Impact of Agronomic Factors on Aflatoxin Contamination in Preharvest Field Corn in Northeastern Mexico

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ABSTRACT

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Infection by *Aspergillus flavus* and aflatoxin accumulation in field corn, *Zea mays*, were studied in relation to single and combined cultural practices in northeastern Mexico during the spring and fall growing seasons of 1991, 1992, and 1993. Aflatoxin contamination was greater during the spring when high temperatures occurred during corn reproduction and maturation. Crops grown with the INIFAP crop management system consistently had high yields and low aflatoxin levels (0 to 6 ppb). The INIFAP system included: (i) early planting; (ii) a well-adapted hybrid (H-422); (iii) 55,000 plants per ha; (iv) adequate irrigation; and (v) ear insect control by insecticides. In contrast, crops in the control management system had management practices opposite to the INIFAP system (late planting, hybrid Growers-2340, 75,000 plants per ha, drought, and no insect control) and had lowest yields and significantly increased aflatoxin (63 to 167 ppb). The two factors most associated with enhanced aflatoxin contamination were late planting and ear insect damage. Cultivar and plant density did not significantly affect aflatoxin contamination when combined with the remaining components of the INIFAP system. Irrigation was not fully explored because of rainfall during the experiments. Artificial ear wounding with a nailboard device significantly increased aflatoxin contamination and interacted with high temperatures, which further demonstrated the importance of both temperature stress and ear injury on preharvest aflatoxin contamination.

Aflatoxin B_1 is a potent hepatocarcinogenic secondary metabolite produced by the fungi *Aspergillus flavus* Link:Fr. and *A. parasiticus* Speare. Both fungi occur worldwide on a number of agricultural commodities, including corn, peanuts, cottonseed, and tree nuts (21), although *A. flavus* appears to be most associated with corn (*Zea mays* L.) (5). In the United States, aflatoxin contamination of preharvest corn is chronic in the southeastern states. Contamination can also be serious in the corn belt states of the Midwest when high temperatures and drought stress occur during the growing season (21).

In Mexico, most corn grain is made into tortillas; therefore aflatoxin contamination in corn is a threat to human health (4,23,27,30). The use of corn containing more than 20 ppb (μg/kg) aflatoxin for human consumption is prohibited in Mexico and other countries, including the United States. Research on aflatoxin contamination of corn in the United States has addressed the influence of cultural practices, including tillage, fertilization, cultivars, plant density, irrigation, insect control, and planting and harvest dates (9,12,17,21,22,29). Although there are some reports on the factors that favor aflatoxin production in stored corn (19), there is no information on the impact of environmental conditions and crop management on aflatoxin contamination in preharvest corn in Mexico.

Field corn has been cultivated commercially in northern Tamaulipas, Mexico, since the early 1960s, after cotton production collapsed due to insect pest resistance to insecticides (1). Currently, more than 250,000 ha (80% irrigated, 20% dryland) of corn are grown during the spring, and about 50,000 ha are planted during a second growing season in the fall. In 1989, when temperatures and incidence of ear insect pests were higher than normal, *A. flavus* was abundantly present in the field prior to harvest and high levels of aflatoxin contamination caused significant decrease in corn commercialization in this region (25). In response to this problem, a series of recommendations were issued by the Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP) (National Institute for Forestry, Agricultural and Livestock Research), based mainly on preliminary observations in this region during 1989 and 1990 and on published information. These recommendations, known locally as "paquete tecnologico INIFAP" (INIFAP technological package) included as its main components: (i) early planting (20 January to 15 February) to avoid the higher ambient temperatures during reproductive and maturation normally occurring when corn is planted later in the season; (ii) use of well-adapted cultivars; (iii) low plant densities (maximum 55,000 plants per ha); (iv) adequate irrigation (10 cm as needed during each of the vegetative, tasseling, and ear development plant stages, in addition to the 15-cm preplant irrigation); and (v) strict monitoring and control of insects infesting the ear. All of these cultural practices were intended to minimize aflatoxin contamination by avoiding plant stress and ear damage, while maximizing yield. In this paper, the impact of the INIFAP crop management system and the individual influences of planting date, varieties, irrigation, plant density, and insect damage on preharvest aflatoxin contamination in field corn in Tamaulipas are reported.

MATERIALS AND METHODS

Field studies were conducted from 1991 to 1993 at the Campo Experimental Rio Bravo (Rio Bravo Experiment Station) near Rio Bravo, Tamaulipas, Mexico. The experiment station (100 ha) is surrounded by commercial fields planted with either field corn or grain sorghum. Soil at the station is a sandy clay loam. In all experiments, tillage, fertilization, control of soil and seedling insect pests, and other agricultural practices (other than those used as study variables) were applied according to the Rio Bravo Station recommendations for field corn (24).

Spring experiments. Experiments were conducted during each of the spring growing seasons of 1991, 1992, and 1993 using a randomized complete block design with nine treatments and four replicates. Plot size was six rows 0.8 m wide and 10 m long. Treatments were designed to test the overall impact of the INIFAP management system, some two-factor combinations, and the individual effect of five cultural practices on aflatoxin contamination (Table 1).

Treatments were: (1) INIFAP crop management, which included the cultivar H-422 (a high-yielding hybrid developed by INIFAP for this region), planting during 10 to 14 February, density of 55,000 plants per ha, adequate irrigation as explained above, and insecticide (deltamethrin, 12.5 g a.i./ha) (Agrevo, Chimalistac, Mexico) applied to the ears using a back-pack man-

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ual sprayer every 5 days from 50% silking to dough stage (four to five applications); (2) use of the hybrid Growers-2340 (highly susceptible to ear insect pests and ear rots according to preliminary observations during 1989 and 1990) plus the remaining four factors in treatment 1; (3) high plant density (75,000 plants per ha) plus the remaining four factors in treatment 1; (4) no irrigation (drought stress) during flowering and ear development (only the preplant and vegetative irrigations) plus the remaining four factors in treatment 1; (5) no insecticide application to the ears plus the remaining four factors in treatment 1; (6) late planting (10 to 11 March) plus the remaining four factors in treatment 1; (7) late planting + no insecticide application to the ears plus the remaining three factors in treatment 1; (8) drought stress + 75,000 plants per ha plus the remaining three factors in treatment 1; and (9) control or "stressed crop management" with Growers-2340, late planting, 75,000 plants per ha, drought stress, and no ear insect control.

All data were obtained from the center four rows of each experimental plot. Dates were recorded for 50% tasseling, dough stage, and physiological maturity. All experiments were hand harvested when grain moisture was 20 to 25%. Each of 25 arbitrarily selected ears was examined for ear insect damage and visible *A. flavus* (frequency of infection). All ears in each plot were hand shelled, and grain yield was estimated (converted to kg/ha at 12% moisture). Minimum and maximum temperatures and precipitation were monitored daily throughout each growing season.

Fall experiments. Similar experiments were conducted during each of the fall growing seasons of 1991, 1992, and 1993, except that late planting was not included as a treatment and the cultivar in the INIFAP crop management system was HV-1 (a nonconventional hybrid developed by INIFAP for this region, particularly for the fall growing season). Planting dates were 23, 24, and 5 August in 1991, 1992, and 1993, respectively.

Treatments (Table 2) were: (1) INIFAP crop management, with the cultivar HV-1, 55,000 plants per ha, irrigation, and insecticide application as explained above; (2) use of the hybrid Growers-2340 plus the remaining three factors in treatment 1; (3) high plant density (75,000 plants per ha) plus the remaining three factors in treatment 1; (4) drought stress plus the remaining three factors in treatment 1; (5) no insecticide application to the ears plus the remaining three factors in treatment 1; (6) drought stress + 75,000 plants per ha plus the remaining two factors in treatment 1; and (7) control or "stressed crop management" with Growers-2340, 75,000 plants per ha, drought stress, and no ear insect control. Data were as those for the spring experiments.

Artificial damage. Fifty ears (dough stage) from the outside rows in each plot of treatments 5, 7, and 9 during spring, and treatments 5 and 7 during fall, were artificially wounded with a nailboard, a modified pinboard device (2,11). The nailboard (18 cm long, 9 cm wide, and 4 cm deep) had 18 steel nails (7.5 cm long and 3 mm diameter) in three rows of six nails. Each ear was wounded three times with the nailboard in different locations, penetrating through the husk cover and wounding an average of nine kernels. After wounding all ears in a replicate plot, the nailboard was cleaned with 70% ethanol prior to wounding ears in the next plot.

Insect monitoring. Insect infestation of corn ears was monitored each year in a plot (10 rows by 40 m long) adjacent to the experiments. Each week from milk stage to physiological maturity, 50 plants were arbitrarily selected, and the ears were removed and transported to the laboratory to inspect for lepidopteran larvae. Observations were also made on abundance and diversity of microcoleopterans in 20 ears only at harvest in each growing season.

Aflatoxin analysis. After harvest, grain in each plot was mixed in 20-liter plastic containers. A 5-kg grain sample was dried at 75°C in a paper bag for 24 to 48 h in a forced-air oven until grain moisture was <15%. Then a 500-g subsample was finely ground in a Wiley mill (Model 4 with a 20-mesh screen) (Artnu H. Thomas, Philadelphia) and placed in a paper bag. After mixing again, a 50-g subsample was weighed and extracted for aflatoxin by using the Aflatest (Vicam, Watertown, MA) immunoaffinity column (3,31). Aflatoxin level (ppb) was measured in a Torbex fluorometer, Model FX-100 (Vicam).

Statistics. Differences in yield, insect damage, frequency of *A. flavus* infection, and aflatoxin concentration among treatments were determined with analysis of variance (ANOVA) (SAS ver. 6.03, SAS Institute, Cary, NC) followed by the Fisher protected least significant difference (LSD) tests ($P < 0.05$) for each growing season and the overall (3-year) analysis for spring and fall ($n = 3$). Before analysis, aflatoxin, insect damage, and *A. flavus* infection data were square root transformed in order to stabilize variances; however, untransformed data are presented. The relationship between all variables was tested by linear regression analysis of SAS.

RESULTS AND DISCUSSION

Spring experiments. Agronomic factors (individual and combined) significantly affected yield, insect damage, and aflatoxin contamination throughout the study. Visible differences in *A. flavus* infection were only detected in 1991, when the incidence was highest (Table 3). Overall, the INIFAP management system consistently obtained high yields (5.9 to 6.7 t/ha) and low aflatoxin contamination (0 to 6 ppb). In contrast, the control or stressed treatment resulted in the poorest yields (2.5 to 3.7 t/ha) and highest aflatoxin levels (63 to 167 ppb).

The two factors most often associated with yield loss, insect damage, frequency of *A. flavus* infection, and aflatoxin contamination were late planting and no insect control. Late planting exposed plants to

Table 1. Treatments for the spring experiments. INIFAP represents maximum management practices for irrigated corn. A susceptible cultivar, high plant density, drought stress, no insect control, and late planting were evaluated as components of the INIFAP system

Treatment	Hybrid	Plant density/ha	Drought stress	Ear insect control	Planting date
1 (INIFAP)	H-422	55,000	no	yes	Early
2	G-2340[z]	55,000	no	yes	Early
3	H-422	75,000[z]	no	yes	Early
4	H-422	55,000	yes[z]	yes	Early
5	H-422	55,000	no	no[z]	Early
6	H-422	55,000	no	yes	Late[z]
7	H-422	55,000	no	no[z]	Late[z]
8	H-422	75,000[z]	yes[z]	yes	Early
9 (control)	G-2340[z]	75,000[z]	yes[z]	no[z]	Late[z]

[z] Agronomic practices imposed as stressing factors.

Table 2. Treatments for the fall experiments. INIFAP represents maximum management practices for irrigated corn. A susceptible cultivar, high plant density, drought stress, and no insect control were evaluated as components of the INIFAP system

Treatment	Hybrid	Plant density/ha	Drought stress	Ear insect control
1 (INIFAP)	HV-1	55,000	no	yes
2	G-2340[z]	55,000	no	yes
3	HV-1	75,000[z]	no	yes
4	HV-1	55,000	yes[z]	yes
5	HV-1	55,000	no	no[z]
6	HV-1	75,000[z]	yes[z]	yes
7 (control)	G-2340[z]	75,000[z]	yes[z]	no[z]

[z] Agronomic practices imposed as stressing factors.

higher minimum (night) temperatures during the reproductive and maturation stages (Table 4), a condition commonly associated with a higher incidence of *A. flavus* and aflatoxin contamination, not only in corn but in other susceptible crops (18,21). Average incidence of ears affected by insects in each crop management treatment was positively correlated with aflatoxin concentration during the period of study (Fig. 1). Insect damage is recognized as a factor enhancing aflatoxin contamination in preharvest corn; insects act as vectors, facilitating spore entry into the cobs and increasing infection by damaging the kernel pericarp (8,21,32). As expected, a higher incidence of ears with insect damage was observed in those treatments without insecticide applications. However, insect damage also increased in some tests under high plant density, drought stress + high plant density, and late planting, all of which received insecticide applications (Table 3). The higher incidence of insect damage might be attributed to differential insect moth preference for oviposition sites or differential insecticide efficacy among treatments.

Treatments producing higher yields, a reflection of better crop management, were

Table 3. Yield, insect damage, *Aspergillus flavus* incidence, and aflatoxin concentration in corn kernels from different corn management systems for spring grown corn

Year Treatment	Yield (t/ha)	Ears damaged by insects (%)	Ears with *A. flavus* (%)	Aflatoxin (ppb)
1991				
1. INIFAP system	6.7 a^z	20 a	1 ab	2 a
2. Growers-2340	6.3 ab	20 a	0 a	2 a
3. 75,000 pl/ha	6.7 a	33 b	2 b	2 a
4. Drought stress	5.7 bc	37 b	2 b	19 ab
5. No insecticide	6.5 ab	64 c	5 c	7 ab
6. Late planting	5.5 cd	61 c	2 b	53 ab
7. No insecticide + late planting	4.7 d	86 d	10 c	63 b
8. Drought + 75,000 pl/ha	4.7 d	35 b	1 ab	19 ab
9. Control	2.5 e	78 cd	1 b	167 c
C.V. (%) =	8.0	12.8	55.1	82.3
1992				
1. INIFAP system	6.0 a	6 b	0 a	0 a
2. Growers-2340	6.7 a	0 a	0 a	0 a
3. 75,000 pl/ha	5.9 ab	12 bc	0 a	4 a
4. Drought stress	5.8 b	6 b	0 a	2 a
5. No insecticide	5.8 b	12 bc	0 a	6 a
6. Late planting	4.7 c	16 cd	1 a	66 ab
7. No insecticide + late planting	4.1 cd	37 e	3 a	241 c
8. Drought + 75,000 pl/ha	5.8 b	10 bc	0 a	3 a
9. Control	3.7 d	26 de	1 a	148 bc
C.V. (%) =	7.9	39.9	178.7	85.3
1993				
1. INIFAP system	5.9 b	18 b	0 a	6 a
2. Growers-2340	6.8 a	3 a	0 a	2 a
3. 75,000 pl/ha	5.6 b	46 cd	0 a	14 ab
4. Drought stress	4.6 c	43 cd	1 a	39 abc
5. No insecticide	4.9 bc	75 ef	1 a	126 c
6. Late planting	4.3 c	59 de	0 a	72 bc
7. No insecticide + late planting	4.1 cd	98 f	0 a	60 bc
8. Drought + 75,000 pl/ha	4.7 c	33 c	0 a	58 abc
9. Control	3.2 d	93 f	0 a	63 bc
C.V. (%) =	9.4	16.2	196.9	67.5
Average (1991-1993)				
1. INIFAP system	6.2 ab	15 ab	0.3 a	3 a
2. Growers-2340	6.6 a	8 a	0.0 a	1 a
3. 75,000 pl/ha	6.1 b	30 bc	0.7 a	7 ab
4. Drought stress	5.4 cd	29 bc	1.0 a	20 ab
5. No insecticide	5.7 bc	50 cde	2.0 ab	46 ab
6. Late planting	4.8 e	45 cd	1.0 a	64 bc
7. No insecticide + late planting	4.3 f	74 e	4.3 b	121 cd
8. Drought + 75,000 pl/ha	5.1 de	26 bc	0.3 a	26 ab
9. Control	3.1 g	66 de	0.7 a	126 d
C.V. (%) =	8.4	13.8	143.9	77.1

[z] Means (within a particular column and year) followed by the same letter are not significantly different (LSD, $P < 0.05$).

Table 4. Average minimum temperatures (C) during reproductive and maturation stages for different planting dates and growing seasons

	Early planting			Late planting		
Growing season	50% tasseling to dough stage	Dough stage to physiol. maturity	Ave.	50% tasseling to dough stage	Dough stage to physiol. maturity	Ave.
1991 Spring	22.8	24.0	23.4	23.3	24.2	23.8
1992 Spring	20.0	23.3	21.6	21.9	24.3	23.0
1993 Spring	20.6	24.1	21.9	22.4	24.0	23.2
1991 Fall	16.3	15.4	15.9	...	...	...
1992 Fall	18.3	13.3	15.7	...	...	...
1993 Fall	21.8	14.5	17.8	...	...	...

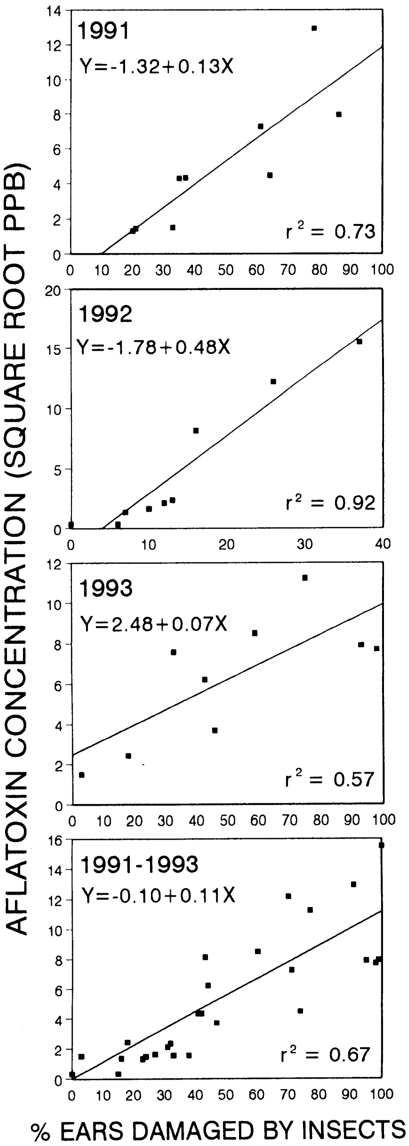

Fig. 1. Relationship between ear insect damage and aflatoxin contamination in preharvest corn during the spring growing season. X-axis represents a standardized scale based on the maximum percentage of ears damaged each year. Each point represents the average (four replicates) of each of nine management practices.

less likely to be contaminated with aflatoxin (Fig. 2). Evidently the stressed plants were more susceptible to aflatoxin contamination (10,17,34). Smith and Riley (29) found the highest aflatoxin levels in field corn exposed to a combination of stressing factors during the growing season. Jones et al. (10) concluded that stress conditions that reduce yield may play a role in predisposing corn to increased aflatoxin contamination.

Apparently cultivar selection and high plant density did not significantly influence aflatoxin contamination when used in combination with the other variables of the INIFAP system (Table 1). Growers-2340 was selected for this study based on its historic susceptibility to ear insects and rots in this region, a condition not expressed in this study. Drought stress was associated with moderate aflatoxin contamination (19 to 39 ppb) in 1991 and 1993, when precipitation was limited. In 1992, frequent rainfall did not allow the drought treatment to be imposed.

High variability in percentage of ears infected with *A. flavus* was observed in all years, which probably caused this parameter to be a poor indicator of aflatoxin contamination. For instance, in 1991, treatment 9 (control) had 1% ears with *A. flavus* and 167 ppb aflatoxin, whereas treatment 7 (no insecticide + late planting) had 10% ears with *A. flavus* and only 63 ppb aflatoxin (Table 3). Because kernels can be infected and yet show no visible *A. flavus* sporulation, aflatoxin contamination may not be associated with visible *A. flavus* (21).

Fall experiments. Differences in yield and insect damage were also observed among crop management treatments during the fall growing seasons (Table 5). Again, the higher and lower yields were obtained by INIFAP system and control, respectively. However, *A. flavus* infection and aflatoxin concentration were undetectable in all treatments during 1991 and 1992, when average minimum temperatures were <16°C during reproduction and maturation (November to December), a condition unfavorable for *A. flavus* infection (20). In 1993, aflatoxin contamination was higher in the control and in the drought + high plant density treatments (Table 5). During this year, planting was almost 3 weeks earlier than in the previous years, and temperatures were higher between tasseling and dough stage (Table 4). This suggests that earlier planting dates during the fall season would be equivalent to the late planting in the spring season, with higher temperatures during critical corn phenological stages. When planted early (late July to early August) in the fall season, corn reproductive stages would coincide with the higher temperatures of October rather than the cooler temperatures of November in the later planting. In addition, the greater aflatoxin contamination observed in 1993 might have been enhanced by severe bird damage during maturation, when about 70% of the ear tips experienced grain loss ranging from 5 to 10%.

Abundance and diversity of ear insects. Five lepidopteran species were collected from the ears in the plots adjacent to the experiments: the noctuids *Helicoverpa zea* (Boddie) and *Spodoptera frugiperda* (J.E. Smith), and the stalkboring pyralids *Diatraea lineolata* (Walker), *D. saccharalis* (Fabricius), and *Eoreuma loftini* (Dyar). However, *H. zea* comprised nearly 90% of all specimens collected from milk to dough stages, when ear-infesting *Lepidoptera* larvae were most abundant. The remaining species were only occasionally collected, regardless of year and growing season. Samples at later stages of ear growth (hard dough to physiological maturity), when *H. zea* densities were sharply decreasing, had slightly more *D. lineolata* and *D. saccharalis* larvae in the ears, a likely indication that individuals were moving from ear shots (secondary ears) and the stalk, the preferred feeding sites (26). The impact of such late ear damage by these stalkborers on aflatoxin production is unknown. However, the higher densities and earlier damage by *H. zea* suggest this species is more important in *A. flavus* contamination of preharvest corn in this region than are the remaining species.

The association of damage by *H. zea* larvae with *A. flavus* and aflatoxin contamination in preharvest corn has been well demonstrated elsewhere (7,8,13,17,32,33). In addition, *H. zea* moths transport *A. flavus* spores (16). In Louisiana, Smith and Riley (29) reported that ear insects other than *H. zea* were insignificant to *A. flavus* and aflatoxin contamination, and that *H. zea* damage and drought stress had a synergistic effect in enhancing aflatoxin contamination.

Microcoleopterans were commonly observed in the ears, particularly in those injured by lepidopteran larvae, similar to the report by Lussenhop and Wicklow (14). More than 85% of the specimens collected at harvest were sap beetles, *Carpophilus* spp. (*Nitidulidae*), possibly comprising a complex of up to five species. The remaining beetles included some *Curculionidae, Mycetophagidae, Anthribidae, Bostrichidae,* and *Cucujidae* species. Beetles, including nitidulids and curculionids, have been associated with aflatoxin contamination in preharvest corn (6,14,15).

Artificial damage. Although physical damage inflicted by the nailboard did not appear severe (an average of nine kernels were wounded), artificial damage significantly enhanced aflatoxin contamination, particularly during the spring seasons (Table 6). A high incidence of *A. flavus* and other ear rots, including unidentified species of *Fusarium*, *Penicillium*, and *Rhizopus,* was observed in the artificially wounded ears. This further demonstrates the importance of maintaining ears free of insect damage to minimize the propensity for aflatoxin contamination in preharvest corn. Artificially wounding the ear was reported to be a valuable tool for screening germ plasm by minimizing escapes and reducing the high variability commonly observed in naturally occurring *A. flavus* infections (11,28,32). Aflatoxin production was significantly higher in late-planted spring treatments (7 and 9) compared to the early planting (treatment 5), suggesting an interaction between damage and high temperatures, similar to the findings by Smith and Riley (29).

During the fall, artificial damage did not

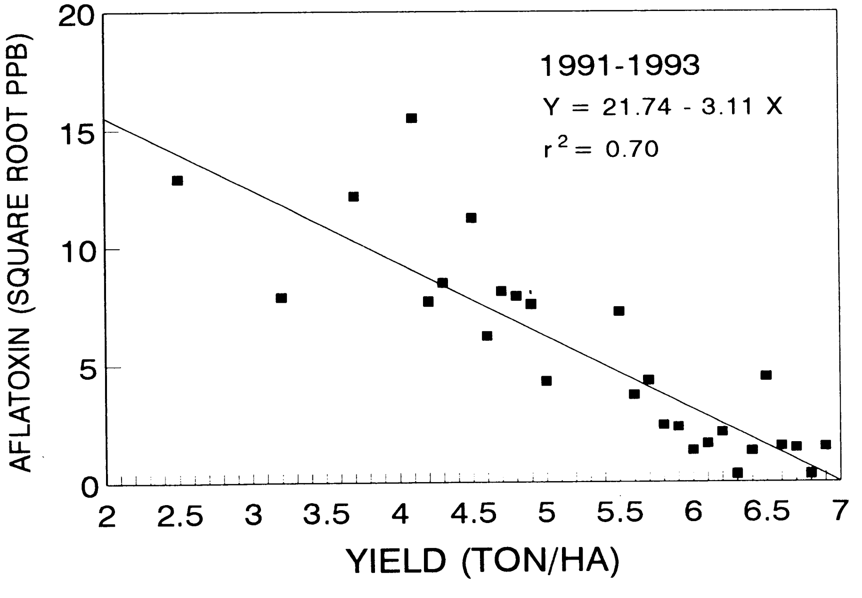

Fig. 2. Relationship between grain yield and aflatoxin contamination in preharvest corn during the spring growing season. Each point represents the average (four replicates) of each of nine management practices. 1991-1993 represents combined data.

increase aflatoxin contamination in 1991 and 1992, when temperature was not conducive for *A. flavus* infection. Although aflatoxin was significantly greater in the nailboard-wounded plants in the fall of 1993, aflatoxin concentrations were not as high as those observed during the spring seasons (Table 6). Overall, the nailboard-wounded ears had aflatoxin concentrations greater by seven- and fivefold during the spring (1991 to 1993) and fall (1993) seasons, respectively, in comparison to those ears without artificial damage.

In summary, these data demonstrated a close relationship between agronomic practices, particularly planting date and damage to ears on aflatoxin contamination in preharvest corn in northeastern Mexico. The INIFAP system, implemented as a mandatory corn management system in this region since 1991, has produced consistent high yields and low risk of aflatoxin. Recent analysis on the economic impact of the INIFAP production system demonstrated high benefits in this area; each dollar invested in research on aflatoxin has yielded a profit of $583 (25).

Table 5. Yield, insect damage, *Aspergillus flavus* incidence, and aflatoxin concentration in corn kernels from different corn management systems for fall grown corn

Treatment	Yield (t/ha)	% ears damaged by insects	% ears with *A. flavus*	Aflatoxin (ppb)
1991				
1. INIFAP system	4.6 a	29 ab	0	1 a
2. Growers-2340	3.6 c	21 a	0	1 a
3. 75,000 pl/ha	4.2 ab	36 b	0	1 a
4. Drought stress	4.4 ab	30 ab	0	1 a
5. No insecticide	4.1 b	52 c	0	4 a
6. Drought + 75,000 pl/ha	4.3 ab	34 b	0	2 a
7. Control	3.5 c	27 ab	0	1 a
C.V. (%) =	7.8	23.8	...	89.5
1992				
1. INIFAP system	4.8 a	32 b	0 a	0 a
2. Growers-2340	4.7 a	11 a	0 a	1 a
3. 75,000 pl/ha	4.3 c	30 b	0 a	1 a
4. Drought stress	4.7 a	30 b	0 a	0 a
5. No insecticide	4.4 bc	56 c	0 a	0 a
6. Drought + 75,000 pl/ha	4.7 ab	32 b	0 a	0 a
7. Control	4.2 c	29 b	1 a	0 a
C.V. (%) =	5.5	16.9	529.2	84.8
1993				
1. INIFAP system	4.3 a	30 ab	0 a	9 a
2. Growers-2340	4.5 a	18 a	0 a	18 a
3. 75,000 pl/ha	4.4 a	31 b	0 a	11 a
4. Drought stress	1.4 b	32 b	0 a	16 a
5. No insecticide	4.3 ac	62 c	0 a	6 a
6. Drought + 75,000 pl/ha	1.2 bc	35 b	3 b	103 b
7. Control	0.7 c	32 b	3 b	100 b
C.V. (%) =	12.7	28.3	204.7	20.6
Average (1991-1993)				
1. INIFAP system	4.6 a	30 b	0 a	3 a
2. Growers-2340	4.3 b	17 a	0 a	6 a
3. 75,000 pl/ha	4.3 b	32 b	0 a	4 a
4. Drought stress	3.5 c	31 b	0 a	6 a
5. No insecticide	4.3 b	57 c	0 a	3 a
6. Drought + 75,000 pl/ha	3.4 c	34 b	1 a	35 b
7. Control	2.8 d	29 b	1 a	34 b
C.V. (%) =	8.3	9.6	240.5	34.9

[z] Means (within a particular column and year) followed by the same letter are not significantly different (LSD, $P < 0.05$).

Table 6. Impact of ear artificial damage by nailboard on aflatoxin contamination of preharvested corn

	Aflatoxin concentration (ppb)				
Treatment	1991	1992	1993	Ave. (1991–1993)	Increase[y] (%)
Spring					
5. No insecticide	104 a[z]	370 a	350 a	275 a	598
7. No insecticide + late planting	904 b	1,060 b	1,100 b	1,021 b	844
9. Control	744 b	900 b	495 a	713 b	566
C.V. (%) =	14.1	24.5	19.6	13.6	
Fall					
5. No insecticide	13 a	0	213 a	75 a	250
7. Control	1 a	0	351 a	118 a	347
C.V. (%) =	75.3	...	34.6	37.8	

[y] In relation to average aflatoxin levels in nonwounded ears presented in Tables 3 and 5 for spring and fall, respectively.

[z] Aflatoxin levels (within a particular year and season) followed by the same letter are not significantly different (LSD, $P < 0.05$). Aflatoxin data are the means of four replicates obtained from ears damaged in the outside rows (1 and 6) of experimental plots (see materials and methods).

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