Reduction of Aflatoxin Contamination in Corn by Irrigation and Tillage

G. A. Payne, D. K. Cassel, and C. R. Adkins

First and third authors, associate professor and research assistant, respectively, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616. Second author, professor, Department of Soil Science, North Carolina State University, Raleigh 27695-7619.

Paper 9946 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh 27695-7601.

Use of trade names in this article does not imply endorsement by the North Carolina Agricultural Research Service of the products named or criticism of similar ones not mentioned.

We wish to thank Ellis Edwards (1980 and 1981) and Indrek Porro (1982 and 1983) for managing the day-to-day field operations for this study. We also thank Wallace Baker for his help in conducting these studies.

Accepted for publication 15 January 1986 (submitted for electronic processing).

ABSTRACT

Payne, G. A., Cassel, D. K., and Adkins, C. R. 1986. Reduction of aflatoxin contamination in corn by irrigation and tillage. Phytopathology 76:679-684.

The influence of irrigation and subsoiling on infection and aflatoxin production by *Aspergillus flavus* in corn kernels was studied over a 4-yr period. Corn was grown on the Atlantic Coastal Plain in a humid environment on a soil with a tillage-induced pan. The corn crops were under natural drought stress each year, and stress was alleviated with a normal or delayed irrigation regime. Corn ears were either silk-inoculated, wound-inoculated, or naturally infected. Aflatoxin levels in naturally infected corn exceeded 80 $\mu g\ kg^{-1}$ in the nonirrigated, nonsubsoiled plots each year and exceeded 1,200 $\mu g\ kg^{-1}$ in the year with the least rainfall. Kernel infection and aflatoxin contamination were always greater in silk-inoculated plots than in naturally infected plots. Wound inoculation resulted in the greatest amount of aflatoxin, but this procedure was so severe that few treatment differences were observed. In silk-inoculated corn, aflatoxin contamination was less each year in plots that were either irrigated or subsoiled. Although several factors may contribute to high amounts of aflatoxin in the field, water stress appears to be a major factor affecting aflatoxin contamination, because subsoiling as well as irrigation reduced aflatoxin contamination.

Additional key word: maize.

Aflatoxin contamination of preharvest corn is a serious problem in the southern and southeastern United States. Currently, no corn hybrid is available with resistance to aflatoxin accumulation, and control recommendations have involved cultural practices. In order to recommend effective cultural control procedures, it is important to understand the influence of environmental factors on the accumulation of aflatoxin. Several factors have been associated with high levels of aflatoxin in preharvest corn, including high temperatures, insect damage, and plant stress (1,6,8,10,11,17,18,20). Ever since aflatoxin contamination has been recognized as a preharvest problem, most of the field reports have come from areas that have experienced drought stress (20). There have been few reports, however, of studies on the influence of water stress on aflatoxin accumulation in corn. Jones et al (6), in a 2-yr study, showed a reduction in aflatoxin contamination by irrigation. Associated with this reduction were fewer airborne conidia of the fungus and fewer infected kernels. Their study indicated that irrigation could be an effective control procedure for aflatoxin contamination.

The objective of our study was to further examine the influence of irrigation, including irrigation scheduling, and to study the influence of subsoiling on infection and aflatoxin contamination. Data are presented that show that aflatoxin levels are lower in either irrigated or subsoiled corn.

MATERIALS AND METHODS

The study was conducted from 1980 to 1983 on the Atlantic Coastal Plain at the Central Crops Research Station near Clayton, NC. The soil was a Wagram (loamy, siliceous, thermic Arenic Paleudult)-Norfolk (fine-loamy, siliceous, thermic Typic Paleudult) complex. Immediately below the 25-cm-deep AP horizon lay an E horizon with a dense, tillage-induced pan that prevented root penetration of the subsoil. The same field was used all 4 yr.

Corn (*Zea mays* L.), cultivar Pioneer Brand 3369A in 1980 and cultivar Pioneer Brand 3320 the remaining 3 yr, was seeded in 95-cm-spaced rows in mid-April each year. Plant population was about $60 \pm 2 \times 10^3$ plants per hectare. All treatments received nitrogen, phosphorus, potassium, and sulfur fertilizer at seeding on the basis of soil test recommendations; in addition, all plots received nitrogen topdressed at the rate of 196 kg ha^{-1}. Individual plot size was 12×12 m.

Experimental design, tillage, and irrigation. The experimental design in 1980 and 1981 was a randomized complete block with four replicates. After the entire field was disk-harrowed once to a nominal depth of 12 cm, the following four tillage-irrigation treatments were imposed. The control treatment (conventional tillage, not irrigated) was disk-harrowed two additional times before planting. The second treatment (conventional tillage, irrigated) was also disk-harrowed twice before planting but was irrigated during the growing season whenever the soil water pressure (SWP) measured by tensiometers at 25 cm deep in the corn row reached −40 kPa (−0.4 bar). The third treatment (subsoiled, not irrigated) was in-row subsoiled to a depth of 45 cm with 2-cm-wide shanks spaced 95 cm apart; loose soil was bedded to a height of 15 cm over the subsoil slit. The subsoiling operation was performed within 3 days of seeding. The fourth treatment (subsoiled, irrigated) was subsoiled as described but was irrigated throughout the growing season whenever the SWP at 30 cm deep reached −40 kPa. Usually, 2–3 cm of water was applied at each irrigation with a solid-set overhead sprinkler system at the rate of 7 mm hr^{-1}.

In 1982 and 1983, the experimental design was a randomized complete block in a split-plot arrangement with three replicates. Tillage treatment was the main plot and irrigation treatment was the subplot. Conventional and subsoil tillage treatments as described earlier were included. The following four irrigation levels were imposed for the conventionally tilled soil: 1) optimum, i.e., irrigated when SWP measured with tensiometers at 25 cm deep

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

reached −40 kPa; 2) a 2-day delay in irrigation, i.e, water was applied 2 days after SWP at 25 cm deep was −40 kPa; 3) a 4-day delay; and 4) no irrigation. Irrigation treatments for the subsoil tillage treatments were identical to those for the conventionally tilled ones except that SWP was measured at 30 rather than at 25 cm deep.

Inoculation procedures. Plants were inoculated with a spore suspension of *Aspergillus flavus* Link ex Fries NRRL 3357 grown on potato-dextrose agar for 10 days at 28 C. Culture plates were flooded with 0.05% Triton X-100, the spores were dislodged with a glass rod, and the concentration of spores was adjusted to 5×10^5 ml^{-1} in 0.05% Triton X-100. Spore suspensions were prepared the day before inoculation and stored at 4 C.

Silk and wound inoculation methods were used in 1981–1983. No inoculations were performed in 1980. Silks were inoculated using a hand-held spray bottle (J. H. Anderson Co, Raleigh NC 27606) to spray 1 ml of spore suspension on silks. In 1981 and 1982, silks in all plots were inoculated when silks in the irrigated plots were yellow-brown (9). Because drought stress delayed silk emergence and development, silk inoculations in 1983 were staggered and done in each plot when silks were yellow-brown. Silk inoculations were done between 1 and 13 July for the 3 yr. After inoculation, ears were enclosed in a plastic bag, then covered with a paper bag. Three days later, the plastic bag was removed, but the paper bag remained on the ear until harvest. For the wound inoculation method, kernels were wounded by peeling back the husks on one side of the ear and applying a pinboard with a line of 23 pins spaced 5 mm apart once to four adjacent rows to injure the kernels. The wounded area was sprayed with 1 ml of conidial suspension. Husks were repositioned, secured with rubber bands, and covered with plastic and paper bags to maintain humid conditions. After 3 days, the plastic bags were removed, but the paper bag remained until harvest. Ears were wound-inoculated in the late-milk to early-dough stage. For each method, all plants (about 50) in a 9-m section of row were inoculated.

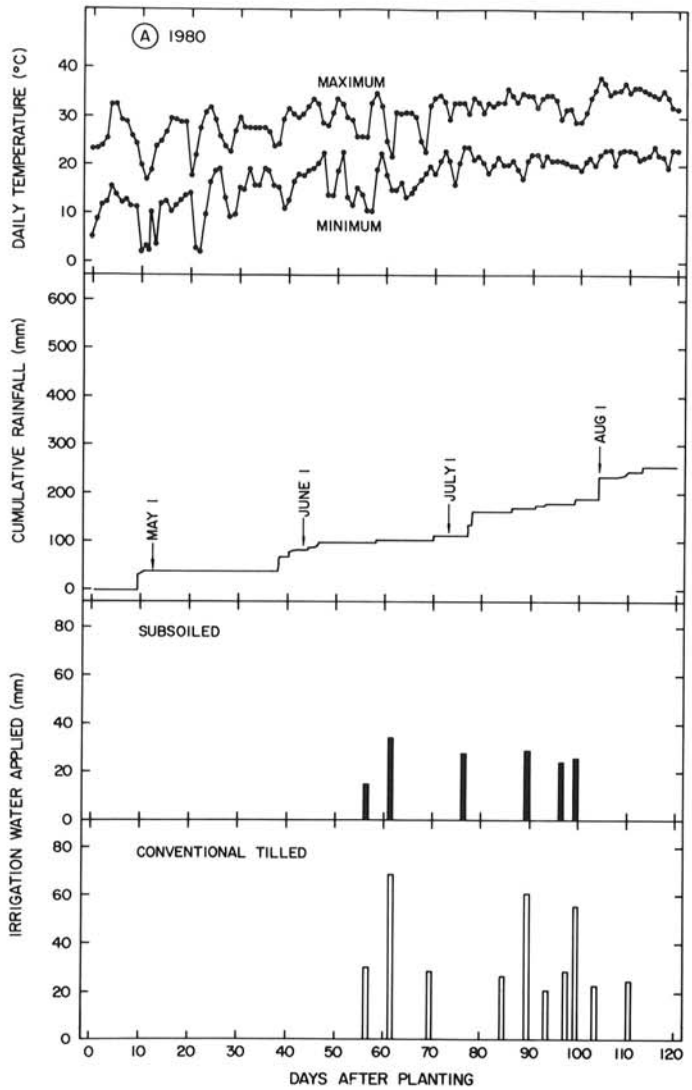

Fig. 1. Daily temperature, cumulative rainfall, and irrigation water applied to research plots at the Central Crops Research Station during 1980.

Aflatoxin analysis. All ears in each 9-m-long row were hand-harvested, bulked, and machine-shelled, then yield was recorded and a 4.5-kg subsample was dried at 60 C (kernel moisture = 10%) and stored in a low-humidity room. The entire sample was ground to pass through a 20-mesh screen and thoroughly blended, then a 20-g subsample was extracted for aflatoxin. Aflatoxin extraction and analysis were done as described by Hutchins and Hagler (5). Aflatoxin amounts are the sum of aflatoxin B_1 B_2. Aflatoxin levels were not determined for silk-inoculated ears in 1981.

Kernel infection. In 1981–1983, all ears in each 9-m-long row were hand-harvested, bulked, machined-shelled, and a 600-ml subsample withdrawn. The subsample was dried at 60 C (kernel moisture = 10%) and stored in a low-humidity environment until assayed. Two hundred kernels were randomly selected from each sample, rinsed thoroughly with running water, surface-sterilized for 3 min in a solution of 95% ethanol/Clorox/water (10:20:70), and plated on malt agar containing 6% NaCl. Only kernels free of visible injury and sporulation of the fungus were plated. The plates were incubated at 34 C for 4 days and the seeds with visible *A. flavus* were counted. No kernels were plated in 1980, and kernels from naturally infected plots were not plated in 1983. No kernels were plated from the wound-inoculated treatments.

RESULTS

1980 and 1981. Drought occurred in both 1980 and 1981. From June to August 1980, only 120 mm of rain fell; consequently, 186 and 156 mm of irrigation water was applied to the conventionally tilled and subsoiled plots, respectively (Fig. 1). Total growing-season rainfall was 44 mm less in 1981 than in 1980 (Fig 2). Irrigation was required eight times for the conventionally tilled and six times for the subsoiled plots. In both years, irrigation or subsoiling increased corn yields (Table 1). Subsoiling was not as effective in increasing corn yields in nonirrigated plots in 1981 as it was in 1980.

Aflatoxin contamination was higher in 1981 than in 1980, but in both years, aflatoxin concentrations in all naturally infected plots except one exceeded 20 $\mu g\ kg^{-1}$, the FDA guideline for aflatoxin contamination (Table 1). Either irrigation or subsoiling led to lower aflatoxin contamination in naturally infected plots in each year. In 1981, irrigation of conventionally tilled plots reduced aflatoxin contamination 16-fold even though the reduction was not significant ($P = 0.05$).

Aflatoxin contamination was high in wound-inoculated kernels, and although treatment differences were not significant, the highest concentration of aflatoxin was in the nonirrigated, conventionally tilled plots (Table 1). The percentage of infected kernels was greater in silk-inoculated plots than in naturally infected plots (Table 1). Irrigation did not significantly reduce the percentage of infected kernels, but the percentage of infected kernels was lower in irrigated plots than in nonirrigated plots in all treatments except the subsoiled, silk-inoculated plots.

1982 and 1983. The growing seasons in 1982 and 1983 differed considerably. The 1982 season was unusually favorable for corn production; rainfall was adequate and timely throughout most of the season (Fig. 3). For conventional tillage, irrigation was required twice for the optimum irrigation treatment, once for the 2-day-delayed irrigation treatment, and once for the 4-day-delayed treatment. For the subsoiled land, both the optimum and 2-day-delayed irrigation plots received one irrigation. In contrast to 1982, the 1983 growing season was dry, especially during July (Fig. 4). Irrigation of conventionally tilled corn was required 10 times to maintain soil moisture at or above −40 kPa for the optimum irrigation treatment. Seven and two irrigations were required for

the 2- and 4-day-delayed irrigation plots, respectively. The subsoiled land required only five irrigations to maintain SWP at 30 cm deep at or above −40 kPa. Four and three irrigations were required for the 2- and 4-day-delayed irrigation plots, respectively.

In 1982, yield was high in all plots except the nonirrigated, conventionally tilled plots (Table 2). In contrast, either no irrigation or a 4-day delay in irrigation of conventionally tilled plots resulted in reduced yields in 1983. Yields were higher in the subsoiled plots than in the conventionally tilled plots, but either optimum or a 2-day-delayed irrigation resulted in the highest yields (Table 2).

Surprisingly, aflatoxin levels in naturally infected plots were similar for 1982 and 1983 (Table 2). Even though the levels were lower than those found in 1981, they exceeded 45 $\mu g\ kg^{-1}$. In contrast to 1980 and 1981, neither irrigation nor subsoiling significantly influenced aflatoxin contamination.

Aflatoxin levels were much higher in the silk-inoculated plots than in naturally infected plots in both 1982 and 1983 (Table 2). In both years, irrigation and subsoiling resulted in lower levels of aflatoxin. In the conventionally tilled plots, the trend was for aflatoxin levels to increase with increased delay in irrigation. In 1982, aflatoxin levels in the nonirrigated treatment were

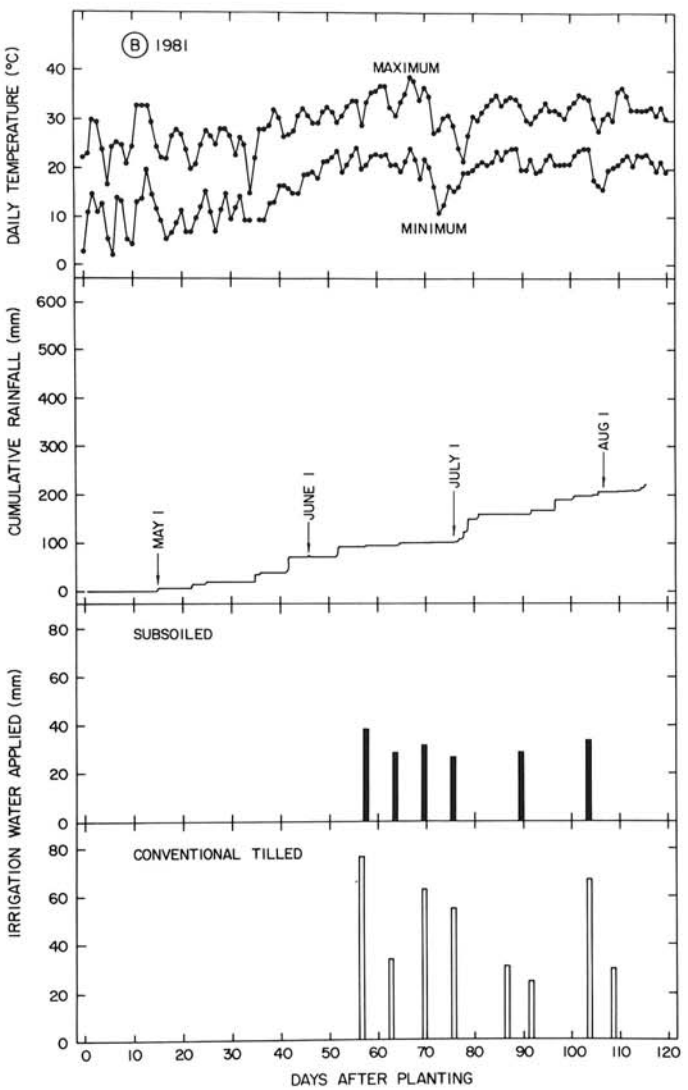

Fig. 2. Daily temperature, cumulative rainfall, and irrigation water applied to research plots at the Central Crops Research Station during 1981.

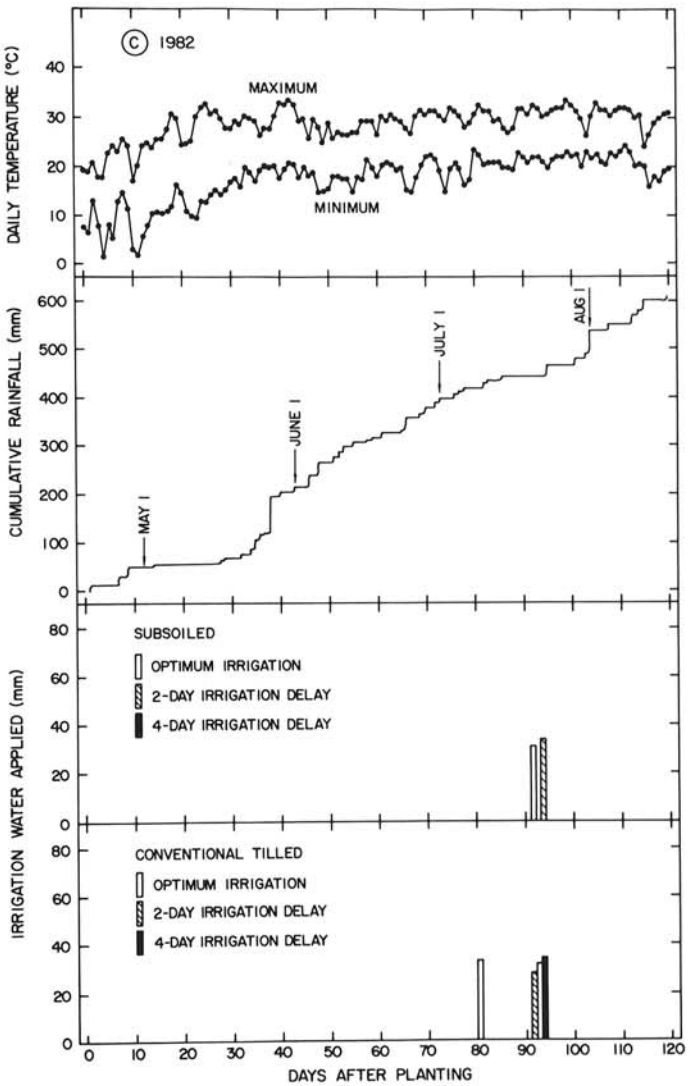

Fig. 3. Daily temperature, cumulative rainfall, and irrigation water applied to research plots at the Central Crops Research Station during 1982.

TABLE 1. Aflatoxin contamination and percent infected kernels of corn not inoculated or inoculated with *Aspergillus flavus* and yield of corn subjected to two tillage treatments and two irrigation regimes in 1980 and 1981

		1980		1981				
		No inoculation		No inoculation		Silk inoculation	Wound inoculation	
Tillage	Irrigation	Aflatoxin[a]	Yield (Mg ha^{-1})	Aflatoxin	%I[b]	%I	Aflatoxin	Yield (Mg ha^{-1})
Conventional	+	25	8.59	78	7	16	5,562	8.73
	−	94	1.86	1,249	18	25	14,323	1.51
Subsoiled	+	22	10.14	42	6	22	7,606	9.36
	−	10	8.14	238	22	18	6,071	4.92
LSD ($P = 0.05$)		35	1.35	1,175	NS[c]	NS	NS	1.67

[a] Aflatoxins $B_1 + B_2$ ($\mu g\ kg^{-1}$).
[b] Percentage of 200 surface-sterilized corn kernels plated on malt agar + 6% NaCl that had visible growth of *A. flavus*.
[c] NS = no significant differences between treatment means.

significantly higher than in all of the irrigated treatments. In 1983, both the 4-day-delay and the nonirrigated treatments had higher aflatoxin levels than either the 2-day-delay or the optimum irrigation plot. Aflatoxin contamination was lower both years in the subsoiled plots than in the conventionally tilled plots. There was a trend for increased aflatoxin as irrigation was delayed in the subsoiled plots, but irrigation did not have a significant effect.

Aflatoxin levels were high in wound-inoculated plots in both 1982 and 1983, and neither irrigation nor subsoiling reduced the levels.

The percentage of infected kernels was low in 1982, and there were no significant differences among treatments. In contrast, infection by *A. flavus* was greater in 1983. Infected kernels in the conventionally tilled plots ranged from 9% for the optimum irrigation to a maximum of 31.5% for the nonirrigation treatments. The number of infected kernels was lower in the subsoiled plots, and irrigation had no effect.

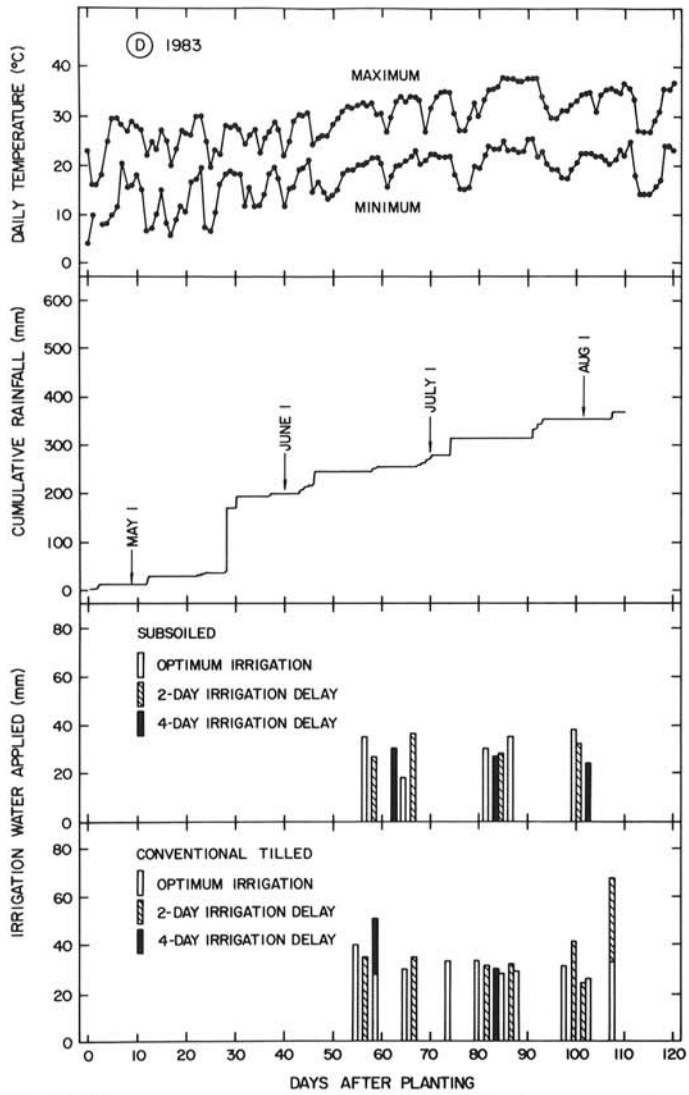

Fig. 4. Daily temperature, cumulative rainfall, and irrigation water applied to research plots at the Central Crops Research Station during 1983.

DISCUSSION

The basic assumption that directed the design of these studies is that aflatoxin levels are greater during years when drought stress occurs, and any procedure that alleviates drought stress should result in lower levels of aflatoxin. The results obtained in this study clearly show that high levels of aflatoxin do occur during years with drought stress and that either irrigation or subsoiling, both of which reduced stress and increased yield in this study, lead to reduced contamination with aflatoxin.

In 1980, 1981, and 1982, irrigation or subsoiling reduced aflatoxin contamination. An exception to this trend occurred in 1983 in plots with natural infection. Even though 1983 was hotter and drier than 1982, aflatoxin contamination in naturally infected plots was no greater than in 1982 and neither irrigation nor tillage reduced aflatoxin contamination. Our data show that 1983 was a conducive year for aflatoxin because contamination was greater in silk- and wound-inoculated plots in 1983 than in any other year. A possible explanation for the lower than expected levels of aflatoxin is that inoculum of the fungus was limited in 1983. This explanation is supported by the striking increase in aflatoxin contamination observed in plots that were silk-inoculated. For conventionally tilled corn, silk inoculation resulted in a sixfold increase in aflatoxin in irrigated corn and a 13-fold increase in aflatoxin in nonirrigated corn.

The source of inoculum for kernel infections is not known, but increased levels of airborne conidia have been associated with increased kernel infection (6). Furthermore, the number of airborne conidia of *A. flavus* has been shown to vary from year to year (4). Because *A. flavus* is predominately soilborne, plant debris in the soil is assummed to be the primary source of airborne inoculum. *A. flavus* can also produce sporogenic sclerotia (15), and sclerotia of *A. flavus* have been found in infected corn seeds (16). The role that these sclerotia play in the epidemiology of the fungus is not known, but Wicklow (16) has shown, however, that the

TABLE 2. Aflatoxin contamination and percent infected kernels of corn not inoculated or inoculated with *Aspergillus flavus* and yield of corn subjected to two tillage treatments and three irrigation regimes in 1982 and 1983

		1982						1983				
		No inoculation		Silk inoculation		Wound inoculation	Yield	No inoculation	Silk inoculation		Wound inoculation	Yield
Tillage	Irrigation	Aflatoxin[a]	%I[b]	Aflatoxin	%I	Aflatoxin	(Mg ha^{-1})	Aflatoxin	Aflatoxin	%I	Aflatoxin	(Mg ha^{-1})
Conventional	Optimum	63	0.7	110	4.0	10,295	8.26	45	288	9.0	13,437	9.89
	2-Day delay	45	2.5	184	3.8	8,443	8.99	104	403	14.2	11,868	9.07
	4-Day delay	132	1.8	263	4.2	10,197	8.07	88	1,391	23.5	10,694	6.98
	No irrigation	95	2.3	517	5.2	12,871	6.83	84	1,111	31.5	15,484	2.48
Subsoil	Optimum	21	0.7	37	2.0	9,071	9.63	64	149	12.5	12,850	9.61
	2-Day delay	43	0.7	119	2.7	6,369	9.91	54	174	8.8	12,324	10.12
	4-Day delay	34	2.5	122	3.0	7,797	9.50	41	293	13.5	12,921	8.47
	No irrigation	42	2.2	113	5.3	6,648	9.29	119	315	8.5	10,793	7.35
LSD ($P = 0.05$)												
Tillage		NS[c]	NS	111	NS	NS	0.79	NS	299	6.2	NS	0.91
Irrigation		NS	NS	157	NS	NS	1.12	NS	422	8.8	NA	1.28
Tillage × irrigation		NS	NS	222	NS	NS	1.59	NS	597	12.4	NS	1.81

[a] Aflatoxins $B_1 + B_2$ ($\mu g\ kg^{-1}$).
[b] Percentage of 200 surface-sterilized corn kernels plated on malt agar + 6% NaCl that had visible growth of *A. flavus*.
[c] NS = no significant differences between treatment means.

population of *A. flavus* rises dramatically in the soil after harvest.

The factors that influence survival of *A. flavus* in soil are not well understood. Hill et al (3) have suggested that the fungus may not compete well with other fungi in moist soils. The cooler and wetter than normal season in 1982 from June to August may have reduced initial levels of the fungus as well as survival and growth of the fungus during the winter. Either the reduction of initial levels of the fungus in 1982 or the reduction of inoculum during the winter may have been important factors influencing the amount of inoculum present in the field in 1983, because the 1983 corn crop was planted in the same field as the 1982 crop.

The results obtained in this study are consistent with the findings of others studying aflatoxin contamination in the field. In studies done in Georgia (11,18), for example, incidence of *A. flavus* and aflatoxin contamination have been found to be associated with heat and drought stress. In a 2-yr irrigation study, Jones et al (6) found more conidia of *A. flavus*, more infected kernels, and higher levels of aflatoxin in nonirrigated plots than in irrigated plots. They concluded that drought conditions contributed to higher aflatoxin contamination because of direct effects on both the fungus and the plant. They proposed that drought stress conditions contribute to increased airborne conidia, because the fungus survives longer in drier soils and is more easily disseminated by wind blowing dry soil containing the conidia. Drought stress was proposed to affect the plant by reducing leaf area, thus making silks more accessible to conidia of the fungus. We also found that irrigation resulted in fewer infected kernels. The effect of irrigation on kernel infection was significant only for 1983, but we think these data are more representative than those obtained in 1981 or 1982 because of the time when the plants were inoculated. In 1983, we inoculated each treatment when the silks were yellow-brown. Previous data indicate that silk color is a better indication of susceptibility than silk age (9).

We did not trap conidia of the fungus in our study; therefore, we do not know the effect of drought on populations of airborne conidia. When the same number of conidia were applied to the silks of ears in all treatments, however, higher concentrations of aflatoxin were present in the drought stress treatments. Therefore, our data suggest that other factors in addition to levels of conidia and silk acessibility must be involved. Drought stress may also influence either infection by the fungus or toxin production once infection has occurred. We had hoped that our wound-inoculation treatments would help answer some of these questions, because an equal amount of inoculum was applied on a wounded kernel and thus neither inoculum concentration nor active penetration was involved. These treatments did lead to high levels of aflatoxin; however, there were no significant differences among treatments. It is interesting that in all instances, the nonirrigated, nonsubsoiled plots had the highest levels of aflatoxin.

In a study such as this one, it is difficult to separate the effects of high temperature from water stress per se, because both occurred during years with drought. For example, aflatoxin levels in naturally infected plots were the greatest in 1981, when rainfall was the lowest and temperatures were the highest. In 1981, there were 18 days in July when the maximum temperatures exceeded 34 C compared with 1982, when there were no days with temperatures higher than 34 C.

Because *A. flavus* has a high temperature optimum (36–38 C) for growth (2), one would expect the fungus to compete better in soil and on plant debris and to produce greater levels of inoculum at higher temperatures. The parasitic abilities of *A. flavus* also appear to increase at higher temperatures. Temperatures higher than 34 C are important in the colonization of corn silks and the subsequent infection of undamaged kernels (7,12). High temperatures also increase aflatoxin production in damaged or wounded kernels (13,14). In a 6-yr field study, McMillian et al (11) showed a correlation between mean temperatures in May through August and high levels of aflatoxin. However, it should be pointed out that the mean temperatures for these months did not vary much between years. A correlation between mean temperatures for the same months of the 4 yr of our study and aflatoxin contamination was performed, and no consistent correlation existed between the mean, minimum, or maximum temperatures during May through August and aflatoxin contamination. Similiarly, we could not show a consistent correlation with aflatoxin contamination and the mean, minimum, or maximum temperatures in June and July. This does not refute the thesis that temperature is important. More likely, high temperatures are critical at certain times in the infection process, but without knowing the precise periods in which they are important, it is difficult to correlate temperature with toxin production.

It could be argued that irrigation reduced aflatoxin levels by reducing air and soil temperatures. In our study, subsoiling also reduced the levels of aflatoxin; therefore, it is difficult to interpret the reduction of aflatoxin in subsoiled plots to be the result of cooler temperatures. A more tenable explanation is that subsoiling reduces water stress and it is the reduction of water stress that results in lower levels of aflatoxin.

Water stress also appears to be involved in aflatoxin contamination of peanuts. Wilson and Stansell (19) found that water stress during the last 40–75 days of the season contributed to aflatoxin contamination of sound mature kernels in three of four years. High levels of aflatoxin were not always correlated with drought stress, and Wilson and Stansell concluded that other environmental factors interact with water stress to promote aflatoxin contamination. Regardless, irrigation was effective in reducing aflatoxin contamination. They found that in all treatments where irrigation was applied during the last 40 days of the season, no significant aflatoxin contamination was detected in any year. Hill et al (3) attempted to separate the effect of temperature and drought on infection and aflatoxin accumulation by *A. flavus* in peanut. They cooled the soil of drought-stressed plots and heated the soil of irrigated plots and concluded that neither drought stress alone nor elevated temperatures alone lead to high levels of aflatoxin. Apparently, both factors contribute to aflatoxin contamination of peanuts. The same situation is likely with corn. We know that high temperatures are important for silk colonization by the fungus (12), and the findings in this study indicate that water stress per se also contributes to higher aflatoxin contamination.

Aflatoxin contamination of corn is a complex problem and is influenced by several factors. High temperatures, water stress, and insects all play an important role in aflatoxin contamination in preharvest corn. Under drought stress, all three of these factors are often favorable for aflatoxin accumulation. The results of this study indicate that one effective cultural practice for the control of aflatoxin in corn is to avoid water stress; irrigation, where possible, can be a very effective means. Where tillage-induced pans are present, subsoiling may be an effective alternative to irrigation.

LITERATURE CITED

1. Anderson, H. W., Nehring, E. W., and Wichser, W. R., 1975. Aflatoxin contamination of corn in the field. J. Agric. Food Chem. 23:775-782.
2. Davis, N. D., and Diener, U. L. 1983. Biology of *A. flavus* and *A. parasiticus*. Pages 1-5 in: Aflatoxin and *Aspergillus flavus* in Corn. U. L. Diener, R. A. Asquith, and J. W. Dickens, eds. South. Coop. Ser. Bull. 279.
3. Hill, R. A., Blankenship, P. D., Cole, R. J., and Sanders, T. 1983. Effects of soil moisture and temperature on preharvest invasion of peanuts by the *Aspergillus flavus* group and subsequent aflatoxin development. Appl. Environ. Microbiol. 45:628-633.
4. Holtmeyer, M. G., and Wallin, J. R. 1981. Incidence and distribution of airborne spores of *Aspergillus flavus* in Missouri. Plant Dis. 65:58-60.
5. Hutchins, J. E., and Hagler, W. M. 1983. Rapid liquid chromatographic determination of aflatoxin in heavily contaminated corn. J. Assoc. Off. Anal. Chem. 66:1458-1465.
6. Jones, R. K., Duncan, H. E., and Hamilton, P. B. 1981. Planting date, harvest date, and irrigation effects on infection and aflatoxin production by *Aspergillus flavus* in field corn. Phytopathology 71:810-816.
7. Jones, R. K., Duncan, H. E., Payne, G. A., and Leonard, K. J. 1980. Factors influencing infection by *Aspergillus flavus* in silk-inoculated corn. Plant Dis. 64:859-863.
8. Lillehoj, E. B., Kwolek, W. R., Zuber, M. S., Calvert, O. H., Horner, E. S., Widstrom, N. W., Guthrie, W. D., Scott, G. E., Thompson, D.

L., Findley, W. R., and Bockholdt, A. J. 1978. Aflatoxin contamination of field corn; evaluation of regional test plots for early detection. Cereal Chem. 55:1007-1013.
9. Marsh, S. F., and Payne, G. A. 1984. Preharvest infection of corn silks and kernels by *Aspergillus flavus*. Phytopathology 74:1284-1289.
10. McMillian, W. W. 1983. Role of arthropods in field contamination. Pages 20-22 in: Aflatoxin and *Aspergillus flavus* in Corn. U. L. Diener, R. L. Asquith, and J. W. Dickens, eds. South. Coop. Ser. Bull. 279.
11. McMillian, W. W., Wilson, D. M., and Widstrom, N. W. 1985. Aflatoxin contamination of preharvest corn in Georgia: A six- year study of insect damage and visible *Aspergillus flavus*. J. Environ. Qual. 14:200-202.
12. Payne, G. A. 1983. Nature of field infection of corn by *Aspergillus flavus*. Pages 16-19 in: Aflatoxin and *Aspergillus flavus* in corn. U. L. Diener, R. A. Asquith, and J. W. Dickens, eds. South. Coop. Ser. Bull. 279.
13. Thompson, D. L., Lillehoj, E. B., Leonard, K. J., Kwolek, W. F., and Zuber, M. S. 1980. Aflatoxin concentration in corn as influenced by kernel development stage and postinoculation temperature in controlled environments. Crop Sci. 20:609-612.
14. Thompson, D. L., Payne, G. A., Lillehoj, E. B., and Zuber, M. S. 1983. Early appearance of aflatoxin in developing corn kernels after inoculation with *Aspergillus flavus*. Plant Dis. 67:1321-1322.
15. Wicklow, D. T., and Donahue, J. E. 1984. Sporogenic germination of sclerotia in *Aspergillus flavus* and *A. parasiticus*. Trans. Br. Mycol. Soc. 82:621-624.
16. Wicklow, D. T., Horn, B. W., Burg, W. R., and Cole, R. J. 1984. Sclerotium dispersal of *Aspergillus flavus* and *Eupenicillium ochrosalmoneum* from maize during harvest. Trans. Br. Mycol. Soc. 83:299-303.
17. Widstrom, N. W. 1979. The role of insects and other plant pests in aflatoxin contamination of corn, cotton and peanuts—A review. J. Environ. Qual. 8:5-11.
18. Wilson, D. M., McMillian, W. W., and Widstrom, N. W. 1979. Field aflatoxin contamination of corn in South Georgia. J. Am. Oil Chem. Soc. 56:798-799.
19. Wilson, D. M., and Stansell, J.R.1983. Effect of irrigation regimes on aflatoxin contamination of peanut pods. Peanut Sci. 10:54-56.
20. Zuber, M. S., and Lillehoj, E. B. 1979. Status of the aflatoxin problem in corn. J. Environ. Qual. 8:1-5.