taken on each sampling date at 1200–1230 hr in the shade of the canopy. Tagging, inoculation, and temperature and water-potential measurements were performed on a different 4-m section of row in each plot twice weekly from the beginning of flowering in June through late July, for a total of seven and six dates in 1984 and 1985, respectively.

Tagged bolls from each section within a plot were harvested separately as they completed dehiscence in August and September, divided into two subsamples per plot section, ginned, delinted, and rinsed according to the method of Klich et al (10). Fifty seeds per subsample (or all of the seeds, if the subsample contained fewer than 50 seeds) were surface-sterilized in a solution of 2% (w/v) sodium hypochlorite and 0.001% Triton X-100 for 2 min under agitation, rinsed thoroughly in sterile deionized water, placed on petri plates containing potato-dextrose agar, and incubated at 24 C for 7 days, at which time the seeds with emergent colonies of *A. flavus* were counted (10).

For each plot section on each tagging date, the mean of the percentage of infected seed in the two 50-seed subsamples and the mean of the xylem water potentials were determined. Analyses were based on these mean values. The data did not satisfy the equality of variances assumption necessary for statistical analyses. Therefore, to achieve homoscedasticity of variances, and to facilitate analyses with many zero data points, the seed infection data were transformed to the square root of the percentage of infected seed plus 0.5. Data were divided into three water-potential classes and analyzed using analysis of variance, and the differences were partitioned with Tukey's studentized range test (4).

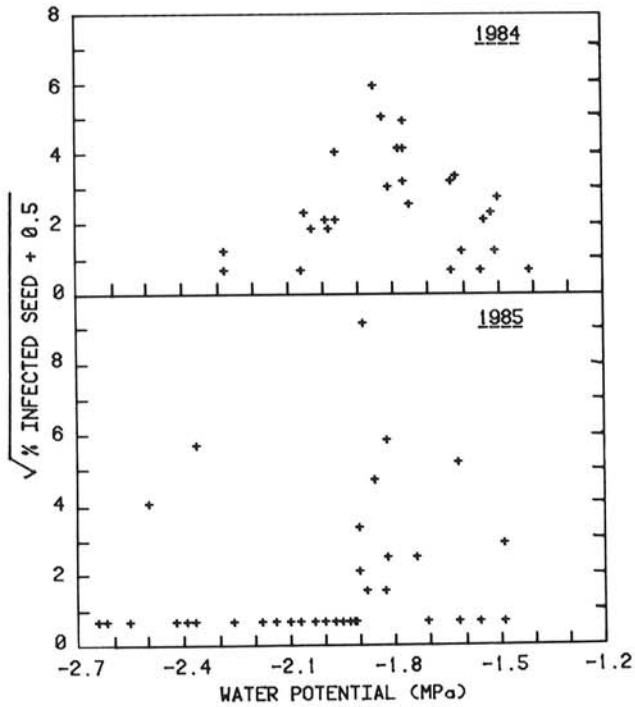

Fig. 1. Proportion of cotton seeds infected with *Aspergillus flavus* as related to minimum xylem water potentials measured on day of anthesis and inoculation. Each data point represents mean transformed percentage of infected seeds of two subsamples from 4-m section of row from bolls tagged and inoculated on involucral nectaries on day of anthesis and harvested at maturity and mean of three water-potential readings taken with pressure chamber between 1200 and 1230 hr on day of anthesis and inoculation.

RESULTS

The relationship between water potential and transformed percentage of infected seed is shown in Figure 1. Generally, the greatest seed infection occurred when the water potential at 1200–1230 hr on the day of anthesis and inoculation was between −1.6 and −1.9 MPa. Only one sample in 1984 and two in 1985 from this water-potential range contained no *A. flavus* in the seeds. Of the 62 observations, there were only two that varied markedly from the general trends. Both of these were 1985 samples with very low water potentials and high seed-infection levels.

Water potential significantly ($P = 0.0011$ in 1984 and 0.0071 in 1985) affected the transformed percentage of infected seed. Differences in the classes occurred for samples in the range between −1.6 and −1.9 MPa, which had significantly greater seed infection ($P = 0.05$) than samples from plot sections with lower water potentials during both years of the study (Table 1). Significantly lower seed infection occurred in plots with water potentials greater than −1.6 MPa, compared with the range from −1.6 to −1.9 MPa, during 1984 but not in 1985. There were only three samples in the higher water-potential range in 1985, which could account for the lack of statistical differences in spite of the fairly large difference in infection levels between these two water-potential classes that year.

On a given date, water potentials within a plot section generally varied by less than 0.1 MPa. There was much greater variation in water potentials among different plots on the same tagging date. The mean variation among plots on any one tagging date was 0.41 MPa, with a range of 0.05 to 0.75 MPa. Temperatures ranged from 34 to 39.5 C during water-potential assessments; however, on a given sampling date, the interplot variation was quite small, generally only one degree. There was no significant relationship between temperature and transformed percentage of infected seed (Pearson product moment correlation [20]; $r = 0.135$, $P = 0.58$ in 1984; $r = -0.206$, $P = 0.23$ in 1985).

DISCUSSION

In cotton, the water potential reaches a minimum at about 1200 hr and remains close to that level until about 1430 hr (7,8). This is the period of maximum diurnal water stress, so the water potential readings reported in this study represent the maximum daily water stress to the plant. The water potentials observed were well within the normal range for cotton grown in this area (16), but lower than the −0.8 to −1.5 MPa observed under normal conditions in other cotton-growing areas (22). Minimum water potentials vary from day to day depending on environmental conditions. Because water potential is so dynamic, the close relationship between water potential on the day of anthesis and inoculation and subsequent seed infection by *A. flavus* observed in this study indicates that the fungus enters the plant fairly rapidly after contacting the entry site.

TABLE 1. Relation of midday water potential (ψ) by class on day of anthesis and inoculation to transformed percentage of mature seed infection by *Aspergillus flavus*

	$\sqrt{\%}$ Infected seed + 0.5[y]	
Water potential class (MPa)	1984	1985
$\psi < -1.9$	1.89 a[z]	1.12 a
$-1.9 \leq \psi \leq -1.6$	3.69 b	3.35 b
$\psi > -1.6$	1.58 a	1.44 ab

[y] Data transformation resulting in homoscedasticity of variances necessary for statistical analyses.

[z] Within year, numbers followed by same letter are not significantly different ($P = 0.05$) according to Tukey's studentized range test.

Propagative units could, conceivably, be drawn into the plant and carried into the boll or seed. Alternatively, the conidia could germinate and enter the developing boll as hyphae. It has been established that fungal entry does not depend on the presence of nectar (9), so the contents of the nectar need not be considered as potential determinants of entry.

Little is known about the physiology of the flower or involucral nectaries at different water potentials. It is known that the leaves reach zero turgor at approximately −1.8 MPa, but how this may relate to *A. flavus* entry into the developing boll is not known (16). Phytoalexin production is influenced by drought stress. Three such peanut kernel phytoalexins have been shown to inhibit germination and hyphal extension of *A. flavus* (23). Future research on *A. flavus* in cotton should include studies of water stress effects on physical and biochemical factors that could influence germination or growth of the fungus.

The temperatures recorded during this study were near the optimum of 36–38 C for the growth of *A. flavus* (19) and did not relate to the seed infection levels. The water-potential readings, however, represented a fairly wide range that included the optimum conditions for growth of *A. flavus*. Ritchie (17) measured the growth rate of *A. flavus* at six temperatures and six salinities (the nature of the solute does not greatly affect the growth rate of this fungus [15]) and found that the maximum growth rate occurred at 37 C and salinities of 25–30 ppt, which correspond to water potentials of −1.59 to −2.19 MPa. This is very close to the range that yielded the highest seed infection in the present study, indicating that the differences in seed infection observed may have resulted in part from optimized growth conditions for the fungus.

Factors influencing water potential may also influence seed infection by *A. flavus*. Because aflatoxin forms only in the presence of *A. flavus*, formation of aflatoxin in cottonseed may also be influenced by water potential. Slight differences in soil type, or minor variations in irrigation or fertilization practices within a field or among fields in close proximity to one another, could change the water potentials enough to account for the intrafield and interfield differences observed in the occurrence of *A. flavus* and production of aflatoxin in the same year (18,21). Over time, the minimum water potential of an individual plant varies so that a plant would not remain constantly susceptible to fungal infection. This is consistent with the observation by Lee et al (11) that only a few bolls on a plant contain aflatoxin-contaminated seed. On a given day, the mean interplot variation in this study was 0.41 MPa, indicating that not all of the flowers blooming on one day would be equally susceptible to entry by *A. flavus*. This could account for the variation in infection levels observed previously (9) among flowers inoculated on the same day.

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