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ccastro@uady.mx
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Alvarado-Carrillo, M.; Díaz-Franco, A.; Delgado-Aguirre, E.; Montes-García, N.

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IMPACT OF CORN AGRONOMIC MANAGEMENT ON AFLATOXIN (*Aspergillus flavus*) CONTAMINATION AND CHARCOAL STALK ROT (*Macrophomina phaseolina*) INCIDENCE

[IMPACTO DEL MANEJO AGRONÓMICO DEL MAÍZ SOBRE LA CONTAMINACIÓN DE AFLATOXINAS (*Aspergillus flavus*) Y LA INCIDENCIA DE PUDRICIÓN CARBONOSA DEL TALLO (*Macrophomina phaseolina*)]

M. Alvarado-Carrillo, A. Díaz-Franco*, E. Delgado-Aguirre and N. Montes-García

Campo Experimental Río Bravo, INIFAP Apdo. Postal 172, km 61 Carr. Matamoros-Reynosa, Río Bravo, Tamaulipas, México.E-mail: diaz.arturo@inifap.gob.mx
**Corresponding author*

SUMMARY

The purpose of this study was to know corn grain aflatoxin concentration, corn charcoal stalk rot incidence and corn production characteristics. Two planting methods (PM) and two irrigation systems (IS) were compared. The evaluated PM were beds (115 thousand plants ha^{-1}) and furrows (85 thousand plants ha^{-1}). The IS were drip and gravity (surface) irrigation. Ear length and grain protein content were not affected by PM or IS. Grain yield was superior in furrows (P=0.007) with 46% increase compared with beds. Between IS, drip irrigation increased yield (P=0.028) in 20% in relation to gravity irrigation. Drip irrigation registered better water use efficiency (P=0.001; 56%) related to gravity irrigation. Aflatoxin level and charcoal stalk rot incidence were not influenced by IS; but between PM, lower aflatoxin concentration (P=0.009; 11.1 µg kg-1) and charcoal stalk rot incidence (P=0.008; 23.7%) were observed in furrows. It seems that high population densities in bed plantings increased water demand, such factor reduced grain yield, increased aflatoxin content and charcoal stalk rot incidence. Furrow planting and drip irrigation were the agronomic management practices that gave greater yields and less risk with *A. flavus* and *M. phaseolina* in corn.

Key words: *Zea mays* L.; plant and grain characteristics.

RESUMEN

El propósito del estudio fue determinar la concentración de aflatoxinas (*Aspergillus flavus*) en el grano de maíz, la incidencia de la pudrición carbonosa del tallo (*Macrophomina phaseolina*) en maíz y las características de producción del maíz. Dos métodos de siembra (PM) y dos sistemas de riego (IS) fueron comparados. Los PM evaluados fueron en camas (115 mil plantas ha^{-1}) y en surcos (85 mil plantas ha^{-1}). Los IS fueron por goteo y gravedad (superficial). La longitud de mazorca y la proteína de grano no fueron afectadas por los PM o IS. El rendimiento de grano en la siembra en surcos fue superior (P=0.007) con incremento de 46% comparado con el de camas. Entre IS, el riego por goteo incrementó el rendimiento de grano (P=0.028) en 20% con relación al riego de gravedad. El riego por goteo registró una mayor eficiencia del agua de riego (P=0.001; 56%) en relación al riego de gravedad. El nivel de aflatoxinas y la incidencia de pudrición carbonosa del tallo no fueron influenciados por el IS, pero entre PM, los niveles más bajos de aflatoxinas (P=0.009; 11.1 µg kg-1) y la incidencia de pudrición carbonosa del tallo (P=0.008; 23.7%) fueron observados en surcos. Al parecer la mayor densidad de población en camas, incrementó la demanda de agua, factor que abatió el rendimiento de grano e incrementó el contenido de aflatoxinas e incidencia de pudrición carbonosa del tallo. La siembra en surcos y el riego por goteo fueron las prácticas de manejo agronómico que promovieron mayores rendimientos y menor riesgo con *A. flavus* y *M. phaseolina* en maíz.

Palabras clave: *Zea mays* L.; características de planta y grano.

INTRODUCTION

Aflatoxins are the mycotoxin group most studied worldwide. They represent a group of toxic metabolites and compounds that are related in their structure and are produced by the filamentous fungi *Aspergillus flavus* Link:Fr. and *A. parasiticus* Speare, and they are associated to several diseases as aflatoxicosis in swine, poultry, domestic animals and humans. There are four principal aflatoxins: B_1, B_2, G_1 and $G_{2,}$ nevertheless B_1 is the strongest and predominant carcinogenic secondary metabolite (Cornell Univ., 2008; FDA, 2008). *A. flavus* and *A. parasiticus* are cosmopolitan fungi with wide incidence associated to agriculture products that include corn (*Zea mays* L.), peanut (*Arachis hipogea* L.), cotton seed (*Gossypium hirsutum* L.) and pecan [*Carya illinorinesis* (Wangenh) K. Koch] (Cornell Univ., 2008; FDA, 2008), nevertheless, *A. flavus* is more related with corn (Diener y Davis, 1987; Hurburgh *et al*., 2005), in which they cause ear rot, with typical symptoms of greenish-yellow powdery masses of development fungi (CIMMYT, 2004; Kuchareck and Raid, 2000).

Corn aflatoxin contamination can occur at pre-harvest, especially when the crop is in the field, or at post-harvest, derived from grain storage after harvest (Hurburgh *et al*., 2005; Kuchareck and Raid, 2000). Aflatoxin presence at pre-harvest is a common phenomena in the productive regions; this problem is more severe when high temperature and drought stress periods are present during the growth cycle (Beltrán and Isakeit, 2004; Cotty and Jaime-García, 2007). In Mexico, 60% of the imported or produced corn is used for human consumption; therefore aflatoxin contamination in this cereal represents a high importance topic in the diet and the population health (Anguiano-Rubalcaba *et al*., 2005; García and Heredia, 2006). The approved regulatory levels of aflatoxin in corn for human consumption in Mexico and in other countries are up to 20 µg kg-1 (ppb) (Secretaría de Salud, 2002; FDA, 2008).

It is well known that the mycotoxin contamination is initiated in the field (pre-harvest), so the more practical and economical actions to reduce the aflatoxin levels are focused to prevent the colonization of *A. flavus* and *A. parasiticus* in the field (García and Heredia, 2006; Munkvold, 2003). Different studies showed that corn contamination with aflatoxin in pre-harvest is influenced by the agronomic management practices (Hurburgh *et al*., 2005; Bucio-Villalobos *et al*., 2001; Beltrán and Isakeit, 2004). It is not clear the impact of the association between population density and the risk of aflatoxin development.

Corn is affected by another fungi called *Macrophomina phaseolina* (Tassi) Goid, that is a soil pathogen that causes charcoal stalk rot and attacks around 100 plant families, in particular in warm-dry conditions (Kending *et al*., 2000; Claflin and Giorda, 2002). The usual symptoms are seen at the end of the growth cycle when soil temperatures are above 32°C and there is drought stress (Almeida *et al*., 2003; Claflin and Giorda, 2002); in corn there is a premature stalk dryness, followed by the presence of black mini sclerotia in the exterior and interior of the stalk base, and a complete rot with dismembered vascular tissues (Kucharek and Raid, 2000; Girón-Calderón, 1993; CIMMYT, 2004). As the earliness of the infection, the yield losses are higher (Girón-Calderón, 1992; Rodríguez-Castillo and Díaz-Franco, 1989). Nevertheless, in later infections the pathogen did not impact yield, plants tend to lodge with the presence of strong winds, which cause losses at harvest where this practice is mechanized (Girón-Calderón, 1993; Kucherek and Raid, 2000; Doubrava and Blake, 2004). In Mexico, corn charcoal stalk rot is present in Tamaulipas, Guerrero and Veracruz states. In northern Tamaulipas, the disease got greater importance since the eighties, where incidences of 60% have been registered in commercial fields. In this same region, the inoculation by the toothpick technique in corn showed that is an inefficient methodology because it gave the same results as the natural infection (Díaz-Franco and Montes-García, 2008; Girón-Calderón, 1988; Rodríguez-Castillo and Díaz-Franco, 1989). Díaz-Franco *et al*. (2008) determined that the charcoal stalk rot incidence and the number of infected internodes had a high correlation (r=0.94). Besides of the seeking of charcoal stalk rot tolerant genotypes, there is the need of information about other agronomic management options that lead to reduce the effects of this disease in corn. Díaz-Franco *et al*. (2008) reported the lowest levels of incidence and severity of *M. phaseolina* with minimum tillage practices, but also there were the lowest corn yields.

In addition, water availability is the principal limiting factor in the arid and semiarid agricultural production ecosystems; therefore the efficiency in the use of water resources in those conditions is a key factor. Crop productivity need to be related with water economy, but a greater knowledge and technology development for a sustainable production is required (Medrano *et al*., 2007). Alvarado-Carrillo and Morales-Beltrán (2002) and Arellano *et al*. (2004), described a drip irrigation method for small areas (around 5 ha) that is low cost compared with the sophisticated systems, and aloud to reduce water consumption up to 50% compared with gravity irrigation. A sustainable agriculture requires of equilibrium between harmless, productivity and the conservation of natural resources. By all the mentioned before, the water use efficiency will be determinant not only in the agronomic management and corn productivity, but also in the consequences with the aflatoxin contamination and charcoal stalk rot.

The main objective of this study was to determine plant characteristics, aflatoxin concentration in corn grain and charcoal stalk rot incidence, and its relationship in two planting methods and two irrigation systems.

MATERIALS AND METHODS

Location description

The field study was developed at the Río Bravo Experimental Station, of the National Institute for Forestry, Agriculture and Livestock Research (INIFAP), in Río Bravo, Tamaulipas, México, localized in 25° 57’ N, 98° 01’ W, 25 masl, semiarid weather, with mean annual temperature of 23 °C and an annual precipitation of 635 mm. The soil is vertisol (FAO, 1988) developed from alluvial sedimentation with clay texture (28 % sand, 31 % mud and 41 % clay), with 1.2 % of organic matter and a pH of 7.8 (1:2 soil/water).

Treatment management

Planting was performed during the autumn-winter cycle with residual soil moisture at February 18 of 2008, and the yellow grain corn hybrid used was “Camino 182”. Before planting, soil was fertilized with 60-40-00, which equals to 65 and 87 kg ha^{-1} of urea and triple calcium superphosphate, respectively. Additionally, 65 kg ha^{-1} of N was applied in the irrigation at initiation of flowering stage. The evaluated planting methods (PM) were beds and furrows (main plots). Twenty beds and 24 furrows were formed, the first ones with width dimensions of 1.6 m and the furrows of 0.81 m, both 150 m length. Planting was mechanized and the beds were planted using a small seed planter with a population density of 115 thousand plants ha^{-1}, and the furrows with a precision planter (Max Emerge®) and a population density of 85 thousand plants ha^{-1}. The utilized irrigation systems (IS) (small plots) were by drip and gravity irrigation (superficial). Each system occupied half of the beds (10) or furrows (12). Three auxiliary irrigations were applied in the gravity irrigation system (tassel initiation, flowering and milk stage). The control was a combination of treatments that represented the northern region, this included the planting in furrows and the application of three irrigations by gravity (Alvarado-Carrillo, 2005). The drip irrigation system used was described by Arellano *et al*. (2004), in which the hose (caliber 8000) was deposited in the center of the bed at 10-15 cm depth, and was operated at 0.56-0.70 kg cm^{-1} pressure, which was regulated with manometers before and after a filter system. Irrigations were applied according to the readings in the tensiometers. The injection of nitrogen fertilization into the water irrigation stream was by using the ‘ventury’ system (Alvarado-Carrillo and Morales-Beltrán, 2002; Arellano *et al*., 2004). The amount of water applied by gravity irrigation in beds was 37 cm, meanwhile by drip irrigation was 27 cm, in furrow-gravity was 46 cm, and in furrow-drip was 38 cm. Daily precipitations were registered from the weather station during the growth cycle. Local indications about the phytosanitary management and other agronomic practices suggested by Rosales *et al*. (2005) were followed.

Measurement of variables and information analysis

In each one of the irrigation systems water use efficiency was measured by using the WUE = kg ha^{-1}/m^3 ha^{-1}, which estimate corn yield produced by each m^3 of applied water (Kirda *et al*., 2005). In each irrigation system, plots of 8 m^2 were marked at random, and were replicated four times. At physiological maturity, in each plot, 10 plants were selected at random and characteristics of plant height, ear length and charcoal stalk rot incidence (percentage of diseased plants), were measured in them. At harvest, all the plant ears were collected and threshed manually, and grain yield was estimated in kg ha^{-1}, with adjusted moisture at 12%. After harvest, grain of each plot were mixed in plastic bags and a subsample of 500 g was milled in a mill Wiley (Model 4, Arthur H. Thomas, Philadelphia) and after this put into paper bags. A sample of 50 g was taken for the total aflatoxin determination by using the Aflatest immunoassay column (Vicam, Watertown, MA). The aflatoxin levels (µg kg^{-1}) were measured in a Torbex (Model FX-100, Vicam) fluorometer. Another 20 g of the milled sample were used for protein percentage determination by the Kjeldahl method. The main differences in each variable were determined by variance analysis using a split plot design distribution. Meanwhile, mean separation was done with Tukey’s test ($P \leq 0.05$). Before variance analysis, aflatoxin concentration and charcoal stalk rot data were transformed to square root to stabilize the variances. The statistical analysis was performed using the program from Olivares-Sáenz (1995).

RESULTS AND DISCUSSION

There were significant statistical differences for plant height, grain yield, and water productivity, charcoal stalk rot and aflatoxin levels. Plant height was affected by planting method and by the interaction between planting method and irrigation system; grain yield was influenced by planting methods and the irrigation system factors; water productivity was only affected by the irrigation system. Meanwhile, charcoal stalk rot and aflatoxin content were affected by the planting method (Table 1). The total rainfall during the growth cycle was 34 mm, therefore, there were conducive weather conditions to have development of both pathogens.

Plant characteristics and water use efficiency

The relationship between plant height with planting methods and irrigation systems, indicated a significant variability (P=0.001); in general plant height increased in the beds planting method, nevertheless, the greatest plant height (P≤0.05) was observed in the combination of beds with drip irrigation (Figure 1). Probably the association between the higher water use efficiency in the drip irrigation system (Table 2) and the high population densities in the bed planting system, promoted the increase in height. Montemayor *et al*. (2006) demonstrated that as the population density increased, there was an increase in plant height. Similar results were reported by Westgate *et al*. (1997), who observed that higher corn population densities induced a high competition for light and stimulated the vertical growth of plants.

Mean ear length was 11.8 cm, and was not influenced by the planting method and the irrigation systems. Meanwhile, grain yield was superior in the furrow planting system (P=0.007) with 2,756 kg ha^{-1} (46%) compared with beds system (Table 2). If we consider that the applied amount of water in beds was 10 cm superior tan in furrows, which appears to be a advantage factor, the differential in population density (30 thousand plants ha^{-1}) with respect to furrows (Table 2), would explain the greater water and nutrient demand under that particular condition and could be a determinant characteristic in the obtained results. These results coincide with different studies that indicate that optimal grain yields fluctuate from 65 to 90 thousand plants ha^{-1} (Cox, 1996; Gutiérrez-Sánchez and Luna-Flores, 2002; Bruns and Abbas, 2003). Between irrigation systems, drip irrigation increased productivity (P=0.028) in 1,318 kg ha^{-1}, 20% more than the gravity irrigation; similarly, water use efficiency was significant (P=0.001) and 56% superior with drip irrigation (Table 2). Díaz *et al.* (2008) concluded that corn grain yield was not influenced significantly by the irrigation systems (drip and gravity), but they found a higher water use efficiency in the drip irrigation system. The information is similar with the one reported by Lamm and Trooien (2003), who mentioned that drip irrigation in corn increased the water use efficiency in 35 to 60%, compared with the traditional gravity irrigation method in furrows.

Under the growing problem of water deficit in the agriculture of the arid and semiarid areas as the northeast part of Tamaulipas (Rymshaw, 1998; Salinas-García *et al.*, 2006), drip irrigation could be an alternative management, not only to increase grain yield but to increase water use efficiency. This irrigation system had shown the benefits of productivity increase and water save when it was evaluated in other crops as okra (*Abelmoschus esculentus* L. Moench), cantaloupe (*Cucumis melo* L.) and watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] (Alvarado-Carrillo *et al*., 2007; Arellano *et al*., 2004).

Grain characteristics and charcoal stalk rot

In corn grain characteristics, the protein content was not affected by both planting methods and the irrigation systems (Table 3). Vázquez-Carrillo *et al*. (2005) concluded that protein content in corn hybrids was increased with the interaction between phosphorus rate and population density. Other study by Uribelarrea *et al*., (2004) indicated that the increase in protein content in corn grain was in the same level as the nitrogen rate increase. When Díaz *et al*. (2008) compared drip and gravity irrigation systems in corn; they registered a significant increase in the protein content in drip irrigation. The protein values registered are in the range of 5.8 to 9.2%, reported by Vázquez-Carrillo *et al*. (2005) and Vasal (2001).

Table 1. Analysis of variance mean squares for plant height (PH), ear length (EL), grain yield (GY), water productivity (WP), charcoal stalk rot (CSR), protein content (Pr) and aflatoxins (Af), in corn 'Camino 182', associated with planting methods (PM) and irrigation systems (IS).

Factor	df	PH	EL	GY	WP	CSR	Pr	Af
PM (P)	1	2420**	2.7	30376640**	0.09	3306**	0.008	375.3**
Error-A	3	45	0.5	780693	0.06	93	0.354	11.6
IS (I)	1	269	0.2	6945856*	3.14**	100	0.697	2.9
P x I	1	1128*	3.8	1417280	0.40	225	0.672	38.7
Error-B	6	121	1.4	842432	0.05	66	0.206	14.7

*, ** Significant at P≤0.05 and 0.01, respectively.

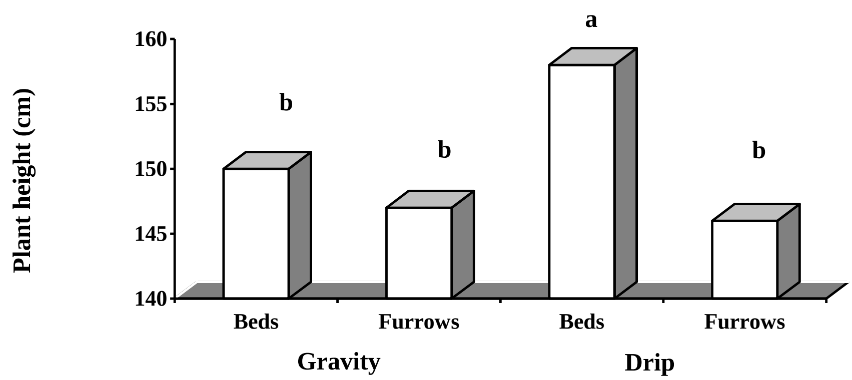

Figure 1. Variation in corn plant height influenced by the combination of two planting methods and two irrigation systems. Values on bars with same letter are similar according to Tukey, P≤0.05.

Aflatoxin contamination registered a similar and not significant level between irrigation systems with average of 16 µg kg-1. There was a significant differential (P=0.003) of 9.7 µg kg-1 between planting methods, being the highest aflatoxin concentration in beds with 20.8 µg kg-1, meanwhile in furrows an average of 11.1 µg kg-1 was registered (Table 3), therefore, planting in beds represented the method with highest risk of aflatoxin contamination. This situation can be due to the highest population density in beds (115 thousand plants ha^{-1}), that increases the water demand, which is a stress phenomena that affects directly grain yield and aflatoxin content. These results coincide with the ones observed by Blandino *et al*. (2008), who conclude that as population density increased, greater was the mycotoxin contamination. It is well documented that several environmental factors have influence in the spore dispersal, penetration, hyphae establishment and aflatoxin production (Cotty and Jaime-García, 2007; Munkvold, 2003). Soil moisture stress is determinant in the expression of high corn aflatoxin contamination (Bucio-Villalobos *et al*., 2001; García y Heredia, 2006; Cotty y Jaime-García, 2007).

Charcoal stalk rot incidence had an average of 38.1% and was not influenced by the irrigation systems, probably because of the irrigation volume difference of 8 cm between both systems that was not sufficient to have an impact on the disease development. Furrow planting method offered the least significant incidence of the disease compared with bed planting method that was 52.5% (Table 3). This had a similar aflatoxin explanation, in which bed planting method have a greater population density that create a greater soil water demand. This phenomenon was observed also for corn grain yield. In the case of *M. phaseolina*, infection is favored by low soil water values (Almeida *et al*., 2003; Claflin and Giorda, 2002; Díaz-Franco *et al*., 2008). Cardona (2006) concluded that in a natural infested soil, microsclerotia populations of *M. phaseolina* increased as temperature rise and soil water is reduced. Since in the present study, lodging was not a problem, it is important to consider the risk that this disease represents in particular when there are strong winds and losses in regions where harvest is mechanized (Girón-Calderón, 1993; Kucherek and Raid, 2000; Doubrava and Blake, 2004; Díaz-Franco *et al.*, 2008).

Table 2. Agronomic characteristics and corn productivity of ‘Camino 182’ subjected to two planting methods and two irrigation systems at Río Bravo, Tamaulipas, Mexico.

Factor	Density (plants ha^{-1})	Ear length (cm)	Yield (kg ha^{-1})	Water efficiency (kg m^{-3})
Planting method				
Beds	115000	11.4	5902 b*	1.93
Furrows	85000	12.2	8658 a	2.08
P>F		*0.103*	*0.007*	*0.333*
Irrigation system				
Gravity		11.7	6621 b	1.56 b
Drip		11.9	7939 a	2.45 a
P>F		*0.719*	*0.028*	*0.001*

*Values with different letter in each treatment are significantly different (Tukey, P≤0.05).

The highest corn grain yields and lowest aflatoxin and charcoal stalk rot levels were obtained in the furrow planting system. This have a particular importance in production regions where aflatoxin and charcoal stalk rot are phytopathological endemic problems. The combination of furrow planting and drip irrigation can be a viable management practice for the arid and semiarid areas, in particular as an efficient water conservation practice in agro ecosystems where there are drought periods that promote water deficits that cause limitations or irrigation cancelations (Alvarado-Carrillo *et al*., 2007; Salinas-García *et al*., 2006). Also, it is essential to mention the relationship between weather and aflatoxin analysis developed by Cotty and Jaime-García (2007), which indicated the need to develop better strategic procedures that include monitoring of weather and actualization of the agronomic practices, as anticipated measures to world climatic change.

Table 3. Charcoal stalk rot incidence (%) and corn grain characteristics of ‘Camino 182’ subjected to two planting methods and two irrigation systems at Río Bravo, Tamaulipas, Mexico.

Factor	Charcoal stalk rot (%)	Grain	
		Protein (%)	Aflatoxins (μg kg^{-1})
Planting method			
Beds	52.5 a*	8.33	20.8 a
Furrows	23.7 b	8.38	11.1 b
P>F	*0.008*	*0.884*	*0.009*
Irrigation system			
Gravity	35.6	8.41	15.6
Drip	40.6	8.15	16.4
P>F	*0.266*	*0.114*	*0.671*

*Data not transformed; values with different letter are significantly different according to Tukey, P≤0.05.

CONCLUSIONS

Ear length and grain protein content were not affected by planting method or the irrigation systems. Furrow planting method gave superior grain yield (46%) compared with beds. Between irrigation systems, drip irrigation increased productivity in 20% with relation to gravity irrigation. Drip irrigation showed greater water use efficiency (56%) with relation to gravity irrigation. Aflatoxin and charcoal stalk rot levels were not influenced by the irrigation systems, but furrow planting reduced aflatoxin concentration to 11.1 μg kg-1 and charcoal stalk rot to 23.7%.

Corn furrow planting and drip irrigation were the agronomic management practices where water use efficiency was greater, grain yields were higher and aflatoxin levels and *M. phaseolina* incidence were low.

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