Aflatoxin synthesis in corn fields in Guanajuato, Mexico

Carlos M. Bucio-Villalobos[1], Doralinda Guzmán-de-Peña[2] and Juan Jose Peña-Cabriales[2]

[1]Instituto de Ciencias Agrícolas, Universidad de Guanajuato, Irapuato, Gto., México and [2]Unidad Irapuato - CINVESTAV, Irapuato, Gto., México

Summary

Aflatoxin contamination of corn is an important problem internationally, particularly in tropical and subtropical conditions that favor infection and synthesis by *Aspergillus*. Environmental conditions (drought) and agronomic practices i.e. N fertilization have been reported as favorable to aflatoxin synthesis in the field. This study was undertaken to investigate whether the contamination of corn commonly observed in stored conditions in this important corn producing region of Mexico known as "El Bajío" is related to infection by *Aspergillus* under field conditions. Results using three corn hybrids of recognized susceptibility to infection showed that corn ears artificially inoculated in the field with a toxigenic strain of *Aspergillus parasiticus* presented a low content of aflatoxin ranging from 13.6 to 24.7 µg Kg^{-1}. No significant differences were observed between the hybrids tested. Similarly, N fertilization practices, 260 Kg N ha^{-1}, applied at sowing did not have an effect on the extent of the contamination observed of 6.2 and 19.3 mg of aflatoxin kg^{-1} in natural infected and inoculated samples with *A. parasiticus* NRRL 2999, respectively. Our data suggest that the cases of aflatoxin contamination of corn in this part of Mexico are not related to infection occurring during the crops growing period but most probably to poor storage conditions of corn.

Key words

Aspergillus parasiticus, Aflatoxin in corn, Mycotoxins, Field contamination, Preharvest contamination

Síntesis de aflatoxinas en campos de maíz en Guanajuato, México

Resumen

La contaminación por aflatoxinas en maíz es un problema internacionalmente importante, especialmente bajo condiciones tropicales y subtropicales donde la infección y síntesis de *Aspergillus* se ven favorecidas. Las condiciones del medio ambiente (sequía) y prácticas agronómicas, por ejemplo la fertilización nitrogenada, han sido reportadas como favorables a la síntesis de aflatoxinas en campo. Este estudio fue realizado para investigar si la contaminación del maíz que comúnmente es observada en condiciones de almacenamiento en esta importante región productora de maíz en México, conocida como "El Bajío", está relacionada con la infección de *Aspergillus* bajo condiciones de campo. Los resultados, usando tres híbridos susceptibles, mostraron que las mazorcas de maíz inoculadas artificialmente en el campo con una cepa toxigénica de *Aspergillus parasiticus* presentaron bajo contenido de aflatoxinas, el cual estuvo dentro de un rango de 13,6 a 24,7 µg Kg^{-1}. No se observaron diferencias significativas entre los híbridos evaluados. Similarmente, la práctica de fertilización nitrogenada, 260 Kg N ha^{-1}, aplicada al momento de la siembra, no tuvo efecto sobre la contaminación observada de 6,2 y 19,3 µg de aflatoxina Kg^{-1} en muestras naturales y contaminadas con *A. parasiticus* NRRl 2999, respectivamente. Nuestros datos sugieren que los casos de contaminación por aflatoxinas en maíz en esta parte de México no estan relacionados con la infección ocurrida durante el desarrollo del cultivo, pero mas probablemente son debidos a las deficientes condiciones de almacenaje del maíz cosechado.

Palabras clave

Aspergillus parasiticus, Aflatoxinas en maíz, Micotoxinas, Contaminación en campo, Contaminación en precosecha

Dirección para correspondencia:
Dra. D. Guzmán-de-Peña
Unidad Irapuato-CINVESTAV
Km 9.6 carretera Irapuato-León,
Irapuato, Gto., México
Tel.: +52 462 39600 / 39648
Fax: +52 462 45846
E-mail: dguzman@ira.cinvestav.mx

Aceptado para publicación el 3 de Mayo de 2001

Aflatoxin contamination of preharvest corn (*Zea mays* L.) has been reported in several countries [1]. In the United States [2], it is a chronic problem in the southern states, and it appears sporadically elsewhere [3]. It may be widespread in developing countries of the tropics and subtropics in which temperature conditions are likely to favor infection of corn by *Aspergillus* spp. [1].

It is well known that environmental conditions strongly influence dispersion of fungal spores [4], penetration and establishment of hyphae in plants and on the production of aflatoxins; also, cultural and agronomic conditions influence the synthesis of aflatoxin in corn [5].

High temperatures and drought conditions are conducive to heavy aflatoxin contamination [6,7], as is insect damage [8]. The interrelationship between soil type and level of aflatoxin contamination in corn requires further research; certainly the soil is important as an inoculum source [9], and altering edaphic factors by fertilization, irrigation or cultivation may affect spore numbers in soil [1]. However, Payne *et al.* [10] demonstrated that deep-ploughing in North Carolina reduced aflatoxin contamination.

There have been several reports that high temperatures and humidity favor fungal growth and aflatoxin synthesis in stored corn [4,5,11-16]. However, since the 1970s it has been accepted that corn kernels become infected with fungi and contaminated with aflatoxin while in the field [4]. In Tamaulipas State, northeast Mexico, early sowing and proper irrigation decreased aflatoxin contamination from 246 to 6 mg kg^{-1} [17]. On the other hand, corn stored in Tamaulipas, 1985 to 1988, revealed only a 2% incidence of *Aspergillus flavus* with unknown toxigenic activity and low levels of aflatoxin B1 [18].

Mexico has one of the highest rates of human consumption of corn in the world (120 kg/year/per capita) [19]. It also represents a mosaic of environmental conditions in which corn is produced and/or stored for various periods of time. Yet information on aflatoxin contamination of corn in the main producing regions is scarce.

In central Mexico, environmental conditions, particularly drought seem to be favorable to aflatoxin synthesis in the field. Furthermore mycotoxicosis in pigs associated to ingestion of contaminated feed are frequently reported for this region. Therefore the present study was undertaken to investigate if the contamination of corn commonly observed in stored conditions in this part of Mexico is related to infection by *Aspergillus* under field conditions.

MATERIALS AND METHODS

Field experiment. The experiments were conducted in the field of CINVESTAV-Irapuato (20° 44' N, 101° 19' W) in Guanajuato, Mexico. The soil is classified as a Pelic Vertisol with pH of 7.2 (1:2 water), organic matter 1.83 %, and a clay texture.

The experiment was a 3 x 2 x 2 factorial with a randomized block design with four replications. The first factor was corn hybrids, the second was *Aspergillus parasiticus* inoculum treatment and the third was nitrogen (N) fertilization, generating twelve treatments. This experiment, sown on May 30 1996, was irrigated the next day. Ploughing, tilling, and leveling were done with machinery methods.

Fertilization. 260 Kg ha^{-1} as urea was applied in the soil in two furrow application: 50 % at sowing and the rest 42 days later. Phosphate also was applied at sowing as calcium superphosphate 80 Kg ha^{-1} in a single furrow application.

Sowing. Two corn seeds were placed over the fertilizer applied in the soil, every 20 cm and then were covered with soil. The row spacing was 0.77 m.

Eight days after emergence, Diazinon 25 E (25% Diazinon, Ciba, México) was applied to control thrips (*Frankliniella occidentalis*) and at 35 days after sowing Cymbush 200 (20% Cipermetrin, Zeneca, México) was applied to control fall army worm (*Spodoptera fugiperda).* Weeds were controlled by hand during 60 days after planting. No measurements of insect damage were made.

The corn hybrids were H-220; H-433 and A-791 (early, mid and late season maturity).

Inoculation. Aspergillus parasiticus strain NRRL 2999 (from the Northeast Regional Researh Laboratory), this species naturally does not infect corn fields in Guanajuato, but was chosen for its stability in aflatoxin production as reported elsewere [20]. The strain was maintained in potato dextrose agar slant tubes. Spores were harvested from 5-day-old cultures: sterile water with 0.01 % triton was added to plates to obtained suspensions of $1.6x10^6$ spores ml^{-1}. Inoculation was achieved by applying 5 ml of suspension with a syringe to each ear through the pollination channel at 15 days after 50% silking, according to each hybrids (72, 78, and 81 days for H-220, H-433, and A-791, respectively). Each treatment was composed of two hundred plants with one ear per plant inoculated. Control plants were similarly inoculated with 5 ml of sterile water.

Harvest. When the ears were mature according to each hybrid: 104, 110, and 113 days for H-220, H-433, and A-791, respectively, plants were harvest.

Fungal population: infestation and identification. Three hundred grams kernel samples from 48 blocks were analyzed for fungal population. Four replicates of one hundred kernel subsamples were placed in 1.5 % sodium hypochlorite for 3 min, rinsed with sterile water three times and placed on a malt salt agar medium and incubated at 30˚C for seven days. The number of forming fungal colonies were counted macroscopic in each grain to determined percentage of incidence. The fungal colonies were isolated on potato dextrose agar and microscopically identification was performed according to Domsch *et al.* [21].

Aflatoxin determination. Samples from each of 48 blocks were harvested and because 12 blocks were loosed in the process, only 36 blocks were analyzed for aflatoxin. Each treatment was harvested separately and kernels weighed to obtain the following samples: 20 samples of 5 Kg; four of 4 Kg; 11 of 2.5 Kg; and one of 2 Kg. Each entire sample was mixed and ground to pass through an 0.8 mm sieve and five subsamples of 60 grams were taken by quartering. From each of these subsamples, 10 grams were taken to obtain a 50 grams composite sample. Thirty six composite samples were analyzed for aflatoxin content; they were extracted according to the modification of the method 1 AOAC as published elsewhere [22]. Quantitative determinations of aflatoxins in the extracts, were made by high pressure liquid chromatography using a Zorbax LC18 column (Dupont, USA). The mobile phase was a mixture of water:acetonitrile:methanol (45 : 15 : 40 by volume). Elution of aflatoxins was recorded at 364 nm. Standard solutions of aflatoxins B1, B2 , G1 and G2 were run under the same conditions as described by Guzmán-de-Peña and Ruíz-Herrera [23].

Other variables measured. Numbers, fresh and dry weights of ears were determined for all treatments. Plant dry weight, grain, and moisture content were also determined.

Environmental data. During the experiment the maximum temperature recorded was 25.2 °C and minimum 12 °C, total rainfall was 837.7 mm and relative humidity of 81.2 % maximum and 25.5 % minimum.

Statistical analysis. Analysis of variance and Tukey`s test were applied to data using SAS (version 6.12; SAS Institute, Cary, NC, USA).

RESULTS

Fungal populations. Various *Fusarium* species predominated in the kernels, with *A. parasiticus* and *Penicillium* spp. present to a lesser extent (Table 1). In fact, the values for *A. parasiticus* were low and there was no statistically significant effect of corn genotype (Table 1). It is important to mention that *A. flavus* and *A. parasiticus* were not found in the soil (data not showed). However *A. parasiticus* was isolated from nonioculated corn.

As a consequence of inoculation, the populations of *A. parasiticus* increased significantly whereas the others were not affected (Table 2). It is important to note that the identified populations corresponded to internal infections, because the kernels were washed with sodium hypochlorite before being placed onto the culture medium.

The application of fertilizer N had no effect on the fungal populations as it is illustrated in Table 3.

Table 1. Fungal populations of kernels of diferent corn genotypes.

Hybrid	Kernels infected with			
	A. parasiticus	*Fusarium* spp. (%)	*Penicillium* spp.	Misc.
H-220	3.3a	34a	1.1a	0.8a
A-791	1.8a	32a	0.7a	0.9a
H-433	3.5a	35a	1.0a	1.2a

Mean of four replicates of each treatment of 100 kernels; values with the same letter in the same column are not significantly different (Tukey at P=0.05).

Table 2. Fungal populations of kernels naturally infected with fungus and inoculated with *A. parasiticus*.

Condition	Kernels infected with			
	A. parasiticus	*Fusarium* spp. (%)	*Penicillium* spp.	Misc.
Naturally infected	0.4a	37a	1.0a	1.1a
Inoculated	9.0b	32a	0.9a	0.9a

Means of four replicates of each treatment of 100 corn grains; values with the same letter in the same column are not significantly different (Tukey at P=0.05).

Table 3. Fungal populations of kernels of corn grown with and without N fertilizer.

N treatment	Kernels infected with			
	A. parasiticus	*Fusarium* spp. (%)	*Penicillium* spp.	Misc.
260 kg N ha-1	2.3a	35a	1.2a	0.9a
Without N	3.4a	32a	0.7a	1.1a

Means of four replications of each treatment of 100 kernels; values with the same letter in the same column are not significantly different (Tukey at P=0.05).

Variables related to yield. The highest yield was obtained with hybrid A-791 and the other hybrids showed lower similar grain production (Table 4). Inoculation with *A. parasiticus* did not affect yield (Table 5). As usually occurs, the application of fertilizer N had a positive effect on yield (Table 6).

Table 4. Variables related to yields of three corn hybrids.

Hybrid	Grain yield (t ha-1)	Ears (ha-1)	Ear wt. (t ha-1)	Stover DM (t ha-1)
H-220	6.5b	54.935b	7.8b	2.1b
A-791	9.1a	61.558a	11.6a	2.9a
H-433	5.9b	49.481b	7.4b	2.2b
Mean Value	7.1	55.325	8.9	2.4

Mean of four replicates of each treatment; values with the same letter in the same column are not significantly different (Tukey at P=0.05).

Table 5. Yield variables of corn naturally infected with fungus and inoculated with *A. parasiticus*.

Condition	Grain yield (t ha-1)	Ears (ha-1)	Ears wt. (t ha-1)	Stover DM (t ha-1)
Naturally infected	7.1a	55.325a	8.9a	2.4a
Inoculated	7.2a	56.364a	9.0a	2.3a

Means of four replicates of each treatment; values with the same letter in the same column are not significantly different (Tukey at P=0.05).

Table 6. Yield of corn with and without N fertilizer.

Treatment	Grain yield (t ha^{-1})	Ears (ha^{-1})	Wt. of ears (t ha^{-1})	Stover DM (t ha^{-1})
260 kg N ha^{-1}	8.3a	56.623a	10.4a	2.9a
Without N	6.0b	54.026a	7.4b	1.9b

Means of four replicates of each treatment; values with the same letter in the same column are not significantly different (Tukey at P=0.05).

Aflatoxin contamination. The procedures used in this study allow us to detect aflatoxins B1, B2, G1 and G2 however only B1 was observed, and the levels of aflatoxin detected in the three corn genotypes were low (Table 7). With N-fertilizer applied, under natural infection, aflatoxin levels were below 8.8 µg Kg^{-1}, whereas inoculation of ears with spores of *A. parasiticus* resulted in 13.6 to 24.7 µg Kg^{-1} (Table 7). A similar trend prevailed with the unfertilized corn, but with generally lower levels of aflatoxin (Table 8).

Table 7. Aflatoxin levels in the kernels of three corn hybrids with N applied, naturally infected with field isolates fungus or inoculated with *A. parasiticus.*

Hybrid	Aflatoxins content (µg kg^{-1})	
	Naturally infected	Inoculated
H-220	5.7a	19.8a
A-791	8.8a	24.7a
H-433	4.1a	13.6a
Mean Value	6.2	19.3

Means of three replicates of each treatment; values with the same letter in the same column are not significantly different (Tukey at P=0.05).

Table 8. Aflatoxin levels in the kernels of three corn hybrids, without fertilizer N, naturally infected with field isolates fungus or inoculated with *A. parasiticus*.

Hybrid	Aflatoxins content (µg kg^{-1})	
	Naturally infected	Inoculated
H-220	3.1a	13.2a
A-791	10.2a	14.6a
H-433	0.6a	11.5[a]
Mean Value	4.6	13.1

Means of three replicates of each treatment; values with the same letter in the same column are not significantly different (Tukey at P=0.05).

DISCUSSION

Fungal populations. The natural fungal populations in the kernels were mainly species of *Fusarium* and *Penicillium*, with very low incidence of *Aspergillus parasiticus*; *Fusarium* spp. predominated. There were no significant differences among the hybrids. Comparison of the fungal populations of naturally infected kernels with kernels from inoculated ears showed a significant difference in the *A. parasiticus* population, which indicated low natural incidence of this species and confirmed that inoculation was effective. It is noteworthy that our values were low in comparison with those reported before, e.g. a 5.2 % natural infection rate for *A. flavus* [24] and 83% recorded by Widstrom *et al.* [25]. Our data show only 3.5 % of grain infected with *A. parasiticus*; it can be suggested that this fungus is poorly infective in the field under the conditions of maximum temperature of 25.2 °C, minimum of 12 °C, rainfall of 837.7 mm and relative humidity of 81.2 % maximum and 25.5 % minimum prevalent during the test period in this region.

The fungal populations of kernels from fertilized corn were not significantly different from those of plants not treated with N. These data differ from those of other reports in which low levels of N fertilizer increased the incidence of *A. flavus* [13, 26-28]. *Aspergillus flavus* was not isolated from any of the uninoculated samples indicating absence from the corn fields of Guanajuato.

Variables related to yield. Grain yield, number of ears, weight of ears and stover dry matter of hybrid A-791 were higher than those of H-220 and H-433. These results were expected, in view of the fact that A-791 was developed to perform well in optimal conditions, which were met in these experiments. The yield-related variables were not affected by inoculation, which is consistent with the fact that there was no visible damage to the ears or plants as a whole. Differences in yield were observed when nitrogen was applied.

Aflatoxin contamination. The amounts of aflatoxin B1 contamination were low. With natural infection and N applied, levels below 8.8 µg kg^{-1} were recorded. Although, inoculation with *A. parasiticus* increased the values, they never reached values higher than 24.7 µg Kg^{-1}. No significant differences were found among the hybrids. Similar trends were observed in the hybrids without N levels, in natural infected and inoculated kernels, in which case the levels of aflatoxins were 10.2 mg Kg^{-1} and 14.6 mg Kg^{-1}, respectively. Again, no significant differences were found among hybrids. It is noteworthy that hybrid H-433, which is considered as susceptible material for aflatoxin synthesis in the northeast of the country, by contrast, showed the lowest level of aflatoxins in this experiment at Guanajuato.

No significant effect of N fertilization was observed on aflatoxin synthesis in the field. Furthermore, the levels were below 8.8 µg Kg^{-1} and below 24.7 mg Kg^{-1} in natural infected and inoculated samples, respectively. These results contrast with those of Wilson *et al.* [28] in Georgia, who found that high amounts of applied N increased aflatoxin levels in corn, and with those of Payne *et al.* [27] in North Carolina, who found that low amounts of fertilizer similarly increased aflatoxin.

The low levels of aflatoxin found in this experiment indicate that environmental conditions were more important in aflatoxin synthesis than toxigenic fungal strain or corn genotype. Payne [5] has pointed out that the most favourable temperature for infection of ears through the pollenation channel is in the range of 28 to 38 °C. The temperatures during this experiment were in average 18.6 °C. By contrast, in the northeast (Tamaulipas), temperatures of 28 to 30 °C or higher are common during the growing period [18] and aflatoxin levels of 250 µg Kg^{-1} have been recorded. Another potentially important factor is relative humidity, which in "El Bajío" was low, around 56 %, when the ears were inoculated. In Guanajuato State the relative humidity is low all year, even during the rainy season. Sauer [15] reported that a relative humidity of at least 85 % is necessary for effective infection of corn.

The results indicate that infection of corn in the field by *A. flavus* and *A. parasiticus* is not common in this region of Mexico due to the unfavorable climatic conditions i.e. low temperature and relative humidity, even if the number of propagules of the toxigenic strain is increased by artificial inoculation. Thus, the outbreaks of aflatoxin contaminated corn occasionally observed in this part of Mexico [29] seem to be related to poor storage conditions and not to field contamination.

We thank Yolanda Rodríguez for technical support with the HPLC equipment during the aflatoxin determination and to Dr. Allan Eaglesham for helpful suggestions, and comments on the manuscript.

References

1. Widstrom NW. The aflatoxin problem with corn grain. Advances Agron 1996; 56: 216-280.
2. Widstrom NW, McMillian WW, Wilson DM. Contamination of preharvest corn by aflatoxin. In: Proceeding of the 39th Annual Corn and Sorghum Research Conference, Chicago, 1984.
3. Wilson DM, Payne GA. Factors affecting *Aspergillus flavus* group infection and aflatoxin contamination of crops. In: Eaton DL, Groopman JD (Eds.) The Toxicology of Aflatoxin. New York, Academic Press, 1994: 309-325.
4. Widstrom NW. Aflatoxin developing maize: interactions among involved biota and pertinent econiche factors. In: Bhatnagar D, Lillehoj EB, Arora DK (Eds.) Handbook of Applied Mycology Vol 5: Mycotoxins in Ecological Systems. Marcel Dekker, New York, 1992: 23-58.
5. Payne GA. *Aspergillus flavus* infection of maize: silks and kernels. In: Zuber MS, Lillehoj EB, Renfro BL (Eds.) Aflatoxin in Maize: Proceedings of the Workshop. CIMMYT, Mexico, 1987: 119-129.
6. Davis ND, Clifford GC, Diener UL. Response of corn hybrids to aflatoxin formation by *Aspergillus flavus*. In: Bulletin 575. Alabama Agricultural Experiment Station, 1985: 1-23.
7. Widstrom NW, McMillan WW, Beaver RW, Wilson DM. Weather associated changes in aflatoxin contamination of preharvest maize. J Prod Agric 1990; 3: 196-199.
8. Fortnum BA. Effect of environment on aflatoxin development in preharvest maize. In: Zuber MS, Lillehoj EB, Renfro BL (Eds.) Aflatoxin in Maize: Proceedings of the Workshop. Mexico, CIMMYT, 1987: 145-151.
9. Lillehoj EB, McMillan WW, Guthrie WD, Barry D. Aflatoxin producing fungi in preharvest corn: Inoculum source in insects and soil. J Environ Quality 1980; 9: 691-694.
10. Payne GA, Cassel DK, Adkins CR. Reduction of aflatoxin contamination in corn by irrigation and tillage. Phytopathology 1986; 76: 679-685.
11. Jones RK, Duncan HE, Payne GA, Leonard KJ. Factors influencing infection by *Aspergillus flavus* in silk-inoculated corn. Plant Dis 1980; 64: 859-863.
12. Diener UL, Davis ND. Biology of *Aspergillus flavus* and *A. parasiticus*. In: Zuber MS, Lillehoj EB, Renfro BL (Eds.) Aflatoxin in Maize: Proceedings of the Workshop. Mexico, CIMMYT, 1987: 33-40.
13. Lillehoj EB. Aflatoxin in maize problem: the historical perpective. In: Zuber MS, Lillehoj EB, Renfro BL (Eds.) Aflatoxin in Maize: Proceedings of the Workshop. Mexico, CIMMYT, 1987: 13-32.
14. Bradburn N, Bluden G, Coker RD, Jewers K. Aflatoxin contamination of maize. Trop Sci 1993; 33: 418-428.
15. Sauer DB. Conditions that affect growth of *Aspergillus flavus* and production of aflatoxin in stored maize. In: Zuber MS, Lillehoj EB, Renfro BL (Eds.) Aflatoxin in Maize: Proceedings of the Workshop. Mexico, CIMMYT, 1987: 41-50.
16. Surekha M, Reddy SM. Influence of temperature and humidity on biodeterioration and aflatoxin production in groundnut fodder by *Aspergillus flavus*. J Toxicol 1989; 8: 291-297.
17. Rodríguez-del-Bosque LA, Reyes-Méndez CA, Acosta-Nuñes S, Girón CJR, Garza-Cano I, García-Villalnueva R. Control de aflatoxinas en maiz en Tamaulipas. In: Folleto técnico Nº. 17. Instituto Nacional de Investigaciones Forestales y Agropecuarias. Mexico, 1995.
18. Guzmán-de-Peña D. Las aflatoxinas en maíz: un reto a los mexicanos. In: Memorias de la IV Mesa Redonda Latinoamericana sobre Prevención de Pérdidas Postcosecha de Granos. CONASUPO, Mexico, 1989: 281-288.
19. Figueroa JD. La tortilla vitaminada. Avance y Perspectiva 1999; 18:149-158.
20. Guzmán-de-Peña D. El estudio de las aflatoxinas en México. In: Ruíz-Herrera J, Guzmán-de-Peña D, Peña-Cabriales JJ (Eds.) Perspectivas de la Microbiología en México. Instituto Politécnico Nacional, México, 1997: 181-199.
21. Domsch KH, Gams W, Anderson HT. Compendium of soil fungi Vol. I. New York, Academic Press, 1980.
22. Guzmán-de-Peña D, Anguiano GL, Medina JJ. Modification of the Method 1 AOAC (CB Method) for the detection of aflatoxins. Bull Environ Contam Toxicol 1992; 49: 485-489.
23. Guzmán-de-Peña D, Ruíz-Herrera J. Relationship between aflatoxin biosynthesis and sporulation in *Aspergillus parasiticus*. Fungal Gen Biol 1997; 21: 198-205.
24. Peña-del-Rio MA. Aspectos epifitiológicos de *Aspergillus flavus* Link ex-Fries y detección de su aflatoxina en maiz en el norte de Nuevo Leon. Doctoral thesis. México, Instituto Tecnológico y de Estudios Superiores de Monterrey, 1996.
25. Widstrom NW, Wilson DM, McMillan WW. Aflatoxin contamination of preharvest corn as influenced by timing and method of inoculation. Appl Environ Microbiol 1981; 42: 249-251.
26. Jones RK, Duncan HE. Effect of nitrogen fertilizer, planting date, and harvest date on aflatoxin production in corn inoculated with *Aspergillus flavus*. Plant Dis 1981; 65: 741-744.
27. Payne GA, Kamprath EJ, Adkins CR. Increased aflatoxin contamination in nitrogen-stressed corn. Plant Dis 1989; 73: 422-424.
28. Wilson DM, Walker ME, Gascho GJ. Some effects of mineral nutrition on aflatoxin contamination of corn and peanuts. In: Engelhard AW (Ed.) Soil-borne Plant Pathogens: Management of Diseases with Macro and Microelements. APS Press, St. Paul Minnesota, 1989: 137-151.
29. Guzmán-de-Peña D. Micotoxinas en el bajío guanajuatense. Avance y Perspectiva 1989; 40: 15-20.