International Journal of Tropical Insect Science Vol. 29, No. 1, pp. 31–39, 2009 doi:10.1017/S1742758409391511

Propagation of mycotoxigenic fungi in maize stores by post-harvest insects

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(Accepted 28 March 2009)

Abstract. Maize pests feeding on grains can transmit with their movement fungi harmful to human and animal health. The aim of the present work was to study the immigration and the dynamics of storage pests in traditional African maize granaries and the fungal spectrum associated with these insects. Treatments were (i) maize cobs protected just after pollination with gauze and stored thereafter, and (ii) unprotected maize cobs as controls. Eight different species of insects were identified in stores. No *Prostephanus truncatus* (Horn) was found in 'protected' maize during the 6 months of storage, but their mean number reached 239 individuals per kilogram after just 3 months of storage in the 'unprotected' stores. Similarly, significantly more *Sitophilus zeamais* (Motschulsky) were recovered from the unprotected than the protected maize treatment. Nine fungal species were found to be associated with the storage insects. On 'non-protected' cobs the genus *Fusarium* (36.05%) was the most frequently identified, followed by *Penicillium* (23.50%), *Rhizoctonia* (5.65%) and *Aspergillus* (3.95%). On protected cobs, *Rhizoctonia* sp. was most frequent (16.76%), followed by *Fusarium* spp. (16.62%), *Penicillium* spp. (8.24%) and *Aspergillus* spp. (2.33%). The toxigenic species encountered were *Aspergillus flavus* Link, *Aspergillus parasiticus* Speare and *Fusarium verticillioïdes* (Sacc.). *Cathartus quadricollis* (Guérin) appeared to carry more fungi towards the store, mainly *Penicillium* spp. (51.47%), *Aspergillus* spp. (46.56%) and *Fusarium* spp. (32.01%). Storage pests, in particular *C. quadricollis* and *S. zeamais*, play an important role in the contamination of maize with fungi, especially those that produce toxins.

Key words: maize, beetles, storage pests, toxigenic fungi, stores

Introduction

Maize (*Zea mays* L.) is one of the major staple grain foods grown throughout Africa. In developing countries, much of it is produced by smallholder farmers, for their own consumption and for sale. Maize yields are low as a result from a combination of biotic and abiotic stresses. In Africa, more than 13% of crop losses are due to insect pests, the others being cryptogamic, bacterial and viral diseases, weeds and rodents (Pantenius, 1988). The most damaging storage pest that infests maize in sub-Saharan Africa is the larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), causing losses of up to 40% after 6 months of storage. It is followed by *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), *Tribolium* spp. (Coleoptera: Tenebrionidae), *Cathartus quadricollis* (Guérin) (Coleoptera: Silvanidae), *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae), *Oryzaephilus* spp. (Coleoptera: Cucujidae), *Gnatocerus* sp., *Palorus* spp. (both Coleoptera: Tenebrionidae) and

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Cryptolestes ferrugineus (Stephens) (Coleoptera: Laemophloeidae) (Pantenius, 1988).

Storage pests create an environment favourable to mycotoxigenic fungi through physical damage on maize grains (Dowd, 1998). The presence of fungi can lead to physical and nutritional deterioration, as well as modifying the organoleptic qualities of the infected grains (Wright, 1998). The prevailing microflora on maize in the tropics belong to the genera *Aspergillus*, *Fusarium* and *Penicillium* (Cardwell *et al.*, 2000). In these genera, some species are mycotoxin (e.g. aflatoxin, fumonisin, ochratoxin, citrinin) producers. Mycotoxins are toxic to humans and most animals (Marasas, 1988). *Aspergillus flavus* Link and *Fusarium verticillioïdes* (Sacc.) have been reported to be the most prevalent fungi on maize in Africa, with aflatoxin and fumonisin co-occurring (Cardwell *et al.*, 2000). There are two basic mechanisms for *A. flavus* contamination on maize: (1) airborne or insect-vectored inoculum contaminates the silk and grow into the cob or, more often, (2) damaged grains become colonized with the fungus (Miller, 1995). The distribution of these fungi in stores in Benin is promoted by insect damage to maize in the field: a fungal infection starts from the field and is often compounded by damage from lepidopteran pests (Schulthess *et al.*, 2002). According to King and Scott (1981), damage by post-harvest pests predisposes maize to fungal infection.

This study aimed to investigate and make an inventory of mycoflora associated with maize storage pests in Benin. The dynamics of these pests inside the stores and their immigration towards the storage structures were evaluated. The potential of each insect pest to convey fungal spores to maize stores and the relationship of certain insects with fungi were assessed.

Materials and methods

Experimental site

The trial was conducted at the Benin station of the International Institute of Tropical Agriculture in Abomey–Calavi, located in the forest mosaic savannah (FMS) zone. The FMS spreads between the sixth and seventh parallel and has two annual maize-growing seasons; one from April to July and the second from September to November, with average rainfall from 1300 to 1500 mm/annum and mean temperatures between 25 and 35 °C.

Experimental procedure and layout

The local maize variety 'Toga' was sowed on 2 ha at the beginning of the first rainy season (80 cm between lines, 40 cm within line) with an application of fertilizer (NPK, 15:15:15) at a dose of 125 kg/ha. Up to the peduncle, some maize cobs were completely covered ('protected') in the field just after pollination by a gauze cloth (0.25 mm mesh size), to avoid infestation by insects. Cobs from the 'non-protected' treatment were left to be naturally infested. At harvest, three replicates per treatment of 'Adja' granaries of 2 m diameter and 0.5–0.75 m high were randomly built in the field using palm baskets (Meikle *et al.*, 1998). The distance between the granaries was approximately 100 × 150 m. The granaries were wrapped with mosquito netting coated with neutral glue (Tanglefoot®, Grand Rapids, Michigan, USA) to capture storage pests immigrating toward the stores. The netting was changed monthly and every 2 weeks insects were collected from the glue for species identification and counting. After harvest, the gauze cloth was removed and protected and non-protected cobs were stored in three replicates each with at least 500 maize cobs in each granary. Ten maize cobs, on average 1 kg, were sampled monthly from each granary from September 2000 to March 2001 to determine pest and pathogen populations.

Assessment of storage pests and their associated fungi

The sampled maize cobs were dehusked and shelled on a set of sieves. All insects were collected, counted and identified to species level using the keys of Dobie *et al.* (1991) and Délobel and Tran (1993). Grain moisture content (mc) was determined according to the method of the International Standards Organization (ISO, 1980). Three separate subsamples of about 10 g of maize grains were ground (Tekmar IKA-A10, Analytical Mill, Staufen, Germany), transferred to a metal container, weighed and dried in an oven for 2 h at 130 °C. In three replicates for all the six treatments, maize losses were evaluated using the count and weight method described by Boxall (1986). For the determination of fungal spectrum on the maize, five replicates per treatment of five maize grains were surface sterilized in 10% sodium hypochlorite, and plated in Petri dishes on filter paper slightly wetted with a mixture of water and 0.05% lactic acid. Petri dishes were incubated for 7 days at 27 °C under 12 h light and 12 h darkness. Fungi present on maize grains were identified using the method described below and from the 25 grains incubated, the percentage of grains infected by each fungus species was calculated to determine their incidence on maize grains.

Assessment of fungi present on insects

All insects found in the granaries and on the glue coated netting were collected, identified and counted. Out of each species group a subsample was plated on potato dextrose agar (PDA) and incubated for 7 days at 27 °C under 12 h light and 12 h darkness. From each treatment, 10 *S. zeamais*, 10 *C. quadricollis*, 10 *Palorus subdepressus* (Wollaston), 5 *P. truncatus*, 5 *Carpophilus dimidiatus* (F.) (Coleoptera: Nitidulidae), 5 *Tribolium castaneum* (Herbst), 5 *C. ferrugineus* and 5 *Gnathocerus cornutus* (Fab.) were plated. Fungi were first isolated, identified up to genus level and again cultured on specific media for species identification. The species identification was done by the single spore method on malt extract agar and Czapek yeast agar for *Penicillium* spp., on potassium chloride and PDA for *Fusarium* spp. and on PDA for *Aspergillus* spp. and other species. Keys used for species identification were those of Klich and Pitt (1998) for *Aspergillus* spp. and Watanabe (1994) for *Fusarium* and *Penicillium* spp. The percentage of insects infested by each fungus species was calculated to determine their relative incidence on immigrants' pests.

Data analyses

Number of insects, losses and incidence of fungi were subjected to ANOVA using the mixed model procedure in SAS version 9.1 (SAS Institute Inc., 2003). Variances were stabilized with the following transformations: $x' = \log\ (x + 1)$ for pest populations; $x' = \arcsin\sqrt{p}$, with $p = x/100$ for fungal percentages. Averages were separated with the *t*-test at 5%. Correlations were computed to establish the interactions between number of insects in stores and fungi and within immigrant insects. Means are presented untransformed in the tables.

Results

Insect infestation in maize stores

The storage insects found in the stores were *P. truncatus*, *S. zeamais*, *C. quadricollis*, *C. dimidiatus*, *P. subdepressus*, *T. castaneum*, *C. ferrugineus* and *G. cornutus*. *S. zeamais* was the most abundant insect on stored maize. On protected maize, *S. zeamais* was found after 1 month of storage, while *P. truncatus* was not encountered during the entire 6 months of storage (Fig. 1). On non-protected maize, *S. zeamais* was present at harvest and its population increased during storage, while *P. truncatus* appeared after 2 months of storage (Fig. 2). The number of all other storage pests increased during storage, except for *C. quadricollis*, which peaked until 3 months of storage and decreased thereafter in both treatments. On protected maize, only *Tribolium* spp. was present at harvest; *C. quadricollis* and *Carpophilus* spp. appeared after 1 month of storage (Fig. 3). On non-protected maize, *C. quadricollis* and *Carpophilus* spp. were present at the beginning of storage, while *Tribolium* spp. and *Palorus* spp. were observed after 1 month of storage (Fig. 4).

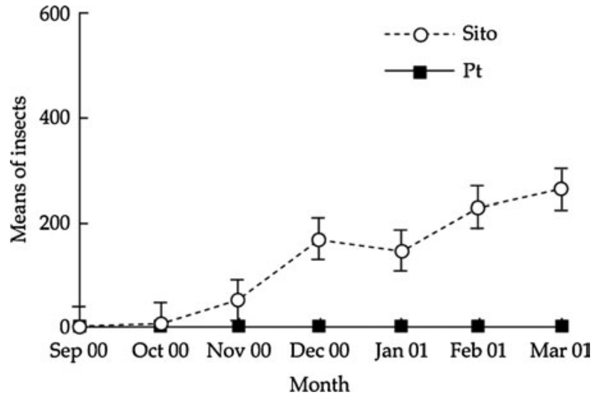

Fig. 1. Mean numbers (± SE) of *Sitophilus zeamais* (Sito) and *Prostephanus truncatus* (Pt) on 'protected' maize

Significant differences between the two treatments were found for *S. zeamais* ($P < 0.0001$), *P. truncatus* ($P = 0.0002$) and for all other insect pests, except for *Cryptolestes* spp. and *Gnatocerus* spp.

Grain losses in stores and its mc

Figure 5 shows the evolution of grain losses due to storage pests during the 6 months of storage. During the first 3 months of storage, there were no significant differences between protected and non-protected maize. After 4 months of storage, losses between treatments differed significantly ($P = 0.0051$), reaching 36.4 and 6.4% after 6 months on non-protected and protected maize, respectively. mc levels evaluated in the two treatments were similar. It started with 18.5% at the beginning of storage, to decrease to 14.0 and 10.9% after 3 and 6 months, respectively.

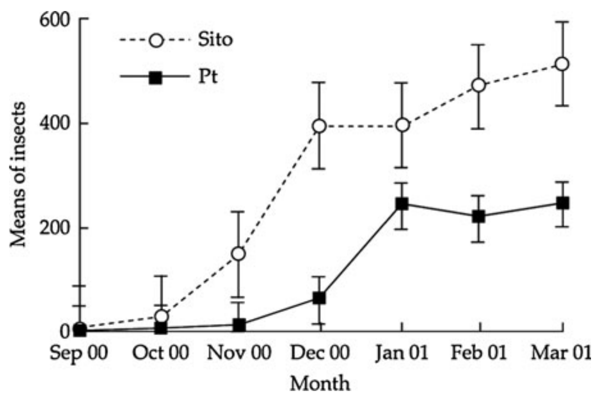

Fig. 2. Mean numbers (± SE) of *Sitophilus zeamais* (Sito) and *Prostephanus truncatus* (Pt) on 'non-protected' maize

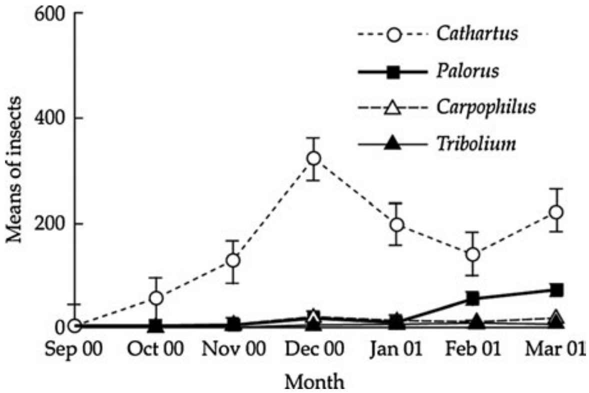

Fig. 3. Mean numbers (±SE) of secondary storage pests on protected maize

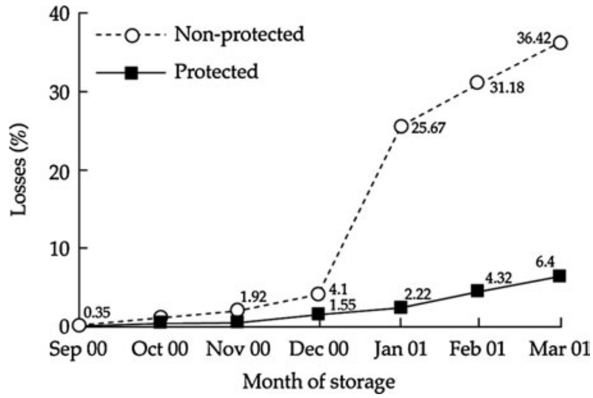

Fig. 5. Evolution of maize losses (%) during 6 months of storage in the two treatments

Fungal incidence on maize grains

Nine different fungal genera were identified on the maize grains: *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus*, *Curvularia*, *Trichoderma*, *Pestalotia*, *Mucor* and *Rhizoctonia*. Fungal incidence, independently of storage time, differed significantly between the two treatments for *Aspergillus* ($P = 0.0067$), *Fusarium* ($P < 0.0001$), *Penicillium* ($P = 0.0007$) and *Rhizoctonia* ($P < 0.0001$; Table 1). The relationship between grain losses caused by storage pests, the number of storage pests and fungi is summarized in Table 2. Per cent losses due to insects were significantly correlated with *Fusarium* spp. and *Penicillium* spp. ($P < 0.001$), but not with *Aspergillus* spp. incidence. Only *C. quadricollis* numbers were significantly correlated with *Aspergillus* spp. ($P = 0.0019$), but not with *Fusarium* spp. incidence. Except for *Cryptolestes* sp., all other storage pests' densities were significantly correlated with *Penicillium* incidence ($P < 0.0054$).

Toxigenic fungi

The aflatoxin producer *A. flavus* appeared on maize after 1 month of storage, whereas *Aspergillus parasiticus* was found on non-protected and protected maize after 4 and 5 months of storage, respectively. The fumonisin producer *F. verticillioïdes* colonized maize from the field; it was more abundant at the beginning of storage and its incidence decreased with storage time. Significant differences between both treatments were found for *A. flavus* and *F. verticillioïdes* incidence, while no such significant differences were recorded for *A. parasiticus* (Table 3). The incidence of *A. flavus* was positively correlated with the number of *P. truncatus*, *Cathartus* sp. and *Tribolium* sp.; the incidence of *A. parasiticus* with the number of *Sitophilus* sp., *Tribolium* sp., *Palorus* sp. and *Cryptolestes* sp., whereas the incidence of *F. verticillioïdes* was only negatively correlated with the number of *Cathartus* sp. (Table 4).

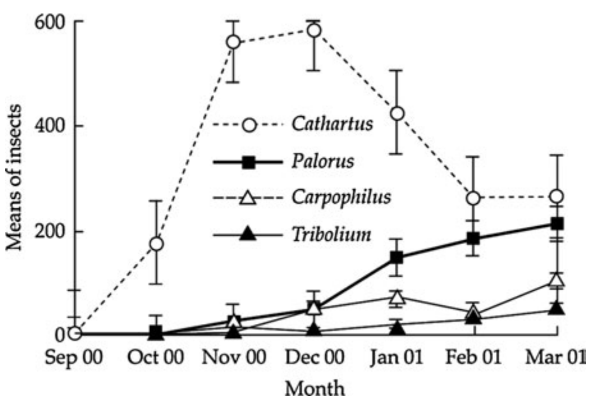

Fig. 4. Mean numbers (±SE) of secondary storage pests on non-protected maize

Fungi on storage pests

Fifteen different fungal genera were identified on the storage pests. The principal storage fungi *Aspergillus*, *Fusarium* and *Penicillium* were present. Other fungi such as *Curvularia*, *Trichoderma*,

Table 1. Effect of maize protection on incidence of different fungi on maize

	Non-protected	Protected
Aspergillus spp.	3.95 ± 0.82a	2.33 ± 0.62b
Fusarium spp.	36.05 ± 3.38a	16.62 ± 1.47b
Penicillium spp.	23.50 ± 3.93a	8.24 ± 1.56b
Rhizopus sp.	0.05 ± 0.05a	0.19 ± 0.11a
Curvularia sp.	0.15 ± 0.08a	0.05 ± 0.05a
Trichoderma sp.	0.10 ± 0.07a	0.05 ± 0.05a
Pestalotia sp.	0.05 ± 0.05a	0a
Mucor sp.	1.05 ± 0.37a	0.95 ± 0.29a
Rhizoctonia sp.	5.56 ± 1.87a	16.76 ± 2.57b

Means (±SE) within a column per fungal genera followed by the same letter do not differ significantly from each other (*t*-test, 5%).

Table 2. Correlation between losses (%), mean number of insects and the principal fungal genera on maize

	Aspergillus spp.	*Fusarium* spp.	*Penicillium* spp.
Losses	0.09	0.59***	0.58***
Prostephanus truncatus	0.16	0.50**	0.57***
Sitophilus sp.	0.14	0.32*	0.43**
Cathartus sp.	0.47**	0.03	0.60***
Carpophilus sp.	0.18	0.32*	0.53**
Tribolium sp.	0.24	0.62***	0.61***
Palorus sp.	0.12	0.56***	0.56***
Cryptolestes sp.	−0.13	0.42**	0.19
Gnatocerus sp.	0.06	0.56***	0.52**

*$P = 0.05$, **$P = 0.01$, ***$P = 0.001$.

Pestalotia, *Mucor*, *Colletotrichum*, *Monilia*, *Syncephalastrum*, *Rhizopus*, *Rhizoctonia*, *Bipolaris*, *Alternaria* and *Candida* were also found. The incidence of *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. present on *S. zeamais* and *C. quadricollis* was significantly different from that found on the other pests. A lower incidence of *Penicillium* spp. was found on *P. truncatus*, *Carpophilus* and *Gnatocerus* spp. (Table 5).

Immigration of pests to stores

The composition of pests found on the glue during the storage period did not differ much from the species found in the stores. *Rhyzopertha* sp. was the only insect identified as an immigrant on both non-protected and protected maize, which was subsequently not found inside the stores. There was a positive relationship between the immigration of *P. truncatus* and the immigration of all secondary pests, except for *Cryptolestes* sp. and *Rhyzopertha* sp.; *Sitophilus* sp. was significantly correlated only with *Carpophilus* sp. (Table 6).

Fungi on immigrant insects

The fungal spectrum identified on the immigrant pests differed little from that found on the pests inside the granaries. Three additional fungi (*Botryodiplodia* sp., *Plodia* sp. and *Macrophomina* sp.) were identified, but incidence of the immigrant pests was low. Statistical analysis of the incidence of the principal storage fungi on the immigrant pests revealed that *Cathartus* sp. conveys significantly more *Fusarium* spp. towards the stores, while *Cathartus* sp., *Sitophilus* sp. and *Palorus* sp. were the pests that convey significantly *Aspergillus* spp. and *Penicillium* spp. toward the stores (Table 7).

Table 3. Effect of maize protection on incidence of toxigenic species on maize

	Aspergillus flavus	*Aspergillus parasiticus*	*Fusarium verticillioïdes*
Non-protected	2.15 ± 0.59a	0.45 ± 0.19a	22.90 ± 2.82a
Protected	1.29 ± 0.07b	0.19 ± 0.11a	11.33 ± 1.23b

Means (± SE) within a column followed by the same letter do not differ significantly from each other (*t*-test, 5%).

Discussion

Insect infestation in maize stores

The storage trial showed that insect infestation of maize already starts from the field. *S. zeamais*, *C. quadricollis* and *Tribolium* spp. were identified at harvest on non-protected maize. These results corroborate those of Hell (1997), who found that the infestation of maize by storage pests in Benin starts in the field and is carried over into the store. The protection of the maize by gauze in the protected treatment could explain the reduction in the number of insects found on maize cobs at harvest compared with the non-protected ones. The pest spectrum encountered on maize stored in this trial did not differ from the spectrum found in traditional granaries by Borgemeister *et al.* (1994), who reported four out of the eight insect species found in this study. The insects known to infest

Table 4. Correlation between mean number of insects and incidence of toxigenic species encountered on maize during the 6 months of storage

	Aspergillus flavus	*Aspergillus parasiticus*	*Fusarium verticillioïdes*
Prostephanus truncatus	0.36*	0.12	0.23
Sitophilus sp.	−0.21	0.39*	−0.17
Cathartus sp.	0.32*	0.18	−0.31*
Carpophilus sp.	0.21	0.13	0.08
Tribolium sp.	0.33*	0.48**	0.08
Palorus sp.	0.23	0.42**	−0.03
Cryptolestes sp.	−0.08	0.40**	0.09
Gnatocerus sp.	0.23	0.24	0.04

*$P = 0.05$, **$P = 0.01$.

Table 5. Incidence of principal fungi on insects in maize stores

	Aspergillus spp.	*Fusarium* spp.	*Penicillium* spp.	Other fungi
Prostephanus truncatus	9.05 ± 3.14d	2.62 ± 1.41c	18.45 ± 5.56e	8.09 ± 3.13d
Sitophilus sp.	47.61 ± 4.84a	23.09 ± 4.22a	67.06 ± 5.14a	34.60 ± 3.89a
Cathartus sp.	39.86 ± 3.44a	18.49 ± 3.08a	73.17 ± 3.97a	35.65 ± 3.91a
Carpophilus sp.	32.02 ± 2.23b	8.45 ± 2.90b	40.47 ± 6.22cd	28.92 ± 5.21ab
Tribolium sp.	16.35 ± 4.09c	6.27 ± 2.23b	63.29 ± 6.20ab	24.29 ± 4.72bc
Palorus sp.	22.76 ± 3.33c	6.99 ± 2.92b	51.67 ± 5.64bc	29.38 ± 4.54ab
Cryptolestes sp.	8.21 ± 2.66d	0d	52.26 ± 7.07bc	20.24 ± 4.72bc
Gnatocerus sp.	8.57 ± 2.49d	1.87 ± 1.07c	33.23 ± 6.07d	17.46 ± 4.29c

Means (±SE) within a column followed by the same letter do not differ significantly from each other (*t*-test, 5%).

maize in the FMS are *S. zeamais*, *P. truncatus*, *Palorus* spp., *Carpophilus* spp., *Tribolium* spp., *C. ferrugineus* and *Gnatocerus* spp. All these insects were also found on our maize samples during the 6 months of storage, except for *P. truncatus*, which was not recorded on the protected maize. Sources for infestation can be (i) crop residues from the previous harvest; (ii) building material of the granaries that can harbour some of these insects; (iii) neighbouring granaries and (iv) poor hygienic conditions during storage (Pantenius, 1988). Storage pests can also be attracted towards the granaries by maize volatiles or in the case of *P. truncatus* by the male aggregation pheromone (Scholz *et al.*, 1998).

At the beginning of storage, compared with other insect species, high numbers of *S. zeamais* and *P. truncatus* were observed on non-protected maize, probably due to an insect succession by which secondary insects ('scavengers') appear on maize only after primary pests have damaged the grains. Secondary insects feed on flour and debris produced by primary insects. After 3 months of storage, the number of *Cathartus* spp. peaked at 585 per kilogram on non-protected maize. At these high densities, there is high intra- and interspecific competition for both food and space (Gaston *et al.*, 1999). Such interactions can lead to a reduction in the growth rate of certain species and sometimes a population reduction in the granary, as observed for *Cathartus* sp. in our experiment. Such competition between storage pests has previously been observed by Biliwa and Richter (1990) for *P. truncatus* and *S. zeamais*, and Meikle *et al.* (1998) found a negative correlation between the growth rate of *P. truncatus* and that of *S. zeamais* on maize stored in granaries in Benin.

Table 6. Correlation between mean number of primary insects and mean number of secondary insects immigrating toward the stores

	Prostephanus truncatus	*Sitophilus* sp.
Cathartus sp.	0.39**	0.20
Carpophilus sp.	0.59***	0.28*
Tribolium sp.	0.48***	0.07
Palorus sp.	0.76***	0.17
Cryptolestes sp.	0.15	0.02
Gnatocerus sp.	0.26*	0.14
Rhyzopertha sp.	−0.11	−0.15

*$P = 0.05$, **$P = 0.01$, ***$P = 0.001$.

Grain losses in stores

The recorded grain losses of 6.4% on protected maize after the 6 months of storage were mainly due to *S. zeamais*. Average losses of up to 10% have been previously recorded in Benin by Meikle *et al.* (1998) for *S. zeamais*. On non-protected maize, grain losses increased considerably, reaching 36.4% at 6 months of storage. Most of these grain losses can probably be attributed to a *P. truncatus* infestation. The incidence of this pest often increases after 3–4 months of storage (Hell, 1997). Likewise, trials conducted in Togo and Benin recorded grain losses due to *P. truncatus* of 30.2 and 20.5%, respectively, after 6 months of storage in traditional granaries (Pantenius, 1988; Fandohan *et al.*, 1992).

Fungal infection on maize kernels

Lower infection rates on protected than on non-protected maize at the beginning of storage indicated that the gauze significantly reduced fungal contamination. Either the gauze presented a physical barrier for fungal spores or the vectoring by birds and/or insects was impeded. *Fusarium* spp. incidence was high at the beginning of storage; *Aspergillus* spp. occurred only later in stores, reflecting the observations of Pitt and Hocking (1999), who classified *Fusarium* spp. among the field fungi and *Aspergillus* spp. as post-harvest fungi. *Fusarium* spp. incidence increased with time, indicating that insect activity might have led to a spread of the fungal spores, and the metabolic

Table 7. Incidence of principal fungi on immigrant insects

	Aspergillus spp.	*Fusarium* spp.	*Penicillium* spp.	Other fungi
Prostephanus truncatus	8.94 ± 2.69e	5.46 ± 2.26d	10.19 ± 3.13ef	15.46 ± 4.02e
Sitophilus sp.	24.13 ± 4.43bc	15.57 ± 3.61d	34.49 ± 5.02b	39.91 ± 5.29bc
Cathartus sp.	46.56 ± 2.85a	32.01 ± 3.04d	51.47 ± 3.17a	56.59 ± 3.45a
Carpophilus sp.	16.41 ± 3.57cd	13.10 ± 3.33bc	20.76 ± 3.92d	30.07 ± 4.78cd
Tribolium sp.	13.01 ± 5.53de	7.69 ± 2.88cd	23.96 ± 4.61cd	9.54 ± 5.01d
Palorus sp.	26.57 ± 3.88b	14.51 ± 3.20b	30.45 ± 4.15bc	40.24 ± 5.00b
Cryptolestes sp.	5.56 ± 2.72ef	5.56 ± 2.72d	11.11 ± 3.73ef	13.19 ± 3.96e
Gnatocerus sp.	7.87 ± 2.97e	5.09 ± 2.23d	16.67 ± 4.32de	24.77 ± 4.89d
Rhyzopertha sp.	2.71 ± 1.60f	5.00 ± 2.29d	5.56 ± 2.5f	13.19 ± 3.83e

Means (± SE) within a column followed by the same letter do not differ significantly from each other (*t*-test, 5%).

activity of pests might have increased moisture levels (Beti *et al.*, 1995; Schulthess *et al.*, 2002). The positive correlation recorded between the number of the majority of the storage pests and the incidence of *Fusarium* spp. and *Penicillium* spp. indicates that secondary pests, which occurred in high numbers, seem to be the principal disseminators of fungi in the granaries even though they do not cause high losses.

Toxigenic fungi

The principal toxigenic species *A. flavus*, *A. parasiticus* and *F. verticillioïdes* recorded in this study were also observed earlier by Hell (1997) in the same agro-ecologic zone in Benin. Cardwell and Cotty (2002) found that *A. flavus* was the predominant *Aspergillus* species, with 90% belonging to this group. Several authors have pointed out the role that storage pests play in the transmission of spores of toxigenic species in the field or on stored maize. Wright (1998) observed that *S. zeamais* infestations favour *A. flavus* incidence in the granaries through its feeding activity that damages grains, thereby facilitating the transmission of fungal spores into the grains. According to Dowd (1991), high *S. zeamais* populations can change the environmental conditions in the granaries to the advantage of fungi. Also certain toxigenic species of *Penicillium* spp. and *F. verticillioïdes* were found to be vectored by *S. zeamais* (Wright *et al.*, 1980; Munkvold and Carlton, 1997).

Fungal infection on storage pests

Some fungal species were found on certain storage pests but not on the maize. These species might be fungi that have a symbiotic relationship with pests as described by Dowd (1991), or maize is not a good substrate and/or host material for the development of these fungi. *S. zeamais* and *C. quadricollis* seem to be the best disseminators of fungal spores since spores of *Aspergillus*, *Fusarium* and *Penicillium* spp. were more often found on these than on other storage pests. Several authors reported *S. zeamais* vectoring fungal spores into maize stores (see above). In *C. quadricollis* possibly, its high mobility in the store, due to the insect's small, elongated body-shape, enables it to clear a way between the husks, and through intact grains (Gwinner *et al.*, 1996).

Immigration of pests to stores

Attraction plays a role in the migration of pests towards a store, either by pheromones produced by conspecifics already present in the granaries or by the odour of damaged maize grains and maize starch (Scholz *et al.*, 1997). Insects seem to be also attracted to maize infected by fungi. Ako *et al.* (2003) found that *S. zeamais* and *C. dimidiatus* are attracted towards maize infected by *F. verticillioïdes*. Likewise, Schulthess *et al.* (2002) previously suggested that some lepidopteran and coleopteran pests are attracted and survive longer or have lower mortality on plants infected with *Fusarium* spp.

The positive correlation between *P. truncatus* and the majority of the secondary insects is due to the tunnelling behaviour of the beetle that results in the production of large amounts of flour and damaged grains (Pantenius, 1988) and induces fungal growth. That was not the case for *S. zeamais*, in which density was only correlated with *Carpophilus* spp., possibly because of the affinity of both insects towards deteriorating grains (Wright *et al.*, 1980).

Fungal incidence on immigrating pests

The fungal spectrum associated with immigrant insects showed that *Cathartus* spp. are likely to vector more fungi towards granaries, in particular *Aspergillus*, *Fusarium* and *Penicillium* spp., than other post-harvest maize pests. This is a new finding since the role of *Cathartus* spp. in the propagation of fungal spores and in the predisposition of maize to fungal infection has not been previously described.

Conclusion

This paper shows a novel avenue for the control of infestation of maize by toxigenic fungi through the protection of maize cobs with gauze before pollination; whether this is a practical solution for small-scale farmers in Africa needs to be verified in future studies.

Acknowledgements

We thank Pascal Gbelissanon for field assistance, Josephine Hotegni and Georg Georgen for laboratory assistance and the late Saka Gounou for critical review of this manuscript. This work was supported by the Aflatoxin Project of the International Institute of Tropical Agriculture (IITA), funded through BMZ/GTZ.

References

Ako M., Schulthess F., Gumedzoe M. Y. D. and Cardwell K. F. (2003) The effect of *Fusarium verticillioides* on oviposition behaviour and bionomics of lepidopteran and coleopteran pests attacking the stem and cobs of maize in West Africa. *Entomologia Experimentalis et Applicata* 106, 201–210.

Beti J. A., Phillips T. W. and Smalley E. B. (1995) Effects of maize weevils (Coleoptera: Curculionidae) on production of aflatoxin B by *Aspergillus flavus* in stored corn. *Journal of Economic Entomology* 88, 1517–1838.

Biliwa A. and Richter J. (1990) Efficacité d'insecticides binaires en poudre sur du maïs égrené, stocké en sacs, pp. 1577–1585. In *Advances in Stored Product Protection: Proceedings of the 5th International Working Conference on Stored-Product Protection* (edited by F. Fleurat-Lessard and P. Ducom). 9–14 September 1990, Bordeaux, France. Imprimerie du Médoc, Bordeaux.

Borgemeister C., Adda C., Djomamou B., Degbey P., Agboka A., Djossou F., Meikle W. G. and Markham R. H. (1994) The effect of maize cob selection and stored maize losses by the larger grain borer *P. truncatus* (Horn) (Col.: Bostrychidae) and associated storage pests, pp. 909–919. In *Stored Product Protection.* Proceedings of the 6th International Working Conference on Stored Product Protection II, Canberra, Australia (edited by E. Highley, E. J. Wright, H. J. Banks and B. R. Champ). CAB International, Wallingford.

Boxall R. A. (1986) *A Critical Review of the Methodology for Assessing Farm-Level Grain Losses after Harvest*. TDRI, London. 139 pp.

Cardwell K. F. and Cotty P. J. (2002) Distribution of *Aspergillus* section Flavi among field soils from the four agroecological zones of the Republic of Benin, West Africa. *Plant Disease* 86, 434–439.

Cardwell K. F., Kling J. G., Maziya-Dixon B. and Bosqué-Perez N. A. (2000) Interactions between *Fusarium verticillioides*, *Aspergillus flavus*, and insect infestation in four genotypes in lowland Africa. *Phytopathology* 90, 276.

Délobel A. and Tran M. (1993) *Les coléoptères des denrées entreposées dans les régions chaudes*. CTA/ORSTOM, Paris. 424 pp.

Dobie P., Haines C. P., Hodges R. J., Prevett P. F. and Rees D. P. (1991) *Insects and Arachnids of Tropical Stored Products: Their Biology and Identification (A training manual).* 2nd edn. Natural Resources Institute, Chatham. 246 pp.

Dowd P. F. (1991) Nitidulids as vectors of mycotoxin-producing fungi, pp. 335–342. In *Aflatoxin in Corn. New Perspectives.* North Central Regional Research Publication 329 (edited by O. L. Shotwell and C. R. Hurburgh). Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa.

Dowd P. F. (1998) Involvement of arthropods in the establishment of mycotoxigenic fungi under field conditions, pp. 307–350. In *Mycotoxin in Agriculture and Food Safety* (edited by K. K. Sinha and D. Bhatnagar). Marcel Dekker, New York.

Fandohan P., Langner B. and Mutlu P. (1992) Distribution, recherche et contrôle du Grand Capucin du maïs *Prostephanus truncatus* (Horn) au Benin, pp. 18–28. In *Implementation of and Further Research on Biological Control of the Larger Grain Borer* (edited by J. Boeye, M. Wright and G. A. Laborius). GTZ, Eschborn.

Gaston K. J., Gregory R. D. and Blackburn T. M. (1999) Interspecific relationships between abundance and occupancy among species of Paridae and Sylviidae in Britain. *Ecoscience* 6, 131–142.

Gwinner J., Harnish R. and Mück O. (1996) *Manuel sur la manutention et la conservation des graines après récolte*. GTZ, 368 pp.

Hell K. (1997) Factors contributing to the distribution and incidence of aflatoxin producing fungi in stored maize in Benin. PhD Thesis, University of Hannover, Germany. 143 pp.

ISO (1980) Determination of moisture content (on milled grain and whole grain). ISO 6540 180, Geneva, Switzerland.

King S. B. and Scott G. E. (1981) Genotypic differences in maize to kernel infection by *Fusarium moniliforme*. *Phytopathology* 71, 1245–1247.

Klich M. A. and Pitt J. I. (1998) A laboratory guide to the common *Aspergillus* species and their teleomorphs. Commonwealth Scientific and Industrial Research Organisation, Division of Food Processing, North Ryde, New South Wales, Australia. 116 pp.

Marasas W. F. O. (1988) Medical relevance of mycotoxins in southern Africa. *Microbiology Aliments, Nutrition/Microbiology, Food and Nutrition* 6, 1–5.

Meikle W. G., Markham R. H., Holst N., Djomamou B., Schneider H. and Vowotor K. A. (1998) Distribution and sampling of *Prostephanus truncatus* (Horn)

(Coleoptera: Bostrichidae) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in maize stores in Benin. *Journal of Economic Entomology* 91, 1366–1374.

Miller J. D. (1995) Mycotoxins in Asia: policies for the future. *ACIAR Postharvest Newsletter* 32, 5–15.

Munkvold G. P. and Carlton W. M. (1997) Influence of inoculation method on systemic *Fusarium moniliforme* infection of maize plants grown from infected seeds. *Plant Disease* 81, 211–216.

Pantenius C. U. (1988) *Etat des pertes dans les systèmes de stockage du maïs au niveau des petits paysans de la région maritime du Togo*. GTZ, Eschborn, Germany. 83 pp.

Pitt J. I. and Hocking A. D. (1999) *Fungi and Food Spoilage* 2nd edn. Chapman & Hall, Gaithersburg, Maryland. 593 pp.

SAS Institute (2003) SAS User's Guide: Statistics, version 9.1., 9th edn. SAS Institute, Cary, North Carolina.

Scholz D., Tchabi A., Borgemeister C., Markham R. H., Poehling H.-M. and Lawson A. (1997) Host-finding behaviour of *Prostephanus truncatus*: primary attraction or random attack? *Journal of Applied Entomology* 121, 261–269.

Scholz D., Tchabi A., Markham R. H., Poehling H.-M. and Borgemeister C. (1998) Factors affecting pheromone production and behavioural responses by *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). *Annals of the Entomological Society of America* 91, 872–878.

Schulthess F., Cardwell K. F. and Gounou S. (2002) The effect of endophytic *Fusarium verticillioïdes* on infestation of two maize varieties by lepidopterous stemborers and coleopteran grain feeders. *Phytopathology* 92, 120–128.

Watanabe T. (1994) *Pictorial Atlas of Soil and Seed Fungi. Morphologies of Cultured Fungi and Key to Species*. Lewis Publishers, London. 411 pp.

Wright V. F. (1998) Assessment of insect infestation in stored maize and their relationship to *Aspergillus flavus* contamination. In *Mycotoxin Prevention and Control in Food Grains* (edited by R. L. Semple, A. S. Frio, P. A. Hicks and J. V. Lozare). UNDP/FAO Regional Network Inter-Country Cooperation on Preharvest Technology and Quality Control of Foodgrains (REGNET) and Asean Grain Post harvest Programme. Bangkok, Thailand.

Wright V. F., Harein P. K. and Collins N. A. (1980) Preference of the confused flour beetle for certain *Penicillium* isolates. *Environmental Entomology* 9, 213–216.