Preharvest contamination of maize by *Aspergillus flavus*

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Abstract

Aspergillus flavus contamination of field-growing maize plants was studied. Samples of stems, leaves, flowers, silks, husks, cobs and kernels were collected 10 times at one-week intervals. Inside infection and surface colonisation in silks, cobs and kernels were much higher than in other plant parts. Aflatoxin was detected in silks, cobs and kernels 102, 112 and 119 days after maturity, respectively. Numbers of kernels infected with *A. flavus* were much higher than those from cobs.

Introduction

The incidence of aflatoxin contamination of maize in Thailand at various stages in the processing and marketing chain has been investigated (Siriacha et al. 1983; Anon. 1985). Samples were taken from fields, farmers' and merchants' storages, and silos for aflatoxin analysis. These surveys indicated that the problem developed after harvest.

However, significant contamination can occur preharvest when serious drought stress affects the maize during the final month of growth (Anon. 1986). Such droughts are said to happen only once in 10 years, but if weather patterns change then preharvest contamination would become a serious problem in Thailand, as in the United States (Anderson et al. 1975, Shotwell et al. 1977).

This paper reports the results of a study of the occurrence of *Aspergillus flavus* contamination on the surface of, and inside maize plant parts, including leaves, stems, tassels, silks, husks and kernels, at different maturities.

Materials and Methods

Sample collection

The maize used in this study was Suwan-I variety grown at Phraputtabaht Field Crop Experiment Station, Lopburi. It was planted in June 1990 in the area of one rai[1]. Maize samples were collected 10 times at 1 week intervals, when tassels were completely visible at 15–20% silking, until 2 weeks after full maturity.

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[1] 1 rai = 0.16 ha.

Maize samples

Samples of leaf, stem, tassel, silk, husk, cob and kernel were randomly collected in order to obtain 200 pieces of each plant part. Each sample of 200 pieces was divided into two groups to study the internal infection and colonisation on the surface of maize plants. Samples of maize kernels at 102, 112, 119 and 128 days maturity were analysed for aflatoxin.

Internal infection with *A. flavus*

One hundred pieces of each sample were surface sterilised with 1% NaOCl for 1 minute, then rinsed twice with sterile distilled water. They were plated in Petri dishes on DRBC medium (King et al. 1979). The number of plant parts from which *A. flavus* grew was then counted after 5 days incubation at room temperature (25–30°C).

Surface colonisation with *A. flavus*

One hundred pieces of each sample were directly plated in Petri dishes on DRBC medium without surface sterilisation. They were incubated at room temperature (25-30°C) and observed for *A. flavus* after 2–5 days.

Analysis of aflatoxins

Five kg of each maize sample was ground and divided sequentially by a sample divider until around 50 g portions were obtained for the analysis using the modified CB method (Siriacha et al. 1988).

Results and Discussion

Infection and colonisation with *A. flavus*

Internal infection and surface contamination with *A. flavus* in maize plant parts at different maturities are shown in Table 1.

Leaves

There was almost no infection inside leaf tissues in the field. Only 2% of samples were found to be infected at 88 days maturity. Surface contamination was detected at every stage of growth. The number of contaminated samples varied from 1–23% and showed no clear correlation between *A. flavus* contamination and level of maturity.

Stems

There was low infection, with 1–3% infected samples found at 81, 88 and 119 days. Surface contamination was low at most stages of growth, except for 11–47% of samples at 60 and 53 days of maturity. There was no clear indication of *A. flavus* accumulating at any particular level of maturity.

Tassels

Only 1% infection was found at 74 and 102 days of maturity. Contamination on the surface was detected, since tassels were completely visible until they became dry. Surface contamination ranged from 0–22%. There was tendency for *A. flavus* accumulation during 53–80 days of maturity.

Silks

A. flavus was found infecting silks at all sampling times (Table 1). This started from young green silks, yellow–brown silk to dry brown (Table 2). High percentages of infection of 7–10% were found in yellow silks during 67–88 days maturity. Very old dry silks had a lower percentage of infection, about 30% during 88–102 days of maturity. High accumulation of *A. flavus* on the silk surface was detected at 74–88 days of maturity. The highest percentage of contamination was 29%.

Husks

Infection was rarely found during 81 days after planting but increased when maize became mature. During 81–102 days of maturity highest percentages of infection (11–13%) and surface contamination (12–34%) were detected.

Cobs

Maize cobs from 60–119 days maturity were collected. Internal infection in the cob of 1% was found once at 67 days maturity, while from 88 days of maturity until 119 days, the infection ranged from 0.5–5%.

Surface contamination was not found in young cobs. Contamination was detected from 88 through 119 days maturity. The percentage of surface contamination varied from 10–14%.

Kernels

Samples of maize kernels at 80 to 119 days maturity were collected. Infections inside the kernels were detected after 102, 112 until 119 days maturity. The percentages of kernels infected were 11.3, 16.8 and 11.5% with average aflatoxin B_1 concentrations of 19.2, 0.8 and 142 ppb, respectively (Table 3). Average aflatoxin B2 was found at 119 days maturity. Surface contamination with *A. flavus* was first found in immature kernels at 80 days after planting and increased as maize became more mature.

The results from this study showed that leaves, stems and tassels were rarely infected with *A. flavus* even when contamination on the surface was always detected. Infection and

Table 2. Infection and contamination with *A. flavus* in silks of maize plants.

Maturity (days)	*A. flavus* detected in silks	
	Internal infection	Surface contamination
53–60	0–2	2–16
Young green silks		
67–88	6–10	15–29
Yellow brown silks		
88–102	2–3.5	10–11
Dry brown silks		

Table 1. Infection and contamination with *A. flavus* in maize plants at different maturities.

Maturity (days)	*A. flavus* detected in maize parts (%)						
	Leaves	Stem	Tassels	Silks	Husks	Cobs	Kernels
53	0a	0	0	0	–	–	–
	11b	47	22	2	–	–	–
60	0	0	0	2	0	0	–
	19	11	5	16	4	0	1
67	0	0	0	6	1	1	–
	9	1	6	15	4	1	–
74	0	0	1	7	2	0	–
	16	0	19	28	4	0	–
81	0	1	0	10	1	0	0
	23	3	14	10	12	0	4
88	2	3	0	10	11	4	0
	2	3	1	29	34	14	6
95	0	0	0	2	3	3	0
	1	1	3	11	13	10	4
102	0	0	1	3.5	13	4	11.3
	1	0	2	10	15	13.3	22.0
112	0	0	–	–	3	0.5	16.8
	1	2	–	–	5	12	61.0
119	0	1	–	–	0	5	12
	11	1	–	–	0	12	84

[a]Infection
[b]Surface contamination

Table 3. Kernels infected with *Aspergillus flavus* (%) and aflatoxin content (ppb) at different maturities.

Maturity of maize (days)	Moisture content (%)	Infected *A. flavus* kernels (%)	Aflatoxin content (ppb.)			
			B_1	B_2	G_1	G_2
102	30.1	11.3	19.2	–	–	–
112	24.7	16.8	0.8	–	–	–
119	21.3	11.3	14.2	1.1	–	–

contamination in husks were higher than those in leaves, stems and tassels. Inside infection and surface colonisation in silks, cobs and kernels were significantly higher than in other plant parts.

In a previous study of the surface colonisation of Thai maize with *A. flavus* (Kawashima et al. 199), the fungus was detected from leaves, stem, tassels but the population was low. Silks were always found to be contaminated with *A. flavus*. Kernels were contaminated after 90 days and the contamination increased as the maize matured.

The occurrence of aflatoxin in maize plants has also been reported by Shotwell et al. (1980). Fifty maize plants naturally and heavily infested with *A. flavus* were examined for aflatoxin in the kernels, cobs, husks, leaves and stalks. At sampling time, approximately 95% of the ears had visible *A. flavus*. The incidences and levels of aflatoxin in the husks, stalks, and leaves were lower than in the cob. The mean aflatoxin B1 was lowest in the leaves (3 µg/kg). Most of the aflatoxin was in the kernels, and toxin in the kernels accounted for most of the contamination (>75%) in the entire plant.

A. flavus was always found to infect and contaminate silks from first emergence. Highest percent of invasion was found in yellow brown silks. Growth of *A. flavus* on attached and detached maize silks has been reported (Jones et al. 1980). In addition, *A. flavus* has been isolated from silks in the fields (Fennell et al. 1977; Bothast et al. 1978). Colonisation of silk tissue, however, is dependent on the condition of the silks (Marsh and Payne 1981). Unpollinated silks are poor substrates for *A. flavus*. This may be due to inhibitors or lack of available nutrients. Similarly, brown dry silks are not readily colonised by the fungus. This could be the result of a low nutrient base, or perhaps lack of adequate moisture. Shotwell et al. (1980) were able to establish infection on 4-week-old silks, but only if the ears were covered with plastic bags. Growth of *A. flavus* on silks yellow–brown in colour was rapid and extensive. Silks at this stage had begun to senescence but were still turgid.

In our study, silks sample were collected from only outside the maize ear not from internal silks that move from outside into the ear. Marsh and Payne (1981) observed *A. flavus* infection of maize silks with scanning electron microscopy. They indicated that, although internal infection was observed, fungal colonisation of silk appears to be mainly external.

Percentage of kernels infected and contaminated with *A. flavus* in our study was higher than for any other parts. *A. flavus* infected kernels were sometimes detected when there was no infection in the cobs. The result suggested that spores of *A. flavus* might be able to colonise maize kernel by the way of the silks if kernels were damaged by other factors. Goto et al. 1986 studied the effects of preharvest inoculation with *A. flavus* onto maize ears in Thailand. In their study maize ears were inoculated by two methods: without damage, through silks; and mechanically damaged with pinboard through husks. The inoculation was tested during the developing period of maize kernels. High amounts of aflatoxin were detected from only the physically damaged group. In the USA preharvest aflatoxin contamination is widely known and is often associated with damage such as that caused by insects (Rambo et al. 1974.; Shotwell et al. 1980; Goto et al. 1986).

Payne (1986) summarised the evidence for silk and kernel infection by *A. flavus* and characterised the infection process. He pointed out that infection of kernels by *A. flavus* and the subsequent production of aflatoxin is a complex process. No one factor can be singled out as responsible for the variability and inconsistent results obtained in studies with this fungus. A major factor, however, relates to the weak parasitic abilities of *A. flavus*. For infection and aflatoxin contamination to occur many conditions must be met. If one important factor is missing, then little disease occurs. In some years inoculum may be limiting, in other years temperature or insects may be limiting. To understand and solve this complex problem we must understand the biology of the fungus as completely as possible and avoid the temptation to look for a rapid solution to the problem.

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