Distribution of *Aspergillus flavus* in the maize fields and drying facilities in Thailand: An examination in dry season (January–February, 1986)

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後藤 哲久^{*1}, 真鍋 勝^{*1}, Kanoknij PANAWAS^{*3}: タイ国のトウモロコシ圃場
および乾燥場における *Aspergillus flavus* の分布—乾季 (1986年1月～2月) の調査

Summary

In order to clarify environmental factors that contribute to invasion of *Aspergillus flavus*, one of the aflatoxin-producing fungi, into the maize kernels, the distribution of *A. flavus* as the potential inoculum in soil and atmosphere of maize fields and drying or storage facilities was examined in 8 provinces of Thailand during dry season (January–February, 1986). *A. flavus* was isolated from almost all of the soil samples. Although the numbers were quite different among the positive samples, it was concluded that the soil from middlemen's drying facilities and farmer's barn was, in general, more heavily contaminated than those from the fields. No *A. flavus* spore was trapped in 90 L of air samples from the 21 fields examined. On the contrary, *A. flavus* spores were trapped in almost all of the middlemen's drying facilities, although their numbers were smaller than those detected on the same locations in rainy season.

It has been accepted, especially in the U.S.A., that the preharvest infection of maize with *Aspergillus flavus* Link, one of the aflatoxin-producing fungi, results in subsequent contamination of aflatoxins¹⁻⁵). It is, however, not clear in Thailand because there is no approach to study environmental factors which influence invasion of *A. flavus* into maize kernels and subsequent aflatoxin contamination in the field and during harvest and storage. Basing upon the view that inoculum level of a toxin-producer may influence the extent of incidence of contamination, we started the investigation on geographical distribution and seasonal changes of *A. flavus* populations in soil and its air-borne spores at maize fields

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and drying or storage facilities, as a collaborative project between the Department of Agriculture of Thailand and the Tropical Agriculture Research Center (TARC) of Japan.

The first survey during the rainy season has been already reported^{6,7)}. In this paper, the results obtained from a continued examination in dry season (January–February, 1986) are described.

Materials and Methods

Soil and air samples examined for *A. flavus* populations were collected in Chiang Rai, Chiang Mai, Phetchabun, Phitsanulok, Nakhon Sawan, Lop Buri, Nakhon Ratchasima and Rayong provinces during January to February of 1986 (Fig. 1). The soil samples were collected from 20 maize fields (12 of farmers and 8 in the regional research centers or experiment stations), a flower field, 2 cassava fields in Rayong province where maize is rarely planted, and a farmer's barn and 4 middlemen's drying facilities for maize. The middlemen's drying facilities examined in this study consist of a flat concreted open space surrounded with walls, where maize is dried in the sun or warehouses.

1. Chiang Rai 2. Chiang Mai 3. Phetchabun
4. Phitsanulok 5. Nakhon Sawan 6. Lop Buri
7. Nakhon Ratchasima 8. Rayong

Fig. 1. Map showing the provinces for collecting soil and air samples
○: field; ●: middleman's drying facility; ▲: farmer's barn.

The samples were collected at 3 points within each sampling site in the depth of 0-10 cm. From each sample, 0.5 g of soil was suspended in 100 ml sterile water and shaken for 15 min on shaker, then 1 ml of suspension was pipetted into 5 Petri dishes (9 cm in diameter) and molten medium was poured and mixed thoroughly. The medium consisted of 45 g malt agar (Difco), 30 g NaCl, 50 mg chloramphenicol, 30 mg rose bengal, 1 mg 2,6-dichloro-4-nitroaniline and 1000 ml water. The dishes were incubated at 30°C for 5 days and growing *A. flavus* colonies were counted.

Air samples in the 21 fields, 6 warehouses at middlemen's drying facilities and 3 farmers' barns were collected by using an air sampler ("SAS" Bacteriological Air Sampler, Pool Bioanalysis Italiana Co.), operating for 1 min (90 L of air was sucked). The numbers of *A. flavus* colonies which grew from trapped spores on agar plates placed in the sampler were counted. The medium consisted of 39 g PDA (Difco), 30 mg rose bengal, 50 mg chloramphenicol and 1000 ml water.

Results and Discussion

1. Soil

The results of examination on *A. flavus* populations were presented in Table 1. Out of the 27 soil samples, *A. flavus* was detected from 26 samples. One negative sample was from a maize field in Phetchabun province. The populations in soils from fields and middlemen's drying facilities were 0-3,533 cfu/g (mean 335) and 1,688-7,884 cfu/g (mean 3,757), respectively. The soil in the farmer's barn yielded 3,237 cfu/g. These results showed that the soils in both the middlemen's drying facilities and farmer's barn were heavily contaminated with *A. flavus*, although the latter had been examined on only one case. Although the *A. flavus* populations in soils significantly varied with different fields, the detection of higher levels of *A. flavus* populations from some locations was noticeable. However, no evidence of geographical difference in the *A. flavus* populations was observed. In the same locations, moreover, the results obtained from rainy⁶⁾ and dry seasons could not show any seasonal change in the *A. flavus* populations in the soils.

2. Atmosphere

Table 1 shows the results on measurement of the numbers of *A. flavus* spores in the air samples. No *A. flavus* spore was trapped in all the 21 fields surveyed. On the contrary, in 5 of 6 samples from middlemen's warehouses, *A. flavus* spores were detected in range of 9-80 cfu/90 L of air (mean 39). Two samples from 3 farmers' barns yielded the *A. flavus* colonies, but the numbers were very low: 1 and 3, respectively.

Tsuruta et al.⁷⁾, examining the distribution of *A. flavus* spores in rainy season (September, 1985), showed that only one of the 7 air samples from maize fields was positive for *A. flavus* (2 cfu/90 L of air), whereas those of 2 middlemen's warehouses yielded 276 cfu and 482 cfu, respectively. The quite similar results that little or no *A. flavus* spores were detected from the air samples in the fields were obtained in two seasonal examinations (rainy in 1985 and dry in 1986). The *A. flavus* spores were trapped in almost all of the middlemen's warehouses in both examinations, although the numbers detected in

Table 1. Populations of *Aspergillus flavus* in soil and atmosphere

Province	Sampling site	<i>A. flavus</i> colony counts	
		Soil(1 g)	Air(90 L)
Chiang Rai	Maize field (Farmer)	282	0
	Maize field (Research Center* ¹)	208	0
	Flower field (Research Center* ¹)	68	0
	Middleman's drying facility	3,723	0
	Maize field (Farmer)	193	0
	Maize field (Farmer)	229	0
Chiang Mai	Maize field (Farmer)	1,087	0
	Maize field (Farmer)	218	0
	Maize field (Research Center* ²)	79	0
	Maize field (Research Center* ²)	46	—
	Maize field (Farmer)	361	0
Phitsanulok	Maize field (Experiment Station* ³)	9	0
Nakhon Sawan	Maize field (Farmer)	2	0
	Maize field (Research Center* ⁴)	15	0
Phetchabun	Maize field (Farmer)	3,533	—
	Maize field (Experiment Station* ⁵)	14	0
	Farmer's barn	—	3
	Middleman's drying facility	—	9
	Middleman's drying facility	7,884	48
	Maize field (Farmer)	12	0
	Farmer's barn	3,237	0
	Maize field (Farmer)	0	0
Lop Buri	Middleman's drying facility	1,733	80
	Farmer's barn	—	1
	Maize field (Farmer)	291	0
	Maize field (Experiment Station* ⁶)	87	0
Nakhon Ratchasima	Middleman's drying facility	1,686	14
	Middleman's drying facility	—	42
	Maize field (Farmer)	7	0
Rayong	Cassava field (Research Center* ⁷)	188	0
	Cassava field (Farmer)	440	0

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the examination of dry season were much smaller than those detected in rainy season.

Some papers⁸⁻¹¹⁾ on the numbers of *A. flavus* spores in the atmosphere of maize fields in the U.S.A. showed that the numbers of air-borne spores of *A. flavus* might not be so high, even though they may depend on the sampling method, weather and the other factors. This means that the low occurrence of *A. flavus* spores may not always exclude the possibility of preharvest incidence of aflatoxin contamination in maize. Nevertheless, the rather high levels of air-borne spores of *A. flavus* in the warehouses of middlemen's drying facilities suggested that the drying and storage processes should be improved for preventing the aflatoxin contamination of maize in Thailand.

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