Assessment of insect infestation in stored maize and their relationship to *Aspergillus flavus* contamination

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ABSTRACT

An entomological assessment of Thai maize in storage was conducted in relation to Aspergillus flavus infection, aflatoxin contamination and association of stored - product insects. The pattern of insect infestation and A. flavus infection showed strong correlation to high densities of weevils (live or dead) and other secondary species. Fumigations when weevil populations were high produced many cadavers on which *A. flavus* propagated supplying great quantities of inoculum. Training in sanitation and pest control is needed. The National Training program should be implemented by upgrading Department of Agriculture staff for teaching broad-based storage management concepts. A training approach is suggested. Future research by the Stored-Product Insects Research Section was suggested in the following areas:

- Surveys of Thai grain for aflatoxin should include collection and quantification of insects in maize samples from various storage conditions;
- Maize weevil population densities should be correlated to the presence or absence of aflatoxin;
- Dispersion of insects from highly infested warehouses or silos can be documented by the use of various kinds of traps;
- Methods for treating maize with a grain protectant after drying need to be developed for extension use;
- A survey for resistance of insects to grain protectants is needed for baseline data and documentation of later changes during insecticide usage.

1. INTRODUCTION

Within the objectives of the joint project of the Government of Thailand and the United Nations Development Program (UNDP) to reduce postharvest losses in stored grains is an investigation of the entomological factors affecting grain damage especially in their relation to the spread of aflatoxin-producing fungi. The role of certain field and storage pests is known in the inoculation of maize before harvest and in the production of conditions, which promote *A. flavus* growth and proliferation during storage. Climatic conditions in Thailand maize growing regions are especially conducive to both A. flavus growth and stored-grain insect population increase.

Maize is Thailand's second largest grain crop after rice. Dry maize is not for human consumption but for animal feed and export. Because of problems with aflatoxin contamination and insect infestation, recent crops have met with reduced prices and refusals at foreign ports (Walters 1987). Whether or not Thailand's maize crop contains permissible aflatoxin concentrations for export may depend on controlling stored grain insect populations as well as drying and handling the maize properly. The triple effect of losses due to insects, fungi and mycotoxins can be devastating in years when conditions are favorable for grain deterioration.

ASSESSMENT OF INSECT INFESTATIONS IN MAIZE AND THEIR RELATIONSHIPS TO *ASPERGILLUS FLAVUS* CONTAMINATION

The presence of insects associated with both field and storage damage of maize in Thailand has been documented by other consultants in this project. One of the objectives of Eugene Smalley's consultancy (December 1985 - February 1986) was to assess the role of insects and arthropods as vectors of mycotoxin-producing fungi. He sampled first crop maize in the field and found the maize weevil, *Sitophilus zeamais* Motsch, in freshly picked ears. The infestations were usually at the tip of otherwise sound ears and were rarely extensive. No weevil infestation was observed in second crop at harvest. However, first crop maize at middleman warehouses (stored for 3-4 months were heavily infested. Dead weevils were yellow-green with conidia of A. flavus. Large silos also harbored tremendous weevil populations. Dilution plate counts of ground weevils revealed the presence of A. flavus and many other storage fungi. Weevil-bored kernels (first crop) from middleman warehouses were 100% infected with A. flavus while sound kernels were 40-50% infected. Second crop whole kernels without weevil damage from the drying floor contained no *A. flavus*. When weevil tunneling was taken into consideration as well as exit holes, 13-18% of the kernels sampled were damaged in first crop stored at middleman warehouses.

Field Studies

During this consultancy (November 1986), little maize was in storage. Small amounts of second crop maize were found but most farmers' cribs were empty. Middleman warehouses visited by this consultant contained 1 or 2 bags of second crop maize but mainly mung bean was in storage. First crop maize was found only at one port facility. The other two visited were exporting rice and other commodities. Samples of maize and insects taken at the above sites were not representative of the 1986 crops. However, the pattern of insect infestation and A. flavus infection is similar to previous findings. Maize from cobs collected at farms showed no growth of A. flavus after surface disinfection with 2% sodium hypochlorite for 1 min. and 4-5 days on malt-salt (4%) agar plates. No stored product insects were found at the 3 farms visited. Cribs were essentially empty. Cobs present generally were low quality and immature. These were not sampled. They appeared to have been sorted from grain sold or gleaned from the field. One farm had good quality maize, taken as samples. No A. flavus was present (Table 1).

Of the two middleman warehouses visited, the first had only one bag of maize, a small seeded variety that at some time had been treated with an unknown pesticide. The rest of the storage was mung beans which also had been treated with pesticide. The maize sample contained no insects. A. flavus infected 45% of the kernels (Table 1, 3c).

The second middleman warehouse had new second crop maize on the drying floor and 1-2 month old second crop maize in 5 stacks. The Lopburi warehouse is the largest buyest buyer of maize in the province. They have a quota on maize export. Farmers know that they can sell here. The second crop of maize is dried on the concrete drying floor to about 16% MC (Steinlite Moisture Meter). The maize is cleaned (?), bagged and placed in stacks 10-15 bags high and 1015 bags wide (minimum). The maize is dried to 15% MC in these stacks by natural air currents over a 2-3 month period. The dryer is used only when the price of maize allows. Dryer bins are sometimes used for storage. Unused bags are piled behind the warehouse under a roof. These are fumigated as needed. Fumigations are undertaken by their personnel using methyl bromide under tarps. Rolled bags are used to "seal" the tarp to the floor. One stack was under fumigation with methyl bromide.

Table 1. Storage fungi counts from maize kernels collected at four sites in Thailand, November 1986, expressed as percent of total kernels plated (10 kernels/plate)*

	Site	No. of plates	*Aspergillus flavus* (%)	*Aspergillus niger* (%)	*Penicillium* spp. (%)	Others (%)
1.	Farm	5	0	8	2	48
2.	Godown					
	Stack a (no insects)	5	8	4	0	14
	Stack b (few insects)	5	18	18	4	26
	Stack c (many dead weevils)	4	90	15	3	3
3.	Middleman Storage					
	sample a	5	24	10	6	18
	sample b	5	24	16	0	44
	sample c	6	45	18	3	13
4.	Silo (cleaned for export)	6	17	3	53	12

* Malt-salt (4%) agar for 4 days after surface disinfection (Seed and Postharvest Pathology Section, DOA). Data is not representative because of small sample size.

Maize was sampled from three stacks. There were many insect present in the samples and wandering about the facility. The maize samples from areas in the stacks where no insects were found had low *A. flavus* counts (8%, Table 1, 2a). Samples with few insects had somewhat higher counts (18%) and samples with many dead weevils had a high degree *A. flavus* infection (90% of kernels, Table 1, 2c). Insects (both live and dead) from these samples reflected the same pattern (Table 2). The stack with lower infestation levels (shorter time in storage) had insects with lower contamination rates. Dead insects are an excellent substrate for *A. flavus*. This shows well in the high A. flavus counts (76 and 100%) from dead insects of all kinds. Smalley (1986) observed piles of dead weevils in warehouses that were yellow-green with *A. flavus* spores. The spore load is likely to increase after death because the fungus is actively growing on the cadavers. This is an important source of inoculum for maize on the drying floor and insects walking over it, or within fumigated stacks. This silo generally kept maize 2-3 months.

The export silo in Bangkok was heavily infested with several species of stored-product insects. Samples taken at the bin tops were composed mainly of red flour beetle, flat grain beetle, and maize weevil. Samples from the cleaning floor were almost entirely maize weevil. All insects were carrying heavy spore loads of *A. flavus* after surface disinfection. Dead insects were as contaminated as live insects or more so (Table 2, a, b, c).

Inspection of maize samples with a black light showed presumptive evidence of aflatoxin only in one kernel of a maize cob from a farm but in many pieces of maize screenings below the cleaners in the export silo. Personnel at the silo were monitoring the grain as it was loaded on the ship and said that aflatoxin concentrations were less than 20 ppm in their whole kernel samples. The screenings were allowed to accumulate for several months. Mounds of screenings (5 ft. high) beneath the cleaning apparatus were black with millions of live and dead weevils.

Table 2. Microbial counts from stored-product Insects collected at two sites in Thailand, November 1986, expressed as percent of total insects plated.

Site	Sample #	Live or dead	Maize weevil	other spp.	Location	Percent				
						A. flavus	A. niger	Pen. sp	Other **	None
Export	Silo									
	1	live	0	25	Bin tops	60	36	28	0	0
	2	live	0	25	Bin Tops	92	20	12	0	0
	3	dead	0	25	Bin Tops	80	0	8	8	24
	4	live	0	25	Bin Tops	16	0	12	32	40
	5	live	25	0	Cleaning Floor	76	52	12	12	0
	6	frozen (from live)	25	0	Cleaning Floor	76	28	16	4	0
	7	dead	25	0	Cleaning Floor	72	44	32	0	0
	8	live	5	11	Cleaning Floor	60/82	0/9	0/9	20/9	0
Godown										
	a	live	25	0	Stack	32	16	28	8	16
	b	dead	25	0	Stack	72	52	4	4	0
	c	dead	25	0	Stack (fumigated)	100	68	12	4	0
Farms – no stored product insects found in empty storage areas										

* Data cannot be considered representative because of small sample size.
** Mainly *A. glaucus* and *A.terreus.*

Table 3. Black light indication of bright green-yellow fluorescence in maize samples from three sites.

Site	BGYF
Farm Sample	
Maize cobs with damaged kernels	yes (1 Kernel)
Godown	
Kernels	yes
Screenings	no
Silo	
Cleaned for export	no
Sweepings on bin to	no
Screening from cleaner	yes (many pieces)

Discussion

Field infestation of maize before harvest has been strongly associated with A. flavus infection and aflatoxin contamination (many authors, see references-. The main correlation is with field pests, such as European corn borer, because they are capable of initial damage to the cob and sheath. Kernel damage by European corn borer opens sites for infection by *A. flavus* from any inoculum source. Stored-product insects found in the field before harvest generally require previous damage to the husk, or ears that protrude from the sheath (some hybrids), in order to gain entry. They may carry A. flavus spores into the ear and inoculate kernels. However, the maize weevil is considered a poor vector of *A. flavus* in the field (LaPrade and Manwiller, 1977; McMillian 1980). The main role of maize weevil in A. flavus infection and subsequent aflatoxin contamination occurs during storage. Large populations of weevils can change conditions within bagged or bulk grain to encourage the growth of storage fungi (Christensen and Kaufmann 1969). They can also carry inoculums from the warehouse or other storage areas back to field prior to harvest.

In Thailand the maize weevils and other stored product insects are a complicating factor in the production of aflatoxin in maize. The main reason for *A. flavus* growth and aflatoxin production seems to be high moisture maize at harvest. The need for drying the grain has been recognized and steps are being taken to promote rapid drying after harvest at the farm level and first buyer stations (several projects in Ag. Engineering and Extension). Maize weevil populations left unchecked in godowns and warehouses pick up a heavy spore load of *A. flavus* and transmit it to both high moisture and dry grain. They are a continuous source of *A. flavus* inoculums throughout the year. As the population increases, weevils migrate to other areas, such as new maize brought into the warehouse. Even if this maize is dry (15% moisture content), the weevils can change the condition of the grain within the stack or bulk by increasing temperature and moisture through their metabolic activities until the limit for A. flavus growth is reached. That is why stacks that seem to have good storage characteristics can become heavily infected with storage fungi within a short period.

The second crop maize observed at the silo in Lopburi province was a good example. At 15% moisture, maize may be considered dry and storable by some. Actually, it is at its lower limit for growth of storage fungi. Any factor, such as weevil infestation. That can change the condition of the maize and raise the moisture content will cause deterioration sooner than expected (Christensen and Kaufmann 1969). Maize stored at 15% moisture has a somewhat predictable safe storage time as long as other factors do not intercede to change the quality of the grain. The damage to kernels by feeding weevils are primary sites for fungal growth. Storage fungi invade kernels more quickly through breaks in the pericarp.

Stored-grain insects other than maize weevil harbor storage fungi also (Table 2). These insects may dispense A. flavus spores throughout a grain mass as readily as the maize weevil. A further consequence of mouldy grain in storage is the rapid increase in populations of these "secondary" insects which can feed on damaged grains but not on intact kernels. These insects are aided in their growth by additional nutritional value from storage fungi growing on the kernels (Wright et al. 1980a). "Blooms" of these insects are often found in association with deteriorating grain. The effects of known mycotoxins on these insects are minimal (Wright et al. 1980b, 1982).

General Recommendations

Maize at "safe" moisture contents for storage or above is particularly vulnerable to maize weevil attack with subsequent rapid increases in weevil numbers, moisture content, temperature, growth of storage fungi and secondary insects. Controlling storage pests is a necessity for reducting losses in stored grain and maintaining grain quality. Fumigations of stacks or bulks already heavily infested -may control the insect population momentarily, but a great deal of damage has already been done. Kernels have been damaged by insects and opened to fungal attack. Fungi already in the kernels are not affected by fumigation. Dead insects are invaded by A. flavus and large amounts of inoculum are produced. The consequences are obvious.

Warehouses and silos that allow insects to build large populations are producing problems for themselves, for farmers and other storage facilities in their area. Migrating insects infest nearby farm fields and cribs and invade other facilities which may have an adequate control program.

Insect populations should be controlled while the numbers are still low. Fumigating heavily damaged, highly infested, stacks or bulks may lead to control failures because the gas cannot penetrate dust packed, insect-damaged areas. Highly infested warehouses act as harborage for insects in the structure itself so that stacks are rapidly reinfested after a fumigation. Other stacks that are not fumigated (usually not all are done at one time) also act as sources of infestation for recently fumigated stacks.

Fumigation of stacks heavily infested with weevils may induce the massive inoculation of the stack with *A. flavus* spores produced on the cadavers of insects. If populations are kept low, the amount of inoculum is also minimal.
Fumigation should be carried out by trained personnel whether commercial operators or in-house employees. Using the proper techniques are important for both economic and safety reasons. Fumigation failures cost money in wastage of chemicals, continued damage to grain and possible long term effects on resistance of insects to fumigants. With so few "safe" fumigants available to us, we must protect their effectiveness by using proper techniques. This will also insure minimal exposure of personnel to these dangerous gases. Initial training should be followed by refresher courses and updating on new techniques and safety measures.
Sanitation in the warehouses is essential. Cleaning up grain and debris that can harbor insects is part of any good control program. Bags of old grain, screenings from previous harvests or loads should be disposed of immediately. Leaving screenings in a facility for longer than one insect life cycle (a month or less for most insects under tropical conditions) allows exponential increase of insects in subsequent generations. Good housekeeping practices, including the value of sanitation as a control measure and the importance of cleaning schedules should be taught along with fumigation techniques and other control practices.

INSECT CONTROL RESEARCH

Research on insecticides and other control measures for seed have been developed into recommendations published in the Manual on Insecticide Usage (partial copy in Annex 111). Extension personnel recommend pesticides based on this manual. A project on insecticide usage on seed maize was underway. Four insecticides were being tested as sack sprays and by direct application on seed. Pirimiphos methyl was the only treatment still effective at 10 months. Fenitrothion, chlorpyriphos methyl and methacriphos gave only short term protection.
Direct application of insecticides to food grains is not a common practice in Thailand. Rice, the staple grain, is held in high regard, and therefore, is not "adulterated" with pesticides. This practice carries over to other grains to some degree. Maize may be treated by some farmers, but the most likely crop for direct application is mung bean. Educating farmers and middlemen to the safe and proper use of grain protestants will be an important part of the National Training Program. Extension personnel are obvious sources of information to the farmers. At this point in time, they require training themselves.
Fumigation with methyl bromide is a common practice in Thailand. Middlemen, especially larger regional buyers with quotas for maize export, routinely fumigate under tarpaulins. They may or may not contract with a private pest control operator to do the job. Often they purchase the methyl bromide and dispense the gas themselves. Many have had no training in the handling of fumigants. Misuse and unsafe practices are common. SPIRS has worked with phosphine and was planning future projects. More equipment was needed for safe and effective research on fumigants. A project developed with the Australian Centre for International Agricultural Research (ACIAR) was underway using phosphine under plastic covers and CO2 in rice storage.

ACKNOWLEDGEMENTS

The cooperation and hospitality of Mr. Chuwit Sukprakarn, Chief, Stored Product Insect Research Laboratory, and his staff were greatly appreciated. In particular, Mrs. Kusuma, Mrs. Busra, and Mrs. Porntip took time from their busy schedules to inform me of the organization of the laboratory, organize field trips and transportation and assist in preparation of samples. Mrs. Dara Buangsuwon, Project Leader and Chief of Seed and Postharvest Pathology Branch, and her staff were invaluable cooperators in the use of laboratory space, preparation of media, plating of grains and insects, and identification of fungi. Mr. Chamlong Chettanachitara, Chief, Plant Quarantine Technical Branch, Agricultural Regulatory Division, provided staff and transportation for field trips to export facilities. Thanks also to Mr. Vern E. Walters for discussions and cooperation on training issues, his friendship and of his wife.

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