Planting Date, Harvest Date, and Irrigation Effects on Infection and Aflatoxin Production by *Aspergillus flavus* in Field Corn

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ABSTRACT

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Infection and aflatoxin B_1 production by *Aspergillus flavus* were measured in a short-season, midseason, and full-season cultivar at three geographically diverse locations in North Carolina. Significant reductions in aflatoxin B_1 were associated with early planting (April vs May) and early harvest (28% moisture vs 18% moisture). Irrigation (employed at one location) reduced infection and aflatoxin concentration in both 1978 and 1979. The effect of irrigation was more pronounced in 1978 when drought stress (as measured by leaf xylem water potentials) occurred during the silking to late dough stage of grain development. Counts of airborne inoculum and weekly determinations of the mycoflora of developing kernels suggested that cultivar × planting date combinations that silked during periods of high airborne spore loads contained greater numbers of infected kernels. A significant correlation ($P = 0.01$) was observed between aflatoxin B_1 and reduced yield. Damage by corn earworm or European corn borer would account for only 10 and 11% of the variation in the level of aflatoxin B_1 concentration at harvest. This suggests that stress conditions that reduce yield may play a role in predisposing corn to infection by *A. flavus* or to increased aflatoxin production once infection has occurred.

Additional key words: water potential, airborne inoculum, epidemiology.

The detection of aflatoxin in corn prior to harvest has prompted investigation of factors that influence infection and aflatoxin production by *Aspergillus flavus* Link ex Fries in the field. Previous work (18,31) has demonstrated that artificially wounded and inoculated ears grown in the midwestern USA contained less aflatoxin than similarly treated ears grown in Georgia or Texas. Survey results also support the conclusion that aflatoxins are more common in southern regions than midwestern regions of the USA (22,23). However, in some years aflatoxins have been found in field corn grown in Indiana (21) and Iowa (13,14). Increased aflatoxin production within southern production areas has been found to be associated with high temperatures and low average rainfall, particularly during the silking to late dough stage of grain development (19,32).

In a recent review, Zuber and Lillehoj (32) pointed out that many aspects of the field infection process by *A. flavus* remain ambiguous including: (i) availability of inoculum, (ii) method of spore transmission to the infection site, (iii) establishment of initial infection by the fungus in developing kernels, and (iv) distribution of secondary inoculum from initial infection sites to other uninfected kernels of the ear. Various insects have been implicated in steps ii–iv above (3,5,6,15,17). However, attempts to demonstrate the role of corn ear insects as vectors of *A. flavus* in controlled experiments have proven inconclusive (12,29). The role of insects in aflatoxin contamination of corn, cotton, and peanuts has recently been reviewed (28).

Jones et al (11) demonstrated the ability of *A. flavus* to infect kernels via colonized silks and produce aflatoxin in kernels on ears grown in controlled-environment phytotron chambers devoid of insects. They also showed that silk infection was favored by high (32–38 C) vs low (21–26 C) temperatures, providing further explanation for the regionality of preharvest aflatoxin contamination.

In the field, inoculum can be deposited on developing silks by insects (5,6,15) and/or by deposition of airborne spores (2,27). Holtmeyer and Wallin (8) found that 65% of *A. flavus* isolates from airborne spores trapped with air samplers produced aflatoxin in qualitative tests on artificial media.

The objectives of this investigation were to examine the influence of several cultural practices, including planting date and harvest date, on the development of aflatoxin in short-season, midseason, and full-season cultivars at three locations in North Carolina. Records were made at harvest of the number of ears visibly infected with *A. flavus*, damaged by European corn borer (*Ostrinia nubilalis* Hubner), damaged by corn earworm (*Heliothus zeae* Boddie), and with sporulation of *A. flavus* associated with insect damage. In addition, airborne inoculum, leaf xylem water potential, and weekly determinations of the mycoflora of developing kernels were monitored in irrigated and nonirrigated plots at one location during the 1978 and 1979 growing season. Preliminary results have been published (9,10).

MATERIALS AND METHODS

General. In 1978, commercial corn cultivars were grown at the Upper Coastal Plain Research Station (UCPRS) near Rocky Mount, the Central Crops Research Station (CCRS) near Clayton, the Tidewater Research Station (TRS) near Plymouth, and the Piedmont Research Station (PRS) near Salisbury (all in NC) to examine the influence of geographic location, planting date, and harvest date on the development of aflatoxins. Similar tests were conducted at the UCPRS and the CCRS in 1979.

Experimental design used at UCPRS, PRS, and TRS was a 2×3^2 factorial, randomized complete block with six replications. Three cultivars, Pioneer Brand (P.B.) 3780 (a short-season hybrid), P.B. 3368A (a midseason hybrid), and P.B. 3147 (a full-season hybrid) were planted in early April and early May in 12.2-m-long, 8-row subplots in 1978 and 9.1-m-long, 8-row subplots in 1979. Rows were 91.4 cm apart. Subplots were randomly assigned to one of three harvest dates (based on grain moisture content) when the grain from the inner six rows was harvested. The May planting at

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PRS was omitted from the study because drought resulted in irregular stands in these plots.

Plants were thinned to the recommended populations approximately 3 wk after planting. Temperature and rainfall data were collected from weather stations (Weather Measure Corp., Sacramento, CA 95841) located within 1 km of plots at each location. Hours of leaf wetness were measured with a DeWitt leaf wetness meter (Valley Stream Farms, Orono, Ont., Canada) located next to the weather station. Dates of 50% silk emergence were recorded for each planting date × cultivar combination at all locations.

Subplots were harvested at three maturity stages based on kernel moisture contents of approximately 28, 24, and 18% moisture in 1978 and about 30, 24, and 16% moisture in 1979. Moisture content was measured with a Dicky-John Moisture Meter (Model 1S-C, Dicky-John Corp., Auburn, IL 62615).

Plots were hand harvested and all ears formed on plants were included in the sample. Ears were machine shelled, and sample weight and moisture content were recorded. Kernels adhering to the cob were removed by hand and included in the sample. Shelled grain was mixed and poured from a metal tub and a 5-kg subsample was collected from the pouring stream at evenly spaced intervals. Five kilogram subsamples were coarse ground in a Dickens subsampling mill (4) that ground and randomly reduced the sample weight to 500 g. Subsamples were then riffle divided and 250 g was finely ground in a Wiley Mill (Model 4 with a 0.84-mm, 20-mesh screen). Samples were dried at 70 C for 48 hr in a forced-air oven. After mixing, 50 g subsamples were weighed and stored at 2 C in glass snap-top Wheaton jars until extracted for aflatoxin.

Irrigation study. The 1978 and 1979 experiments conducted at CCRS involved a split plot of overhead sprinkler irrigation vs no irrigation. A 16-row buffer zone was planted between the irrigated and nonirrigated treatments in April. Whole plots were a $2^2 \times 3$ factorial randomized complete block with the midseason hybrid being omitted from the study. All treatments were replicated six times and harvested, examined, and sampled as previously described. Airborne inoculum, water stress, and weekly determinations of the mycoflora of developing kernels were monitored at this location in 1978 and 1979.

Xylem water potential. Water stress was quantified with a Model J-14 Plant Press (Cambell Scientific, Logan, UT 84321). Water potential in six plants (one per replicate) of each treatment was measured three times weekly (Monday, Wednesday, and Friday) at 0900 hr. Four-centimeter sections of the dominant ear leaf were excised with a razor blade, placed in the chamber, and pressure was increased until water was observed at the cut edge. Readings (in pounds per square inch, psi) were converted to bars by using the standard equation 1 bar = 4.759247×10^3 N/M^2 (0.689746 psi) (30). Measurements began approximately 2–3 wk prior to silking and continued until senescence. Whole-plot irrigations of 2.5 cm were applied when the mean xylem water potential for any cultivar × planting date subplot was reduced to less than −14 bars.

Inoculum potential. Andersen air samplers (Model 064 Viable type, Andersen 2000 Inc., Atlanta, GA 30336) were used to monitor airborne populations of *A. flavus*. The operation of the samplers has been described (1). One air sampler each was placed in the irrigated and nonirrigated plots on covered tables at a height of 1 m (approximately at ear height). Samplers were adjusted to operate for 30 sec each hr at a volume of 0.028 m^3/min. Airborne populations were monitored for 166 days (13 May–27 Oct) in 1978 and 143 days (24 May–13 Oct) in 1979. Spores were trapped on petri plates containing Czapek Dox agar (Difco) + 6% NaCl and plates were changed daily at 0800 hr. Colonies were identified following incubation for 6 days at 25 C in 1978 and 4 days at 36 C in 1979.

Mycoflora. Isolations were attempted from developing kernels of all treatments during the 1978 and 1979 growing seasons. Six ears of each treatment (one per replicate) were harvested weekly beginning 7 days after midsilk. The outer husks were removed and a rectangular section of the inner husks near the tip region was excised with a sterilized scalpel. Ten kernels were removed from this region, surface sterilized for 4 min in 0.5% NaOCl, double rinsed in sterile distilled water and plated (five per plate) on Czapek Dox agar + NaCl. Plates were incubated for 7 days at 25 C. The same procedure was repeated to examine kernels from the middle and base regions of each ear. In all, 180 kernels were plated weekly from each treatment for approximately 8–10 wk.

Aflatoxin analysis. Samples were extracted for aflatoxin analysis by using a modified Pons' procedure (20). This procedure was selected over the method approved by the Association of Official Analytical Chemists (AOAC) since it is cheaper and quicker, the solvents are less hazardous, and it permits analysis for ochratoxin. The procedure has been compared to the AOAC method (CB) and found to be comparable (238 samples analyzed independently in two laboratories averaged 131 ppb aflatoxins by the AOAC method and 130 ppb by the modified Pons' method [*unpublished*]).

Dried extracts were stored under nitrogen at −5 C. Quantities of aflatoxin were determined on activated thin-layer chromatographic (TLC) plates (No. 6061 Eastman Chemical Products, Inc., Kingsport TN 37662). Plates were developed with benzene:methanol:acetic-acid (90:5:5) in unequilibrated tanks. Aflatoxin B_1 concentrations were estimated visually by comparison with commercially prepared standards (Applied Sciences Laboratories, State College, PA 16801). Diluted sulfuric acid was used as an indicative test for aflatoxins (24).

RESULTS

The occurrence of aflatoxin B_1 in commercial corn hybrids varied in both incidence (numbers of samples containing aflatoxin B_1) and level (concentration of aflatoxin B_1 in micrograms per kilogram).

Aflatoxin incidence. The incidence of detectable levels of aflatoxin B_1 varied across locations within years and within locations between years. A total of 40.2% (253/630) of the samples during the 2-yr study contained aflatoxin B_1 at levels exceeding the Food and Drug Administration action guideline of 20 μg/kg; in 1978, 43.4% (164/378) had more than 20 μg/kg compared to 35.3% (89/252) in 1979. The greatest incidence of aflatoxin-positive samples occurred at the sandy coastal plain sites, UCPRS (44.4%, 48/108 samples) and CCRS (nonirrigated treatments—81.9%, 59/72 samples). Lower incidences occurred at TRS (6.9%, 5/72 samples) and at PRS (39.9%, 21/54 samples).

Cultivars planted in April contained fewer positive samples than the same cultivar planted in May (Table 1). The interaction of cultivar × planting date revealed an increasing number of positive samples for the short-season, midseason, and full-season cultivars within the April plantings while no such increase was observed within the May plantings.

Aflatoxin concentration. Analysis of variance revealed significant differences ($P = 0.01$) between planting dates in 1978 and 1979 experimental plots at UCPRS (Table 2) and nonirrigated plots at CCRS (Table 3). Significant differences between planting dates within irrigated plots at CCRS were observed in 1978 ($P < 0.01$) but not in 1979 ($P > 0.12$). Aflatoxin B_1 concentrations were low in 1978 at TRS and planting dates did not result in significant differences (Table 4).

Aflatoxin B_1 concentration increased with delayed harvest in 1979 but not in 1978 (Tables 2 and 3). The effect of delayed harvest

TABLE 1. Mean percent aflatoxin-positive samples of harvested corn for three cultivars averaged across location, year, and harvest date[a]

	Planting date		
Cultivar[b]	April	May	Mean
P.B. 3780	6.5	63.3	32.9 (85/258)[c]
P.B. 3368A	16.7	68.8	38.6 (44/114)
P.B. 3147	35.5	64.2	48.1 (124/258)
Mean	20.5	63.5	40.2 (253/630)

[a] Positive samples contained ≥20 μg/kg aflatoxin B_1.

[b] P.B. = Pioneer Brand.

[c] Values in parentheses represent the number of positive samples over the total number of samples examined.

was significant ($P = 0.01$) at UCPRS but not ($P = 0.09$) at CCRS in 1979. Heavy rains during the harvest period in 1979 may have favored continued aflatoxin production. Weather conditions during the 1978 harvest period were extremely dry. Total rainfall from 10 August to 30 October was 8.1 and 6.6 cm in 1978 and 40.6 and 26.5 cm in 1979 at UCPRS and CCRS, respectively. Late season rainfall delays drying of corn in the field and favors continued aflatoxin synthesis.

Alfatoxin B_1 concentration (micrograms per kilogram) was regressed against percent infected ears, yield, and damage by corn earworm and European corn borer. Variation in numbers of infected ears accounted for 85% ($r^2 = 0.85$) of the variation in aflatoxin concentration in a simple linear model. A simple linear regression including all 630 samples in this study revealed that variation in damage by either corn earworm or European corn borer would account for only 9 and 10% of the variation in the incidence of *A. flavus*-infected ears and 10 and 11%, respectively, of the variation in the incidence of aflatoxin B_1. Graphic plots of aflatoxin B_1 against CEW damage and ECB damage did not suggest a second-order relationship. A significant correlation ($P = 0.01$) was obtained between aflatoxin B_1 and reduced yield. Variation in yield accounted for 68% of the variation in aflatoxin concentration. This suggests that stress conditions that reduce yield may help predispose plants to infection by *A. flavus* or to increased aflatoxin production once infection occurs.

Irrigation study. Irrigation was employed to reduce plant stress in the CCRS experiment in 1978 and 1979. Irrigated whole plots contained 23.6% (34/144) aflatoxin-positive samples compared to 54.9% (79/144) positive samples in nonirrigated whole plots. The effect of irrigation on levels of aflatoxin B_1 was dramatic. Irrigated subplots contained an average of 7.3 μg/kg compared to 61.9 μg/kg in nonirrigated subplots. Irrigated subplots contained fewer visibly infected ears and higher yields (Table 5).

Xylem water potential. Water stress, exceeding −14 bars required the use of supplemental irrigation on four occasions in 1978 and three occasions in 1979. In 1978, irrigations of 2.5 cm were applied on 22 and 28 June and 5 and 25 July. In 1979, irrigations were applied on 10 July and 2 and 7 August.

Under North Carolina conditions, the critical grain filling period (midsilk to black layer formation) is approximately 48 days for P.B. 3780 (short season) and 54 days for P.B. 3147 (full season). During 1978, water stress was high during the grain filling period of all planting date × cultivar combinations. Irrigated subplots generally had less-negative water potential readings (Fig. 1) and higher yields (Table 5) than nonirrigated subplots.

In 1979, rainfall was more frequent, water stress was much lower, and water potential readings did not fall below −13 bars (the

TABLE 2. Effect of planting date and grain moisture content at harvest on aflatoxin concentration in corn grown at the Upper Coastal Plain Experiment Station near Rocky Mount, NC

Planting date	Cultivar[a]	Harvest moisture[b]	Aflatoxin B_1 (μg/kg)[c] 1978	1979
April	P.B. 3780	1	0	0
		2	0	0
		3	0	0
	P.B. 3368A	1	0	0
		2	0	3
		3	7	0
	P.B. 3147	1	0	5
		2	0	38
		3	7	25
May	P.B. 3780	1	30	250
		2	48	325
		3	50	408
	P.B. 3368A	1	40	192
		2	35	358
		3	30	667
	P.B. 3147	1	135	242
		2	103	267
		3	87	350

[a] P.B. = Pioneer Brand.
[b] Harvest dates were based on percent moisture of grain: 1 = 30–28%, 2 = 24–22%, and 3 = 18–16% moisture.
[c] Mean of six replications.

TABLE 3. Effect of planting date, grain moisture content at harvest, and irrigation on aflatoxin concentration in corn at the Central Crops Research Station near Clayton, NC

Planting date	Cultivar[a]	Harvest moisture[b]	Aflatoxin B_1 (μg/kg) Irrigated 1978	Irrigated 1979	Nonirrigated 1978	Nonirrigated 1979
April	P.B. 3780	1	0[c]	0	3	0
		2	0	0	20	0
		3	0	0	5	0
	P.B. 3147	1	3	0	55	8
		2	5	0	42	0
		3	15	0	67	3
May	P.B. 3780	1	15	3	192	30
		2	17	10	185	30
		3	15	10	207	37
	P.B. 3147	1	25	0	163	0
		2	30	0	227	0
		3	23	0	188	20

[a] P.B. = Pioneer Brand.
[b] Harvest dates were based on percent moisture of grain: 1 = 30–28%, 2 = 24–22%, and 3 = 18–16% moisture.
[c] Mean of six replications.

TABLE 4. Effect of planting date and harvest dates on aflatoxin concentration in corn at the Tidewater Research Station near Plymouth, NC, in 1978

Planting date	Cultivar[a]	Harvest moisture[b]	Aflatoxin B_1 (μg/kg)[c]
April	P.B. 3780	1	0
		3	0
	P.B. 3368A	1	3
		3	0
	P.B. 3147	1	7
		3	0
May	P.B. 3780	1	0
		3	0
	P.B. 3368A	1	8
		3	0
	P.B. 3147	1	0
		3	17

[a] P.B. = Pioneer Brand.
[b] Harvest dates were based on percent moisture of grain: 1 = 30–28% and 3 = 18–16% moisture.
[c] Mean of six replications.

TABLE 5. Effect of irrigation, planting date, and cultivar on aflatoxin B_1 concentration, incidence of visible *Aspergillus flavus* and yield of corn grown at the Central Crops Research Station near Clayton, NC

Treatments and cultivars[a]	Aflatoxin (μg/kg) April	May	Infected ears (%) April	May	Yield (kg/ha) April	May
1978						
Irrigated						
P.B. 3780	0[b]	8	0.7[a]	2.7	7,593[a]	7,971
P.B. 3147	16	26	3.0	7.8	9,602	9,037
Nonirrigated						
P.B. 3780	10	195	2.8	19.9	3,389	2,824
P.B. 3147	54	193	18.8	20.4	5,460	4,644
1979						
Irrigated						
P.B. 3780	0	8	0.5	2.1	6,653	6,088
P.B. 3147	0	0	0.6	1.6	7,845	7,092
Nonirrigated						
P.B. 3780	0	32	0.2	18.4	6,276	4,330
P.B. 3147	4	7	0.2	4.1	6,653	5,774

[a] P.B. = Pioneer Brand.
[b] Mean of six replications.

requirement for supplemental irrigation) until very late in the grain filling periods of the April-planted cultivars. Corn planted in May experienced some water stress during the grain filling period. Little difference was detected between irrigated and nonirrigated treatments with respect to xylem water potential, yield, or aflatoxin B_1 concentration at harvest. The short-season cultivar planted in May experienced some water stress late in the grain filling period (Fig. 1), showed yield reductions averaging 1,946 kg/ha, and the grain from nonirrigated plots contained more aflatoxin than that from irrigated plots (Table 5).

Airborne inoculum. A total of 1,406 colonies of *A. flavus* were identified among colonies from spores collected in air samples in 1978 (465 from the sampler located in the irrigated plot and 941 from the sampler in the nonirrigated plot). In 1979, 606 colonies of *A. flavus* were identified (274 from the irrigated plot and 332 from the nonirrigated plot). Daily totals were low and variable during May and early June in both 1978 and 1979. Weekly totals of viable spores of *A. flavus* trapped (15 May–15 Aug) were negatively corrleated with millimeters of rainfall and hours of leaf wetness ($P = 0.05$) In the field, airborne spores of *A. flavus* increased during both growing seasons. However, the increased frequency of rain during 1979 (Fig. 2) may have served to reduce the total airborne populations in 1979 compared to 1978. Harvesting operations greatly increased the populations of *A. flavus* in the air, particularly in the heavily infected, nonirrigated plots in 1978 (Fig. 2). Nonirrigated cultivar × planting date subplots that pollinated during weeks of heavy airborne spore loads (Fig. 2) contained a higher percentage of ears showing visible growth of *A. flavus* at harvest (Table 5).

Mycoflora. In 1978, 4.4% of kernels plated yielded *A. flavus* after incubation; 1.6% of kernels from irrigated treatments and 7.3% of the kernels from nonirrigated treatments yielded *A. flavus*. The frequency of isolation decreased from the base region to the tip region (54, 27, and 19% of infected kernels originating from the base, middle, and tip regions, respectively). The number of infected kernels followed the same general trends as the percent incidence of visibly infected ears at harvest with the short-season cultivar planted early containing fewer infected kernels that the short-season cultivar planted late or the full-season cultivar at either planting date (Fig. 3).

In 1979, 2.0% of the kernels plated yielded *A. flavus* upon incubation; 1.0% of the kernels from irrigated treatments and 3.0% of the kernels from nonirrigated treatments yielded *A. flavus*. The frequency of isolation did not decrease from base to tip (34, 27, and 39%, respectively). The number of infected kernels again followed the same pattern as the percent incidence of visibly infected ears at harvest; the short-season cultivar (P.B. 3780) planted in May contained the highest number of infected kernels (Fig. 3).

DISCUSSION

The development of aflatoxin in field corn is influenced by factors that increase plant stress, particularly during the pollination and grain filling stage of development. Under NC conditions, early planting resulted in less stress during this period and reduced levels of aflatoxin B_1. Lillehoj et al (15) reported a greater incidence of aflatoxin B_1 in April- and May-planted than June-planted corn in 1976 experiments in Florida and Georgia. Widstrom et al (29) reported an increase in the percent aflatoxin contaminated samples in 19 April- vs 2 May-planted corn in 1974 experiments in Georgia. In both instances, aflatoxin concentrations represented the mean of two replications and the influence of planting date was aggregated over other treatment effects. However, influence of planting date on aflatoxin concentration at harvest in our study was affected by location, within-year climatic factors (ie, drought periods), and maturity factors of the hybrids tested. April-planted corn in

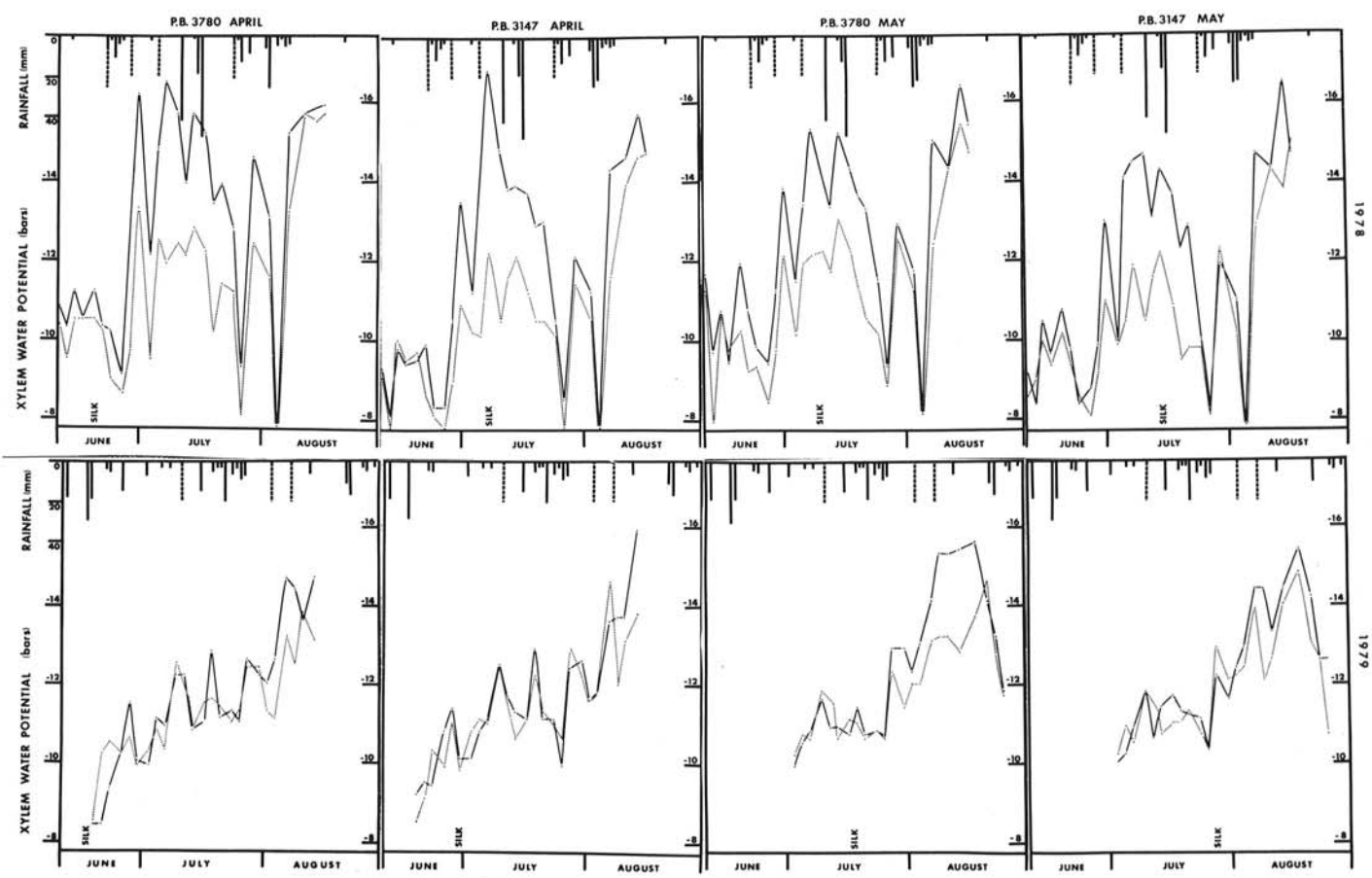

Fig. 1. Xylem water potential of irrigated (dashed line) and nonirrigated (solid line) field corn planted at the Central Crops Research Station near Clayton, NC in 1978 and 1979. Rainfall (millimeters per day) is shown in solid bars, irrigation is represented as dashed bars. Relative dates of midsilk are shown for each planting date × cultivar combination. Measurements represent the mean of six samples taken at 0900 hr.

Georgia may represent a late planting whereas in North Carolina it represents an early planting. In most years, corn planted in early April in North Carolina was less drought-stressed and as planting dates were delayed past 15 April, yield reductions were observed.

Late planting shifted the ear development phase from June/July to July/August. This shift was accompanied by increased temperatures during ear development. The mean number of days with temperatures exceeding 32 C across locations were 7, 15, and 20 days for June, July, and August of 1978, and 0, 7, and 19 days (respectively) for these months in 1979. Temperatures exceeding 32 C are favorable for silk infection by *A. flavus* (11). Delayed planting also exposed developing ears in the CCRS experiment to higher inoculum loads (Fig. 2).

We suggest that drought stress may predispose corn to increased aflatoxin contamination by increasing the exposure of susceptible silks to airborne spores of *A. flavus*. Cessation of leaf expansion is one of the most sensitive indices of plant water deficits. Leaf water potentials of less than −4 bars have a marked effect on halting leaf elongation (7). Vitosh (26) suggested that a major benefit of supplemental irrigation is to provide a full crop canopy and thereby reduce evaporation from bare soil. Drought-affected plants have a lowered leaf area index and increased frequency of drought-induced leaf roll. The high porosity sand and sandy loams of the Coastal Plain region (including CCRS and UCPRS) are more subject to moisture depletions that can result in yield reductions than the clay soils of the Piedmont (including PRS) or the poorly drained, high organic matter soils of the Tidewater region (including TRS) (25).

The hypothesis that inoculum deposition interacts with drought stress and crop canopy could account for the dramatic reduction between irrigated and nonirrigated treatments with respect to the number of ears with visible growth of *A. flavus* at harvest (Table 5), the frequency of isolation of *A. flavus* from developing kernels (Fig. 3), and the aflatoxin B_1 concentration at harvest (Table 5). It may also account for the highly significant correlation between aflatoxin B_1 concentration and reduced yield.

Literature on field development of aflatoxin in corn is replete with attempts to associate insect damage both specifically and quantitatively with infection and subsequent aflatioxin production by *A. flavus* in vivo. Although insects have been implicated in *A. flavus* infection in preharvest corn, experiments designed to determine specific vector relationships have proven inconclusive. In this study we have found that factors influencing plant stress such as lack of rainfall and late planting are strongly associated with increased infection and aflatoxin production by *A. flavus*. These same factors often increase the frequency of damage by ear inhabiting insects. Lillehoj et al (15) reported that the incidence of *A. flavus* on larvae of corn earworm, European corn borer and fall army worm (*Spodoptera frugipenda* J. E. Smith) did not vary significantly between diverse locations from the southern regions to the Corn Belt in the USA. The 1.0% external occurrence of *A. flavus* on larvae was similar to the 1.7 to 3.1% occurrence in corn insects collected at diverse locations in an earlier study (5). In contrast to the uniformity of the insect-*A. flavus* association, aflatoxin levels in corn were much higher in samples from southern test locations than from Corn Belt locations.

We conclude that aflatoxin contamination of field corn can be attributed to (at least) two entirely different mechanisms—high spore loads and increased drought stress during the grain filling period when coupled with favorable temperatures can result in a significant number of kernels infected through silks colonized by *A. flavus*. Severe insect infestations can amplify the aflatoxin problem

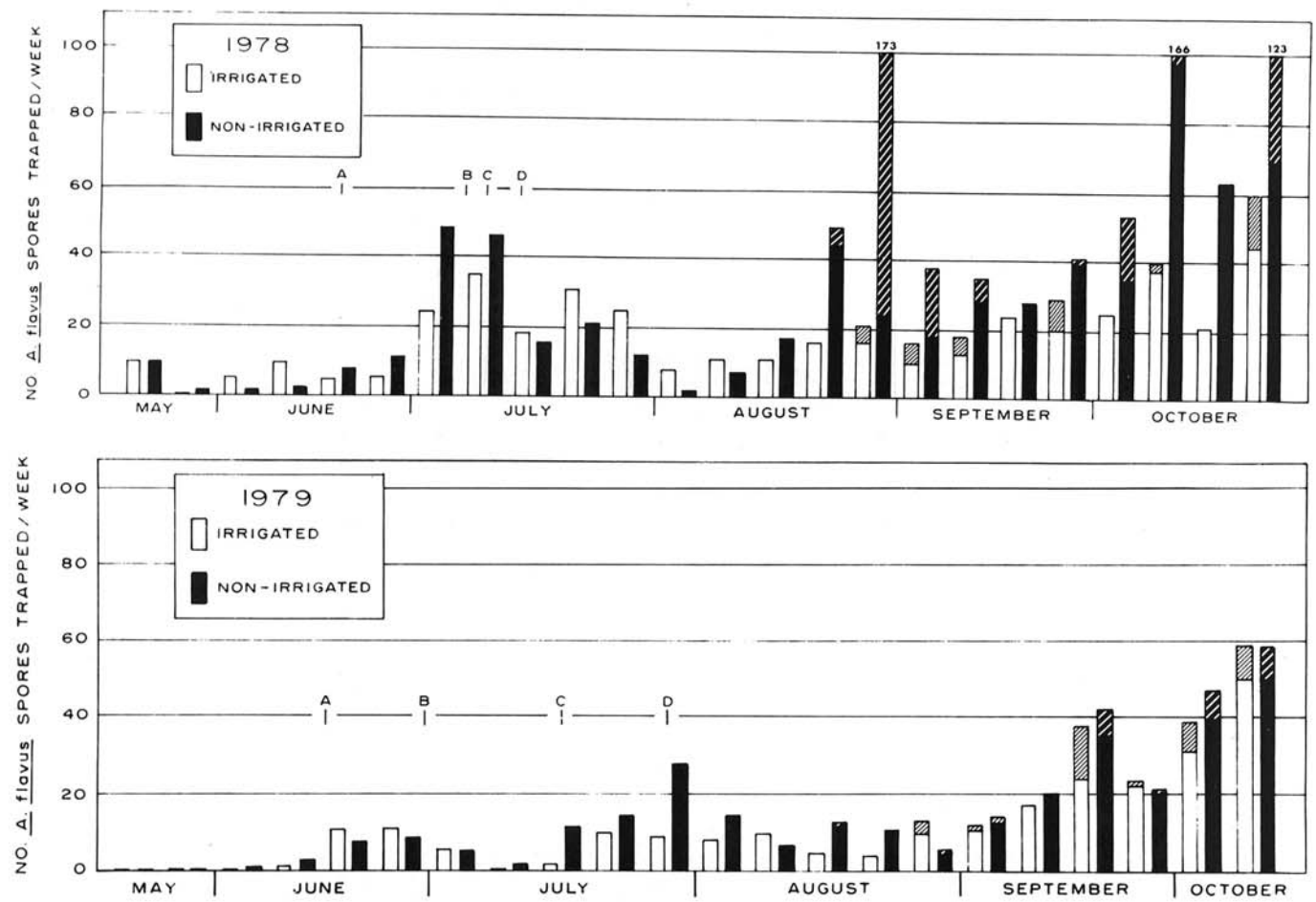

Fig. 2. Weekly totals of airborne spores of *Aspergillus flavus* in irrigated vs nonirrigated plots of corn cultivars Pioneer Brand (P.B.) 3780 and P.B. 3147. Andersen air samplers were operated for 30 sec each hour at a volume of 4.7×10^{-4} m^3/sec. Petri plates were changed daily at 0800 hr. Hatched areas represent spores trapped on harvest dates. Dates of midsilk are **A**, P.B. 3780, April planting; **B**, P.B. 3147, April planting; **C**, P.B. 3780, May planting; and **D**, P.B. 3147, May planting.

through physical damage to kernels in feeding and by allowing for further spread of *A. flavus* from kernel to kernel within ears. When the pericarp of a kernel is broken, the contents are exposed to invasion by microorganisms and the moisture content drops rapidly to levels (≤35% moisture) where *A. flavus* can compete successfully with other microorganisms. Sporulation by *A. flavus* may also be enhanced when silk infected kernels are damaged by insect feeding. Higher levels of aflatoxin and characteristic bright greenish yellow fluorescence have been found in damaged kernels (16,17).

Aflatoxin contamination of field corn can be influenced by factors that increase the number of infected kernels (planting date, moisture stress, and insect-mitigated secondary spread) and by factors that increase the concentration of aflatoxin in infected kernels (delayed harvest, late season rainfall, and pericarp damage). While drought stress may predispose plants to greater

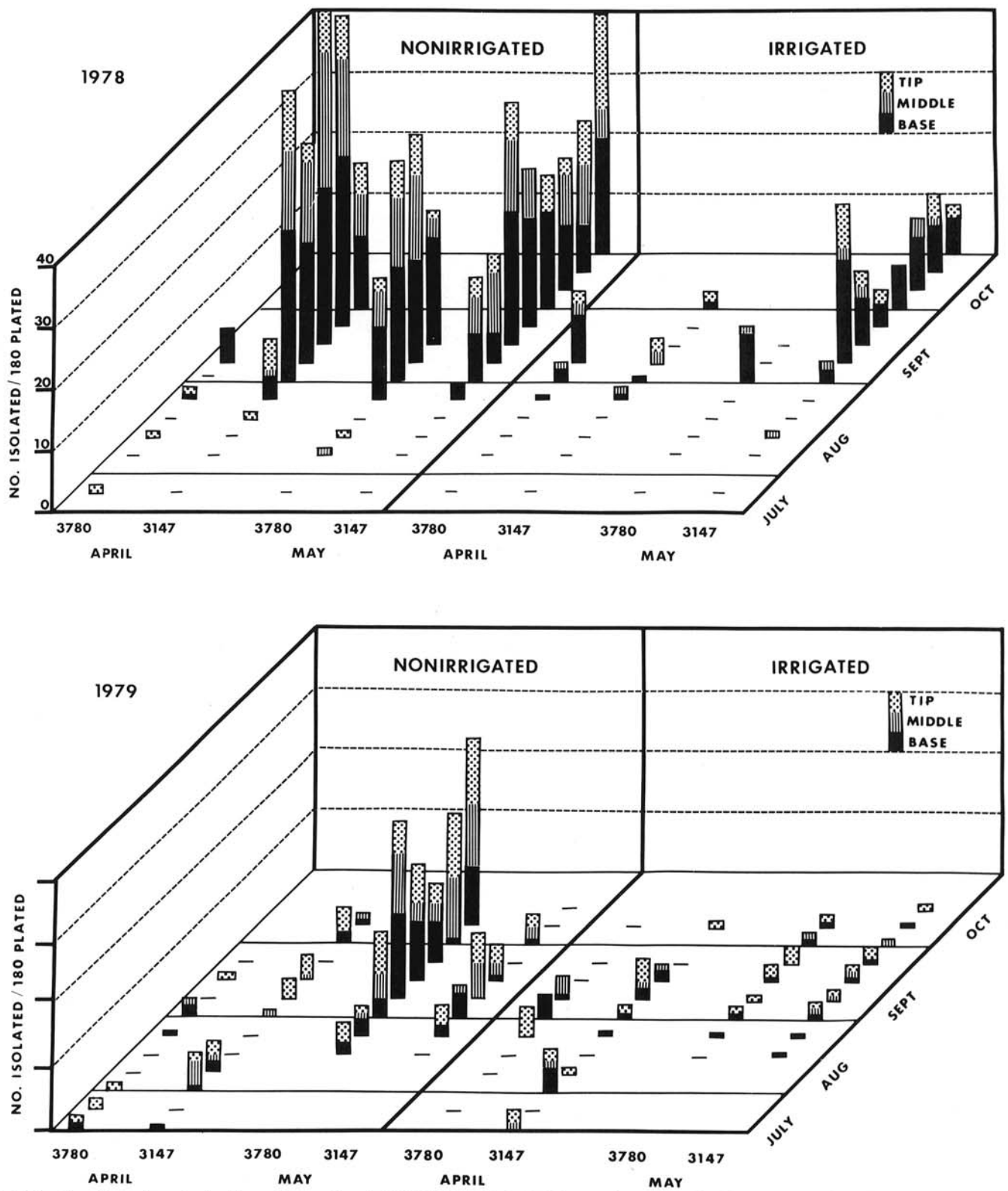

Fig. 3. Number of corn kernels of cultivars Pioneer Brand (P.B.) 3780 and P.B. 3147 from which *Aspergillus flavus* could be isolated during 1978 and 1979 growing seasons. Isolations began approximately 2 wk after silk emergence and continued through harvest. Ten kernels were plated from the tip, middle, and base of portions of six ears in each treatment.

numbers of infected kernels, it may also play a role in altering the nutritional status of developing kernels, thus increasing alfatoxin synthesis in drought-stressed kernels once infection has occurred.

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