THE RED FLOUR BEETLE , *Tribolium castaneum* (Herbst) : EFFECT OF FRASS ON OCHRATOXIN A PRODUCTION AND AGGREGATION PATTERN IN AERATED WHEAT COLUMN

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Namita Goswami

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NAMITA GOSWAMI

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To My Mother

who taught me to value knowledge

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ABSTRACT

The red flour beetle, *Tribolium castaneum* (Herbst) is a common pest of stored grain in Canada. The insects contribute to an increase in the temperature and moisture content of the grain which dramatically influences its stability and quality. In addition, through oviposition and feeding, *T. castaneum* can act as a vector for the dissemination of fungal spores. Aesthetics aside, the excrement or frass produced during feeding is relatively high in nitrogen, (ca. 9-10%) and may serve as a metabolite for fungal growth and or mycotoxin production. In this investigation, the effects of excreta or frass from *T. castaneum* on ochratoxin A (OA) production by *Aspergillus ochraceus* was evaluated at 7, 14, and 21 days of incubation using either whole barley or wheat (30 g). Ground wheat was similarly investigated. Results indicated that the addition of frass (3, 6 or 9%) to barley did not result in enhanced OA production. In whole or ground wheat, however, frass appeared to have an impact on the rate of OA production. In the absence of frass, OA levels peaked, usually at 14 d. In contrast, OA production in wheat containing frass did not peak during the incubation period, but rather increased at a slow steady rate. In the case of whole wheat, a maximum level of OA (26.1μg/g) was obtained at 21 d when 9% frass was employed. OA levels in ground wheat containing frass were significantly higher ($P<0.05$) than in wheat without frass at 21 days. Uric acid, the main nitrogenous component of frass (ca. 87%) significantly increased ($P<0.05$) OA production compared to control. Biomass

accumulation (glucosamine) of *A. ochraceus* in whole or ground wheat peaked at 14 d. The addition of frass appeared to have a moderating effect on biomass accumulation. Levels did not peak during the incubation period, but rather increased at a slow steady rate. This effect was also shown when uric acid replaced frass. OA production in non sterile wheat was greatly reduced. The presence of frass in non sterile wheat further reduced OA production. Aeration which provides for a very effective method of controlling temperature and moisture in stored grain, was investigated with respect to insect movement in stored grain. Using sectioned, simulated grain towers, the distribution profile of *T. castaneum* (100 insects; mixed sexes) released on the uppermost section of either aerated (1.7 L/sec) or non aerated grain columns, was examined. At 12 d, results indicated a difference in insect migration within aerated and non aerated columns. Specifically, in aerated columns, a significant difference ($P<0.05$) in insect density was observed between the uppermost and bottom sections. This distribution profile was not observed in non aerated columns. The distribution profile between male and female beetles within a column was not significantly different ($P>0.05$). Significant differences were also not observed between column types ($P>0.05$).

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INTRODUCTION

Bulk grain can be stored for prolonged time periods with minimum qualitative and quantitative losses. This can only be achieved, however, if the grain is protected from the elements of weather, insects, rodents, birds, and fungi. In particular temperature and moisture must be closely controlled usually in association with aeration. The moisture content of grain is the single most important determinant governing spoilage. Moisture content levels higher than 13-15% (moisture activity 0.70 ; A_w) usually result in deterioration even after relatively short periods (2 to 3 months) of storage and at temperatures as low as 5° C. (Williams, 1991). Higher levels of moisture stimulate fungal germination and insect reproduction both of which are leading causes of damage to stored grain (Christensen and Kaufmann, 1969). With respect to fungi, two main groups are associated with the deterioration of cereals. Field fungi, characteristically colonize ripening grain and include *Alternaria, Cladosporium* and *Fusarium* spp. but seldom develop further since they normally require a high A_w (0.90). By contrast, storage fungi are present in low numbers before harvest but develop rapidly in stored grain when conditions become suitable. Storage fungi include species of *Aspergillus* and *Penicillium* which can develop at low temperatures (5 to 10° C) (Williams, 1991). Storage fungi are capable of growth at A_w levels of 0.65 to 0.90. In many instances the growth of these fungi progress sequentially as the moisture content in the grain increases. For example, at a

moisture content of 13.5 to 14.5% (A_w 0.65-0.70) *A. restrictus* may develop. In creasing the moisture content to 14- 15% (A_w 0.70-0.75) will encourage *A. glaucus* to develop. At an A_w of 0.75 to 0.80, *A. candidus* and *A. ochraceus* will develop, while at an A_w 0.85, *A. flavus* will develop. *Penicillium* spp. usually require a higher moisture content and seldom develop on cereals if the A_w is below 0.80 to 0.90. The increased moisture content in grain is often due to several factors including respiration by grain fungi and insects and by moisture migration due to temperature differentials within the grain bulk which appear more critical in the winter months.

Insects are also a major cause of loss in stored grain (Cotton and Wilbur, 1974). Among grain insects, *Cryptolestes ferrugineus* (rusty grain beetle), *C. pusillus* (flat grain beetle) *Tribolium castaneum* (red flour beetle), *T. confusum* (confused flour beetle) and grain mite, *Acarius silo* are the most tolerant of low temperatures and dry climates of Canada and northern United States. *Tribolium* spp. in particular can survive in very low (less than 8%) moisture environments (Williams, 1991). These insects damage grain by eating the endosperm. They also contaminate grain via fecal pellets or frass, and by their exoskeletons and dead bodies (Cotton and Wilbur, 1974). In addition they serve as substrates for the development of fungi (Sinha and Watters, 1985). Storage fungi usually accompany or follow insect infestation. Insects carry fungal spores via their gut, setaceous body parts, ovipositor and mouthparts. During feeding and reproduction their metabolic activity increases the moisture level and temperature of the grain making it suitable for fungal growth (Dunkel, 1988). Many insects are dependent on fungi for food (fungivores) and or essential nutrients. For example, some insects depend on fungi for the B-group vitamins which are not available in their diet (Van Wyk et al., 1959). The combined activity of both insects

and fungi invariably lead to kernel damage. The damaged kernels are ideal sites for further microbial and fungal activity and mite infestation (Williams, 1991). The fungi also interact or compete with each other for moisture and nutrients (Panasenko, 1967). Among the fungi, the *Aspergillus, Penicillium* and *Fusarium* spp. are prolific mycotoxin producers. Mycotoxins are secondary metabolites produced by fungi which are known to be carcinogenic, mutagenic and teratogenic. These mycotoxins are becoming an increasingly serious problem in the animal and human health area (Bhatnagar et al., 1992) The role of *Aspergillus* spp. and insect damage in preharvest grain, contaminated with mycotoxins was reported by several authors (Anderson et al., 1975; Lillehoj et al., 1975; Smith and Riley, 1992). Toxin formation may occur in the field and or during storage. One important mycotoxin with respect to Canadian prairie grain is ochratoxin. Several species of *Penicillium* and *Aspergillus* produce ochratoxin. Ochratoxin is known to cause kidney and liver damage in farm and laboratory animals and has been suggested as the causative agent of nephropathy, a fatal chronic kidney disease in cows and pigs (Krogh, 1991).

The interaction between moisture and temperature is the most important aspect affecting grain preservation. For ideal grain storage, both the temperature and moisture should be kept as low as practicable. Aeration is an effective method for the control of both temperature and moisture. With a slow constant flow of air, both condensation caused by natural movement of air and hot spots created by insects, fungi or both can be prevented. This requires the action of blowing or suction fans. Blowing is practical especially if the air can be warmed slightly so that the relative humidity can be reduced thereby enabling it to carry more moisture. Air must be efficiently removed from the top of the grain bin to prevent condensation on the roof, particularly

in metal bins (Williams, 1991). Aeration using ambient air is the most cost effective method of protecting grain from both insects and fungi and thereby mycotoxin production.

The purpose of this study was two fold : (1) to assess the affect of insect excreta or frass on the growth and mycotoxin production of fungi in grain. (2) To assess the effects of aeration in a grain column with respect to insect movement and or aggregation and In order to evaluate these effects, a common but important grain infesting insect, the red flour beetle, *Tribolium castaneum* (Herbst) was chosen. *Aspergillus ochraceus*, which is known to produce ochratoxin in storage grain, was selected as the representative fungal organism.

The manuscript will be presented in the form of two sections. The first section will deal with the effects of insect frass on ochratoxin production while the second section will deal with the effects of aeration on insect movement in a simulated grain bin.

SECTION I

ROLE OF INSECT FRASS ON OCHRATOXIN A PRODUCTION

REVIEW OF LITERATURE

INSECTS

Stored grain products are prone to insect infestation which can result in both qualitative and quantitative losses. These losses can be prevented or minimized by storing grain as dry as possible in clean, weatherproof, uninfested, and preferably aerated granaries. In empty granaries, insect hide in cracks and crevices where they can survive in grain residues until they infest newly harvested grain. Storage insects do not hibernate (Cotton, 1954; Sinha and Watters, 1985) therefore their abilities to survive during winter in unheated silos is relatively low (Freeman, 1973 ; Williams, 1991). Some cold-hardy insects, however, do survive. More than fifty species of insects and mites have been reported to infest stored grain in Canada's prairie provinces (Mills, 1986). Only a few species cause severe damage; these include four major types: weevils, moths, beetles and scavengers. The weevils, including the granary weevil, rice weevil and the maize weevil generally develop inside the grain kernel. These insects are widely distributed and are frequently the most destructive of all the insects (Cotton, 1954).

Insect Damage - Direct Effects

Insects directly damage grain through feeding and by oviposition. Both these actions contribute to the deterioration of the seed envelope (pericarp) resulting in enhanced fungal contamination and growth.

Granary weevils, *Sitophilus granarius* (Linnaeus) which are perhaps the most destructive of the pests, are known to damage grain by boring holes in the kernels to reach the soft endosperm. Using their snouts, females excavate holes in the kernels into which they oviposit their eggs (usually one egg per hole). These holes are later sealed with a gelatinous fluid in order to protect their egg (Cotton 1954). During development each weevil larva can consume as much as 30 mg of grain, leaving ca. 6 mg of debris. At 30°C and 70% relative humidity larvae have been reported to consume as much as 64% of the wheat kernel, which represents all of the germ and a large part of the endosperm (Sinha and Watters, 1985). In many cases rice weevils, *Sitophilus oryzae* (Linnaeus) have been reported to develop on two sides of a single kernel at the same time. A single insect can destroy about 30% of a wheat kernel during its development from egg to adult (Sinha and Watters, 1985). During growth in stored wheat at 30°C for 20 weeks, weevil damage to kernels was reported to range from 80-100% (Sinha and Watters, 1985). During its 100 day lifetime, female weevils can reproduce three to four times and are capable of laying several hundred eggs. Within a few weeks an enormous increase in population is therefore possible under favorable conditions (Cotton and Wilbur, 1974). In addition to direct destruction by feeding, contamination of the grain also occurs during larval development from cast off skins or exoskeletons. This process can occur four times during insect development.

The lesser grain borer, *Prostephanus truncatus*, differs from the weevil in that its eggs are deposited on the outside of the kernel. Feeding larvae and adults can reduce the grain kernel to a bran shell and in the process produce an inordinate quantity of fecal pellets. Large quantities of fecal-pellet-dust can accumulate in the grain mass. The pellets have been reported to have a sweetish, musty odor that characterizes their infestation (Cotton and Wilbur, 1974).

Moths generally oviposit on the surface of grain. The developing caterpillars then burrow into the kernels. In the case of meal moth, *Pyralis farinalis*, larvae can cause up to 99 % loss in the wheat kernel (Sinha and Watters, 1985). Undamaged kernels are eaten only when the moisture content is greater than 20%. The Indian meal moth, *Plodia interpunctella* (Hübner) which is found in many grains, cereals and nuts is restricted to the upper 20 cm of the grain bulk. Meal moths usually occur in patches of moldy grain and contaminate grain through their feces and cast skin. Dead specimens and cocoons also contribute to contamination. In addition, the larvae are known to produce a silken substance that often webs grain kernels into clumps.

In Canada, the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) and the red flour beetle *Tribolium castaneum* (Herbst) represent two major pests of stored grain (Mills, 1986). Normally these insects feed outside the kernels. However, the larvae will penetrate into the germ area and remain there during the developmental period. Beetles damage grain and cereal products through feeding and contamination, viz dead bodies, cast skins and fecal pellets or frass (Sinha and Watters, 1985; Cotton and Wilbur, 1974). The red flour beetle, *T. castaneum* and the confused flour beetle, *T. confusum* have odoriferous glands that secrete a pungent, irritating liquid containing quinones. In flour, a pink discolouration can develop due to this secretion.

The secretion has been reported to contain 80-90% 2-ethyl-1, 4-benzoquinone, 10-20% 2-methyl-1, 4-benzoquinone and a trace of 2-methoxy-1, 4-benzoquinone (Loconti and Roth, 1953).

Insects that feed on moldy grains are known as scavengers and do not cause great damage unless they are present in large numbers. The meal worm, *Tenebrio* spp., the hairy fungus beetle, *Typhaea stercorea* (Linnaeus), the foreign grain beetle, *Ahasverus advena* (Waltl.) the corn sap beetle, *Corpophilus dimidiatus* (F.) and the black fungus beetle, *Alphitobius piceus* (Oliv.) are some important scavengers found in stored grain (Cotton, 1954).

Insect Damage -Indirect Effects

Vectors of fungal dissemination

Grain infesting insects, scavengers and mites serve as vectors for the dissemination of fungi and their spores. During feeding and oviposition insects transport fungi via external appendages and hair and or internally via their digestive tract (Mills, 1986; Dunkel, 1988). For example Dix (1984) reported that corn weevils, *S. zeamais* were naturally contaminated with *Aspergillus flavus* spores as well as spores of *penicillia*. Fecal droppings or frass discharged during feeding is also known to contain fungal spores

(Griffiths et al., 1959). Certain insects including confused flour beetle, *T. confusum* are fungivores and therefore partially depend on fungi for their growth and development. These types of insects would be especially important in the distribution of fungi and their spores. In addition some insects have been reported to carry mycotoxins. These mycotoxins have been detected through metamorphosis from larva

to the adult stage (Eugenio et al., 1970). These insects have metabolic adaptations (enzymes) which enable them to deal with fungal metabolites (Wright et al., 1980 b). For example the confused flour beetle, *T. confusum* and cigarette beetle, *Lasioderma serricorne* show little or no ill effects from ochratoxin A when exposed to 1000 ppm. These insects have a longer history of exposure to mycotoxins and thus are relatively resistant to their effects (Dowd, 1992).

Distribution of bacteria

Husted et al. (1969), reported that the rice weevil, *Sitophilus oryzae* (Linnaeus) retained *Salmonella montevideo* both internally and externally for seven days after exposure to contaminated wheat. When exposed to contaminated wheat from 14 to 21 days, the insects retained *Salmonella* for at least five weeks. *Escherichia intermedia, Bacillus subtilis, Serretia marcescens, Proteus vulgaris, Micrococcus spp, Streptococcus spp* and various bacteria in the *Klebsiella-Aerobacter* group have been isolated from the gut and feces of the granary weevil, *S. granarius* and the yellow meal worm, *Tenebrio molitor* (Linnaeus) (Brooks, 1963; Harein and de las Casas, 1968). Large numbers of bacteria have been isolated from the larvae and adults of the confused flour beetle, *T. confusum*. Bacterial numbers appeared more numerous within the insects than in the food from which the insects were taken (Van Wyk et al., 1959). Van Wyk et al. (1959) reported that certain bacteria growing in the gut of insects appeared to supply B vitamins to the host.

Sources of moisture and heat

Insects produce metabolic water and heat both in the larval and adult stages.

Christensen and Hodson (1960) demonstrated that when 200 granary weevils, *S. granarius* were placed into a sealed container with 2-4 kg of wheat after 2-6 months, the moisture content rose by 3%. The temperature also increased by 6° C. Dix (1984) demonstrated that the moisture content of maize, experimentally infested with corn weevils, *S. zeamais* for 16 weeks rose 25%; the temperature also increased by 5° C. Agrawal et al. (1957) also reported that wheat infested with the granary weevil, *S. granarius* increased in moisture content from 18 to 23% within three months. Similar increases in the moisture content of grain due to weevil infestation have been reported by Sinha (1984).

Since insects possess a relatively high metabolic activity, their contribution to an overall increase in heat and moisture is far greater and more important than that contributed by dormant grain or microorganisms (Milner and Geddes, 1954). For example, the rusty grain beetle, *C. ferrugineus* (Stephens) through its metabolic activity can generate sufficient heat and moisture to create hot spots in grain during winter storage (Sinha and Watters, 1985). Burges and Burrell (1964) also reported that the adult saw toothed grain beetle, *O. surinamensis* could produce as much as 677 calories of metabolic heat at 25° C during a 24 hour feeding period in wheat (density of 80 insects/kg wheat). Eighme (1966) concluded that among the four primary grain infesting insects, the granary weevil, *S. granarius* had the greatest ability to initiate and promote the spread of hot spots.

Factors Affecting Growth and Reproduction of Insects

Food

Insects are generally associated with an excess of food, therefore quantity usually

is not a factor. In contrast, the quality of food greatly affects the ability of insects to develop and mature. Abushama and Al-Jeraiwi (1987) studied the food preference and development of the red flour beetle, *T. castaneum* and observed that wheat flour and semolina were more suitable than rice and broken wheat for its development. In general, food materials of relatively small size are preferred over larger particles. Stored grain insects require vitamins of the B group (Van Wyk et al., 1959). However, some stored product insects have lower vitamin requirements than others, possibly owing to the presence of intercellular microorganisms or symbiots that provide accessory food substances (Cotton and Wilbur, 1974).

Temperature and Moisture

Generally the rate of development of stored product insects increases from 20-35°C. Death usually occurs within 5-7 degrees above this range. Death at low temperatures is variable (Sinha and Watters, 1985); development normally ceases at 10°C or lower. Insects require moisture and are attracted to damp grain. However, moisture requirements differ among insect species. Some insects can survive and adapt in a low moisture environment due to their highly developed excretory system (Cochran, 1978). For example, the rusty grain beetle, *C. ferrugineus* and red flour beetle, *T. castaneum* are both well adapted to survive cold Canadian Prairies, due to their low (minimum relative humidity 1%) humidity tolerance (Sinha and Watters, 1985).

Grain dockage

In order to reproduce in whole grain, the red flour beetle, *T. castaneum* requires

a high moisture content. However, the presence of dockage in grain helps them to reproduce even after the moisture has been completely eliminated. McGregor (1964) observed that the development of the red flour beetle, *T. castaneum* in grain was influenced by the presence of dockage; the number of insects present being directly proportional to the amount of dockage.

FRASS

Stored product insects produce relatively dry fecal pellets, with uric acid as the main nitrogenous component. Uric acid and its ammonium salts are insoluble and may be eliminated with very little loss of water. Uric acid makes up 80% or more of the nitrogenous constituents of excreta in terrestrial insects (Stobbart and Shaw, 1974). The measurement of uric acid is therefore a good indicator of insect infestation in grain or grain products. Sen (1968) compared the rate of excretion of uric acid by larvae, pupae and adults of the red flour beetle, *T. castaneum* and reported that excretion of uric acid per mg body weight for larvae was higher than that of the adults.

The amount of frass produced by insects vary. For example, a rice weevil, *S. oryzae* larva converts about 14 mg of grain into carbon dioxide and water and produces 14 mg of frass, during development into a weevil weighing 2.4 mg. van Bronswijk and Sinha (1971), measured the amount of uric acid in grain infested by both the rusty grain, *C. ferrugineus* and the saw toothed beetle, *O. surinamensis*. A similar determination was made in grain infested with both the granary weevil, *S. granarius* and red flour beetle, *T. castaneum*. The authors reported that the combination of the granary weevil, *S. granarius* and the red flour beetle, *T. castaneum* produced 20% more uric acid (ca. 0.11 mg uric acid/mg of grain dust) than the other insect

combination (ca. 0.09 mg uric acid/mg of grain dust). Gupta and Sinha (1960), also reported that the uric acid content in the frass of the granary weevil, *S. granarius* averaged 11.68% as compared to 17.67% in the confused flour beetle, *T. confusum* and 19.58% in the rusty grain beetle, *C. ferrugineus*. The uric acid content is low in the frass of the granary weevil, *S. granarius*. Unlike *T. confusum* (confused flour beetle) the granary weevil, *S. granarius* retains a portion of uric acid and urates in its fat body .

Studies performed by Weaver et al. (1990) showed that frass from mealworm larva, *Tenebrio molitor* (L) contained 0.08 g of butyric acid, 0.03 g of propionic acid, 0.02 g of valeric acid and 0.24 g lactic acid per 100 g frass. These acids were not present in the insect diet; acetic acid (0.25 g /100 g frass) was present both in the diet and in the frass.

Frass can serve as a source of microbial contamination; high concentrations in grain may also act as chemical messengers, since both frass and lactic acid were found to be attractive to larvae. When present in high concentrations frass and lactic acid were shown to attract mealworm larvae. In contrast, butyric, acetic, propionic and valeric acid were repellent to the larvae (Weaver et al., 1989, 1990).

Factors Affecting Frass Production

Factors which assist insects in feeding also contribute to frass production. For example, the saw toothed grain beetle, *O. surinamensis,* cannot attack undamaged grain unless it is moist and soft. Generally the presence of dust, broken kernels and dockage promotes infestation by the saw toothed grain weevil, *O. surinamensis* in warm and often freshly harvested grain. The type of grain is also important. The

granary weevil (*S. granarius*) can reproduce three to four times faster on whole wheat and barley as compared to whole oats at 30°C and 70% relative humidity (Sinha and Watters, 1985). Temperature is also an important factor in frass production since it influences growth and reproduction. For example, the minimum and optimum temperature for development of rusty grain beetle *C. ferrugineus* is 22°C and 33°C respectively (van Bronswijk and Sinha, 1971).

FUNGI

Fungi are a major cause of grain spoilage and rank second only to insects. More than 150 types of fungi have been isolated from cereal grains. These types include both field and storage fungi varieties. Field fungi, including *Alternaria*, *Cladosporium* and *Fusarium* seldom undergo further development in storage due to their high moisture requirement (22 to 23% wet weight basis or 30 to 33% on a dry weight basis; (Christensen and Kaufmann, 1974). Most types gradually die off during storage when the moisture content is below that required for growth (Wallace, 1973; Mills, 1986; Magan and Lacey, 1988; Trojanowska, 1991). Storage fungi, particularly *Penicillium* and *Aspergillus* species, which are generally present in low numbers before harvest, develop during storage when conditions become suitable. The *penicillia* normally invade grain kernels with a moisture content of 17% and higher, whereas *aspergilla* can grow below 17% (Trojanowska, 1991; Magan and Lacey, 1988; Christensen and Kaufmann, 1969). Storage fungi can grow in environments in which the intergranular air is in equilibrium with a relative humidity of 70-90% (Christensen and Kaufmann, 1974).

Conditions Required for Growth of Storage Fungi

Moisture

The moisture content of grain is one of the most important factors influencing stability. For example, grain initially containing 12.6% water (0.60 A_w), has been stored experimentally for up to 16 years (Lacey and Magan, 1991). Humidity limits within which growth of the fungi are possible, are not as wide as those for temperature. For example, *Aspergillus niger* can develop at temperatures from 6° to 45°C, however, its relative humidity range is only 88 to 100% (Panasenko, 1967). During storage small increases in relative humidity especially in the 71-75% range (14.5-15% m.c.) can result in large differences in fungal growth (Christensen and Linko, 1963; Sauer and Christensen, 1966). Christensen and Kaufmann (1974), analyzed cereals from several countries and reported that two fungal species were consistently isolated. The organisms, *A. restrictus* and *A. glaucus* were shown to initiate growth at a relative humidity of 71-75% (m.c. of 13-14%). When a moisture content of 13-14% was reached, the *A. glaucus* group appeared on the grain. Through metabolic activity, the moisture content was raised to 15-16%, which enabled *A. candidus, A. ochraceus* and *A. flavus* to develop. With a further increase in moisture, *A. versicolor, A. niger* and species of *Penicillium* appeared. Although storage fungi germinate at lower A_w values than field fungi, their minimum A_w for germination and growth is influenced by additional factors such as temperature, nutrition and the nature of the solutes used to control A_w (Magan and Lacey, 1988). For example, xerophilic *Penicillium* spp. can germinate at an A_w of 0.81-0.83 at 16°C, 0.81 A_w at 23°C and 0.83-0.86 A_w at 30°C (Magan and Lacey, 1988). The *penicillia* invade kernels with a moisture content of 17% and higher, whereas *aspergilla* can grow below 17% (Christensen and Kaufmann, 1969;

Magan and Lacey, 1988; Trojanowska, 1991).

Temperature and pH

Generally fungi are more tolerant of lower than higher temperatures. Most fungi develop at temperatures from 5° to 35°C, with an optimum of 25°-35°C (Panasenko, 1967). Field fungi generally survive better at 4-5°C than at ambient temperatures although this differs among species. (Lacey and Magan, 1991). The majority of *Penicillium* and *Aspergillus* spp. have a growth range between 10 and 40°C, with an optimum at 25-35°C. On the whole, *Penicillum* species are better suited to grow at a lower temperature and occur more frequently in temperate climates, although *Aspergillus* species can grow over a wider temperature range (-8°C to 58°C). Several fungi, e.g., *Thermomyces lanuginosa, Talaromyces thermophilus*, are thermophilic and can grow up to 60°C. These fungi can cause spontaneous heating in grain (Lacey and Magan, 1991). The temperature of growth of many fungi is often a function of other environmental parameters including pH, nutritional adequacy and moisture (Moss, 1991).

Grain fungi can grow over a wide pH range (1.5-9.8). For example, *A. niger* and *A. candidus* can grow over a pH range of 2.1-7.7 and 1.8-8.5 respectively. Sporulation, germination and/or enzymic activity occur within a much narrower pH range. As with temperature, the pH range of growth is dependant on other environmental factors (Moss, 1991).

Mycotoxin Production

Mycotoxins are secondary metabolites produced by fungi which are toxic to both

man and animal. They are perhaps the most carcinogenic of all known natural compounds (Bhatnagar et al., 1992). Mycotoxins are produced by various fungi including many which grow on cereals and cereal products, both in the field and/or in storage. As a result, stored crops may contain mycotoxins arising from either field infection or from storage or a combination of the two. The main genera of fungi associated with mycotoxin production in grain include species of *Aspergillus, Penicillium, Fusarium, Claviceps, Alternaria, Pithomyces, Stachybotrys, Phoema* and *Diploidia* (Smith and Hacking, 1983).

Factors Affecting Mycotoxin Production

Preharvest mycotoxin production

Mycotoxin production during harvest is affected by various physical factors (temperature, moisture content, rapidity of drying, rewetting, ambient relative humidity) and the degree of exposure of the crop to mechanical injury and extensive spore inoculation (Abramson, 1991).

Damage due to insect feeding can also lead to mycotoxin production. For example, the European corn borer, corn earworm and fall amyworm are involved with aflatoxin production in corn. The maize weevil in particular is associated with chronic aflatoxin contamination since they effectively serve as a vector for the dissemination of *A. flavus*. A study by Wildstrom (1992) indicated the mean concentration of aflatoxin for infested field plots was more than four times higher for weevils (329 ng/g), as for corn earworms (80 ng/g), or fall amyworms (60 ng/g).

Drought stress can also play a major role in the contamination process. For example, corn plants weakened by drought (temp > 30°C) are more susceptible to

fungal invasion. Drought stress and insect damage were shown to have a synergistic effect enhancing aflatoxin levels in corn during the preharvest period (Smith and Riley, 1992).

Post harvest mycotoxin production

(i) Moisture and temperature

Moisture and temperature are the two most important factors affecting mold growth and mycotoxin production in stored grain. Temperature interacts with A_w to influence mold growth and thereby mycotoxin production (Moss, 1991). For example, high levels of aflatoxin production by *A. parasiticus* were favored by temperatures of 25 to 30°C when the A_w was >0.90 whereas below 8 to 10°C, aflatoxin production occured, but the amounts produced were reduced and the time required for production was extended (Bullerman et al., 1984). Northolt et al. (1979) investigated the production of OA in relation to water activity and temperature. At 24°C optimum A_w values for OA production by *A. ochraceus* and *P. cyclopium* were 0.99 and 0.95-0.99 respectively. At the optimum A_w, the temperature range for OA production by *A. ochraceus* was 12-37°C; for *P. cyclopium* temperature range was 4 to 31°C.

A_w and temperature requirements can also differ for dissimilar toxins produced by the same species and for similar toxins produced by different fungal species. For example, OA production by *A. ochraceus* on poultry feed was optimum at 30°C and 0.95 A_w, while penicillic acid production was optimum at 22°C and 0.90 A_w. (Lacey and Magan, 1991).

(ii) Substrate

The A_w and temperature requirements for fungal growth and toxin production also depend on the nutritional adequacy of the substrate. Toxigenic fungi can grow on a wide range of substrates; however, certain substrates are more suitable for optimum toxin production. For example, aflatoxins are produced in high concentrations in various oil seeds such as groundnuts, Brazil nuts, pistachios, almonds, walnuts and in cereals. However, they are not formed in any significant quantity in soybeans (Moss, 1991). Bullerman et al. (1984) reported that phytic acid in soybean was capable of binding zinc, a requirement for toxin production. In addition, toxigenic mold may grow abundantly on various substrates, yet mycotoxin synthesis may not occur. For example, studies have indicated that food substrates high in carbohydrates generally favor higher mycotoxin production compared to those substrates which are high in protein and lipids (Leistner and Pitt, 1977). Substrates such as cheese, which are high in protein but low in carbohydrate favor penicillia growth, but production of mycotoxin including patulin, penicillic acid and ochratoxin production was small or absent (Bullerman, 1981). Trenk et al (1971) reported that OA production by *A. ochraceus* at 28°C was highest in chopped corn and lowest in bleached flour. Optimal time for toxin production was observed to depend on the temperature and the nature of the substrate. Madhyastha et al (1990) reported that highest OA production by *A. alutaceus* (*A. ochraceus*) was highest in peanut meal and lowest in rapeseed. For *P. verrucosum*, however, wheat supported maximum OA production.

The presence or absence of certain ions, and the type and source of carbon and nitrogen can either activate, induce or depress certain enzymes, thereby disturbing the

normal channelling of key intermediates that support balanced growth (Malik, 1982).

(iii) pH

In fungi, enzymatic activity which normally occurs within a narrow pH range can affect mycotoxin production. For example, studies by Ferreira (1968) demonstrated that glutamic acid and proline were excellent nitrogen sources for OA production by *A. ochraceus*. The uptake of glutamic acid is, however, was shown to be temperature and pH dependant. Bacon et al. (1974), demonstrated that optimum uptake for these amino acids occured at pH 6.0 and at 28°C. Patulin, a mycotoxin produced by *Penicillium expansum* is synthesized over a much narrower pH range (3.2 to 3.8) than that supporting active growth (2.8 to 4.0). Also the production of T-2 toxin by *Fusarium sporotrichioides* was greater at pH 5.5 than at either pH 4.0 or pH 7.0 despite its ability to grow over a pH range of 4.0 to 7.0 (Moss, 1991).

Varying the substrate pH has been reported to influence aflatoxin production by *A. flavus* (Venkitasubramanian, 1977). At pH 7.2, 5.8, 4.5, 2.8, aflatoxin production was 5.4 , 5.0, 4.8 and 5.2 respectively.

Sansing et al (1973) reported that maximal OA production in a synthetic medium coincided with alkaline (7.5-8.0) pH values. Ammonium accumulation in the medium as a result of amino acids deamination was cited as the cause.

(iv) Microbial competition

In a natural ecosystem fungal growth may become restricted by the competitive growth of other microorganisms. These microorganisms can also prevent or limit mycotoxin production. For example, decreased levels of aflatoxin have been observed when other fungi were grown in mixed cultures with *A. flavus* and *A. parasiticus* . Mixed fungal cultures were also reported to produce different aflatoxin G_1/B_1 ratios,

even though the total amount of B_1 remained constant (Wildstrom, 1992). Corn invaded by *Fusarium* spp. was shown to prevent aflatoxin production in spite of heavy infestation by *A. flavus* (Sauer and Burroughs, 1980). Competition from *A. chevalieri* and *A. candidus* was reported to reduce aflatoxin production when rough rice was inoculated with *A. parasiticus*. On sterile corn, *A. flavus* produced 24-117 times more aflatoxin, when compared with non-sterile corn (Sauer and Burrough, 1980). Ashworth et al. (1965) reported that aflatoxin production by *A. flavus* was inhibited by the presence of *A. niger*. Chelack et al. (1991) also reported marked reduction in OA production by *A. alutaceus* in the presence of competing mycoflora.

OCHRATOXIN

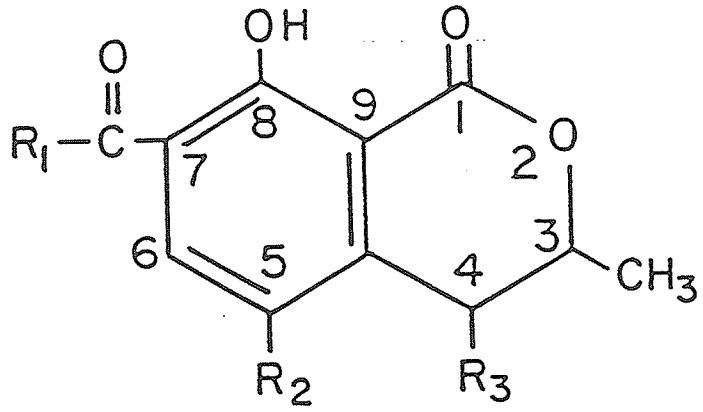
Ochratoxins (Fig. 1) include a group of structurally related secondary metabolites produced by several species of *Aspergillus* and *Penicillium*. Ochratoxin A is a 7-carboxyl-5-chloro-8-hydroxyl 3,4 dihydro-3-R-methyl-isocoumarin which is linked through a carboxy group to L-ß- phenylalanine. Ochratoxin B and C (OB, OC) are the dichlorinated and ethyl ester derivatives of OA. (Chu, 1974).

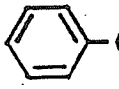
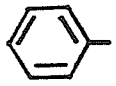
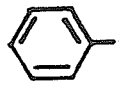
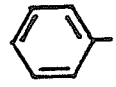
OA is a colorless, crystalline compound. It is highly soluble in polar organic solvents, slightly soluble in water and dissolves in aqueous sodium hydrogen carbonate.

Ochratoxigenic Fungi

In Western Canada, *Penicillium viridicatum, P. cyclopium* and *P. chrysogenum* represent the main ochratoxigenic fungi (Abramson and Mills, 1985). *Aspergillus ochraceus*, the first ochratoxin producing organism was isolated from sorghum grain and is often found in soil and decaying vegetation. *A. alleaceus, A. melleus, A.*

Figure 1. Chemical Structure of Ochratoxins.



	R_1	R_2	R_3
OA	 $-CH_2CH(COOH)NH-$	$-Cl$	$-H$
OB	 $-CH_2CH(COOH)NH-$	$-H$	$-H$
OC	 $-CH_2CH(COO\overset{*}{E}t)NH-$	$-Cl$	$-H$
OAOH	 $-CH_2CH(COOH)NH-$	$-Cl$	$-OH$
Oα	$-OH$	$-Cl$	$-H$
Oβ	$-OH$	$-H$	$-H$

* methyl or ethyl ester

sulphureus, A. sclerotiorum, A. ostianus also produce ochratoxin A and B. Several species of *Penicillium* also produce ochratoxin. *P. palitans, P. commune, P. chrysogenum, P. purpurescens, P. variable, P. verrucosum.* Of these organisms *P. verrucosum* also produces citrinin (Marquardt et al., 1989).

Natural Occurrence of Ochratoxin

Ochratoxin A has been detected in several plant products including cereals, ground nuts and beans in concentrations ranging from 5 to 27,500 μg/kg (Steyn, 1984). Generally oilseeds, peanuts and soybean have been reported to support a higher production of OA and OB by *A. ochraceus* compared to wheat and corn (Madhyastha et al., 1990).

Scott et al. (1972) reported OA in 18 of 29 samples of heated farm grain in concentrations ranging from 0.03 to 27 ppm. Ochratoxin ranging in concentration from 110-115 ppm was detected by Shotwell et al. (1971) in corn and Yoshizawa (1991) detected OA in 23 of 180 barley samples (0.01 to 0.037 ppm). Levi et al. (1974) reported OA in green coffee beans, while Mislivec et al. (1975) and Christensen, (1975) reported its presence in dried beans and black pepper respectively. The natural occurrence of OA in barley was also investigated with respect to its possible transmission to beer (Steyn, 1984). Krogh et al., (1974) demonstrated, however, that ochratoxin was destroyed during malting and brewing. Chu et al. (1975) demonstrated that ochratoxin was partially destroyed in the mashing and brewing process. About 28% of the spiked toxin was found in final beers brewed from starting materials containing 1 and 10 μg/g.OA.

Toxicity

Pigs fed a diet containing OA accumulated ca. 0.5% of the toxin in their tissues (Steyn, 1984). The immune system of the pig appears sensitive to OA below 1 mg/L. with a 10% inhibition of lymphocyte response being produced by ca. 0.06 mg of OA; this level has been identified in blood of slaughtered pigs (Stromer, 1992). Marquardt et al. (1988) reported the presence of OA in the blood of slaughtered pigs from Western Canada. OA is primarily a nephrotoxin which has been shown to cause porcine nephropathy in Sweden and Denmark. Other countries including Finland, Norway, Germany, Hungary and Great Britain have also reported its presence in pigs (Steyn, 1984). OA along with a co-contaminant citrinin, have been shown to be the principal agents in porcine nephropathy, which is comparable to Balkan nephropathy, suggesting a common relationship (Krogh et al. 1977).

The toxicity of OA in hens and chicks was reported by Chu and Chang (1971). Hamilton et al. (1982) reported several outbreaks of ochratoxicosis in chickens associated with feedstuff containing 2 ppm OA. Levels of 1-4 ppm ochratoxin in feed seriously affected egg production, efficiency and mortality of laying hens (Choudhury et al., 1971).

Conditions Necessary for Ochratoxin Production

Production of ochratoxin is influenced by two main factors: temperature and water activity of the substrate. The minimum temperature for OA production by *Penicillium* and *A. ochraceus* is 4 and 12°C respectively. The minimum A_w for toxin production by *A. ochraceus, P. cyclopium, P. viridicatum* is in the range of 0.83-0.87, 0.87-0.90, 0.83-0.86 respectively. On barley meal, *P. viridicatum* produced maximum

quantities of OA at 0.97 A_w and at temperatures as low as 12° C (Northolt et al., 1979).

Substrate

Cracked corn, pearled wheat, winter wheat and soybeans were found to support ochratoxin production by *A. ochraceus* (Chu, 1974). Ferreira (1968) found that among nitrogen sources glutamic acid and proline were best suited for ochratoxin production. Ammonium acetate also supported OA production at a high concentration (10g/l). Media containing yeast extract as a nitrogen source were also well suited for ochratoxin production (Davis et al., 1969). Lai et al. (1970) demonstrated that trace elements (Fe^{3+}, Zn^{2+}, Cu^{2+}, B^{3+},and Mo^{6+}) were required by *A. melleus* (NRRL 3519 and 3520), *A. ochraceus* (NRRL 3174) for ochratoxin production.

MATERIALS AND METHODS

Frass Production

Red flour beetles, *Tribolium castaneum* (Herbst) obtained from Agriculture Canada, Winnipeg, Manitoba were reared in wide mouth glass jars filled with ca. 2.50 kg of whole wheat and barley. The insectory had a screw top lid with a wire mesh center. The wire mesh was covered with filter paper which allowed for gas exchange but prevented insects from escaping. The wheat and barley was adjusted to 14-15% moisture by the addition of sterile distilled water and was kept at room temperature for 48 h in order to reach equilibrium. The beetles were raised only on sound wheat and barley; no wheat germ was added. These precautions were taken in order to facilitate frass collection. About 500-600 adult red flour beetles were introduced into each insectory which were maintained in a humidity chamber in the dark at 30°C. After 30 to 40 days the frass was separated from the grain mass by sieving (ca. 1 mm). The sieve was small enough to remove dead insects, exoskeleton, egg cases and cocoons. The frass was sterilized by electron beam irradiation (Atomic Energy of Canada, Pinawa, Man; 10 KGy).

Insect Population and Frass Production

Whole sieved (2.36 mm) wheat (*Triticum aestivum*, 100 g;) contained in 250 ml flasks was adjusted to a moisture content of 16% (wet weight basis). Red flour beetles

(mixture of sexes; ca. same age group) in populations of 50, 100, 250 were introduced into a series of flasks (insectory) which were subsequently incubated at 22°C for 14 d. The frass was collected by sieving the flask contents using a 20 mesh screen. All experiments were repeated in triplicate.

Organism

Aspergillus ochraceus Wilhelm ATCC 22946 (NNRL 3174) obtained from the American Type Culture Collection, Rockville, Md., was grown on slants of potato dextrose agar (PDA, Difco) containing yeast extract (Difco, 1.0%) and NaCl (3%, PDA-YE-NaCl) for 7-10 d at 22 °C. Slants were maintained at 7°C.

Conidia used as inocula for toxin production were produced on PDA-YE-NaCl medium (10 d, 22-25°C) and harvested using sterile distilled water containing 1% Tween 80. Harvested spores were passed through a series of sterile Pasture pipettes (14.5cm) packed with glass wool. The procedure removed mycelial debris and facilitated the removal of spore clumps. All spore inocula were standardized using sterile distilled water containing 1% Tween 80 (10^6 conidia/ml). Spore numbers were determined using a serial dilution technique employing PDA with incubation at 22°C for 5 d.

Ochratoxin Production in Grain Fortified with Frass

Barley (variety Bedford; 30 g) contained in 250 ml flasks (foam caps overwrapped with aluminium foil) were sterilized (20 min at 121°C) and adjusted to a moisture content ca. 24% (wet weight basis) by the gradual addition of predetermined amounts of sterile distilled water. This protocol involved the periodic shaking of flask contents

and an equilibrium period of ca. 48 h at room temperature. Sterile frass (3, 6, and 9%) was then added to a series of these flasks and allowed to further equilibrate (24 h, 22°C). The moisture content of the flask contents was determined in duplicate using a oven drying method (130°C, for 19 h; American Society of Agricultural Engineers, 1975). All flasks were inoculated with *A. ochraceus* (10^6 spores per flask) and incubated in the dark at 25°C. The experiment was performed in triplicate and samples were analyzed in triplicate at 0, 7 and 14 d of incubation for toxin production. Flasks containing no frass served as controls. At the time of sampling, the entire flask contents was dried (24 h, 100°C) ground (CRC Micro-mill, Chemical Rubber Co. Cleveland, Ohio.) and stored at -20°C in polyethylene bags until analyzed.

The entire procedure was repeated using either whole or ground wheat (red spring wheat) as the substrate. The incubation period was extended to 21 d.

Ground wheat was prepared using a Wiley mill and a 1.0 mm screen. All ground samples were screened twice to ensure an even size distribution.

Ochratoxin Production in Ground Wheat Containing Uric Acid

The protocol followed was similar to that described earlier with the exception that uric acid (0.077, 0.154.and 0.230 g/30 g wheat) in lieu of frass was used. The levels of uric acid added to the moisturized, 1.0 mm ground wheat (30 g) corresponded to the uric acid level in 0.0, 3.0, 6.0 and 9.0% frass respectively.

Effect of Frass on Ochratoxin Production in Sterile and Non Sterile Wheat

Ground (1.0 mm) and sterilized wheat (30 g; 121°C for 20 min) contained in 250 ml flasks was supplemented with sterile frass (5.0%) and adjusted to a moisture

content of 24% as described previously. Flasks were subsequently incubated with a 1.0 ml suspension containing either 10^4, 10^5 or 10^6 spores and incubated at 25°C in the dark. The experiment was performed in triplicate and samples were analyzed in duplicate at 0, 7 and 14 d. Flasks which were similarly inoculated but contained no frass were used as controls. This protocol was repeated using ground, non sterile grain. Prior to use, the non sterile grain was evaluated for yeast and mold (PDA, 5 d at 22°C) and standard plate count (plate count agar, 48 h at 35°C) using a sterile serial dilution technique.

Effect of Frass on The Growth of *Aspergillus ochraceus* on Synthetic Media

PDA containing sterile frass (1%) was surface inoculated with a dilute spore suspension of *A. ochraceus* (0.1 m; ca.10 spore/ml). Following incubation at 22°C for 12 d, the diameter of the resulting colonies were measured. A similar protocol was performed with PDA supplemented with 2 and 3% frass. Inoculated PDA without frass served as the control.

Frass Analysis

The total reducing sugar of the frass was determined using the phenol-sulphuric method as outlined by Dubois et al. (1956). Glucose was used as the standard.

The pH (Accumet; pH Meter Model 910) of the frass was determined by using 1.0 g of frass in 10 ml of distilled water. An iodine test was performed for starch determination. The uric acid content of the frass was determined using a spectrophotometric method described by Marquardt (1983). A standard curve was

prepared by using uric acid (Sigma Chemical Co. St. Louis, MO).

The chitin content of the frass, mainly contributed by insect exoskeletons, was determined in terms of its glucosamine content (glucosamine being a breakdown product of chitin). The method of Rotter et al. (1989) was used. Biomass accumulation by *A. ochraceus* was also assessed in terms of chitin content. When frass was employed in the substrate, biomass chitin was calculated as: the difference between total chitin minus insect chitin.

The total nitrogen content was determined by microkjeldahl (A.O.A.C. 1975).

Ochratoxin Analysis

Ochratoxin was analyzed by the method of Frohlich et al. (1988). Ground samples (10 g) were extracted with $CHCl_3$ -0.1 M H_3PO_4 (12:1) following shaking (30 min) and filtered (Whatman No 4). The filter paper was rinsed with additional $CHCl_3$ (20 ml), combined with the primary filtrate and evaporated to dryness under vacuum using a rotary evaporator at room temperature. The residue was dissolved in $CHCl_3$ (4.0 ml) and subjected to reverse phase thin-layer chromatography (RPTLC).

Reverse Phase Thin-Layer Chromatography

RPTLC plates (ca. 5×10 cm KC-18 reverse phase plates, particle size 200 μm; Whatman) were each spotted with a standard OA solution (10 μL volumes;) and three 10 μL volumes of samples. Following air drying, the plates were placed in a sealed chamber containing methanol-water (70:30; 20 ml) until the solvent front reached the end of the plate. Plates were dried and exposed to UV light (λ = 366 nm) in order to locate the blue fluorescent OA spots (Rf= 0.76). The fluorescent area was marked

with a needle. To ensure complete removal the area outlined was made slightly larger than the fluorescent spot where OA was detected.

OA recovery from RPTLC plates

The OA was recovered from the RPTLC plates using a special recovery device (Frohlich et al., 1988). The toxin was removed from the spotted area using methanol (4 ml) and the eluted samples were dried under N_2 with gentle heating (60-70°C). Samples were stored at -20°C.

Quantitative analysis of OA

Quantitative analysis of OA was performed by high-pressure liquid chromatography on a Beckman Ultrasphere ODS 5-μm column (4.6 mm×25 cm) according to the method of Frohlich et al., (1988). The eluting solvent consisted of 30% H_2O (pH 2.1; adjusted with phosphoric acid) and 70% methanol- isopropanol (90:10). A Hewlett-Packard fluorescence detector 1046 A in conjuction with Hewlett Packard integrator 3390 A was used.

RESULTS

The composition of the frass produced by *T. castaneum* is given in Table 1 . As shown from the results uric acid nitrogen contributed to ca. 87.4 % of the total nitrogen content. Approximately one-third of the frass was composed of chitin (based on glucosamine content) indicating the presence of insect integument and/or fungal biomass. Reducing sugars (1.24%) were also detected in the frass, however, starch (iodine test) was not detected. The pH of the frass was 6.05.

The level of frass produced by various populations of *T. castaneum* in whole wheat at 14 d is shown in Table 2. Increasing the insect population coincided with an increase in the production of frass. The increase however, was not proportional to the initial insect population, signifying perhaps intragroup competition for the substrate.

The time course production of ochratoxin in whole, sterile, barley with frass is shown in Fig. 2. At 14 d no significant difference ($P > 0.01$) in toxin production was observed either between the control (21.9 μg/g) and barley 6% - frass (20.0 μg/g) or between barley - 3% (14.9 μg/g) and barley- 9% frass (12.4 μg/g) (appendix table 1). Toxin production in the former substrates was significantly higher ($P < 0.001$) when compared to the latter two substrates both at 7 and 14 d. The pH of the substrates ranged from 6.05 to 6.07 at the onset of incubation.

The incorporation of frass into whole, sterile, wheat either at the 3 or 6% level

TABLE 1. Composition of frass produced by *T. castaneum.*

Components	Concentration
Total nitrogen	9.76 ± 0.4 %
Uric acid	8.53 ± 1.5 %
Carbohydrate	1.24 ± 0.09 %
Starch	N.D[a]
pH	6.05 ± 0.01
Chitin	33.3 mg/g ± 0.01

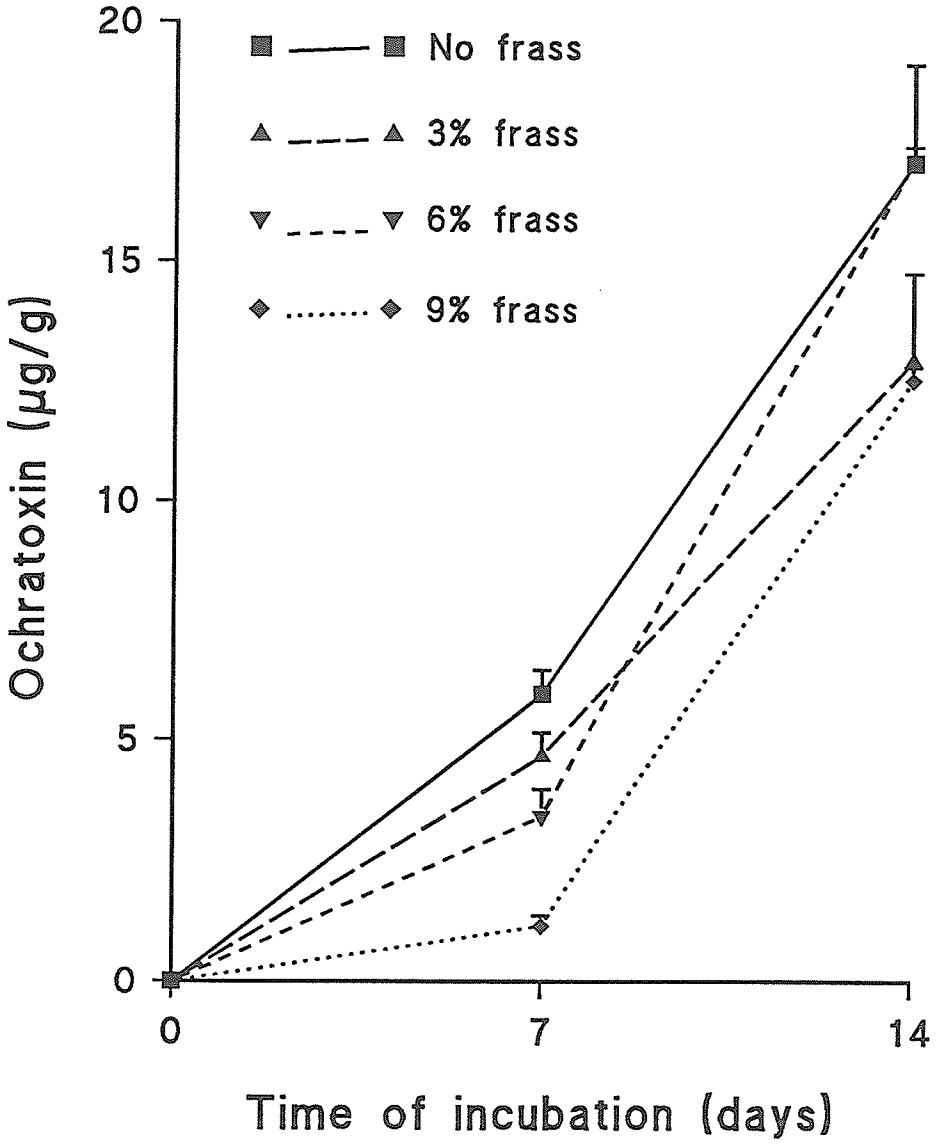
[a] Not detected

TABLE 2. Amount of frass produced by *T. castaneum* in whole wheat with 16% moisture after twelve days.

No of insects[a]	Frass (mg/100g)	Frass (%)
50	46.3 ± 21.1	4.6
100	91.76 ± 16.5	9.1
250	165.23 ± 32.8	16.5

[a] Adults only; mixed sexes.

Figure 2. Effect of 0, 3, 6, and 9% of frass on OA production in whole barley by *A. ochraceus*. Bars represent standard deviation of the means.


20
15
10
5
0
No frass
3% frass
6% frass
9% frass
Ochratoxin (µg/g)
0
7
14
Time of incubation (days)

did not significantly ($P > 0.05$) increase toxin production (Appendix Table 2) when compared to the control either at 14 or 21 d (Fig. 3). At 14 d OA production was highest in the control (17.21 μg/g); this level was significantly higher ($P < 0.05$) than that obtained in wheat 9%- frass (13.35μg/g). At 21 d, however, OA production in the control decreased to 4.18 μg/g; in contrast toxin production with 9% frass increased to 26.15 μg/g which was significantly higher ($P < 0.01$) than that observed among the remaining substrates. Growth (glucosamine concentration) of *A. ochraceus* increased in all substrates during the first two weeks of incubation (Fig. 4; Appendix Table 3). Maximum growth was attained in the control (30.3 mg/g) at 14 d which coincided with maximum toxin production; growth of the control decreased thereafter. The presence of frass in wheat appeared to have no stimulatory effect on the growth of *A. ochraceus*. Increasing the concentration of frass in the substrate appeared to decrease the rate and extent of growth. The pH of the substrates ranged from 6.05-6.07.

Toxin levels increased ca. five to ten fold when ground wheat (1.0 mm) was used as the substrate (Fig.5). At 14 d, toxin production in wheat- 9% frass (222.0 μg/g) and wheat 6%-frass (190.53 μg/g) was significantly higher than in the remaining substrates ($P < 0.001$) (Appendix Table 4). At 21 d maximum toxin accumulation was detected in wheat-3% frass. This level of toxin, however, was not significantly different ($P > 0.05$) from that obtained in the remaining substrates. Maximum growth of *A. ochraceus* in ground wheat occurred in the absence of frass at 14 d, thereafter it decreased. The inclusion of frass (3, 6 or 9%) appeared to alter the pattern of growth for *A. ochraceus*. In most cases the growth pattern appeared protracted but increased steadily.

When the frass component was replaced with equivalent levels of uric acid (0.0,

Figure 3. Effect of 0, 3, 6, and 9% of frass on OA production in whole wheat by *A. ochraceus*. Bars represent standard deviation of the means.

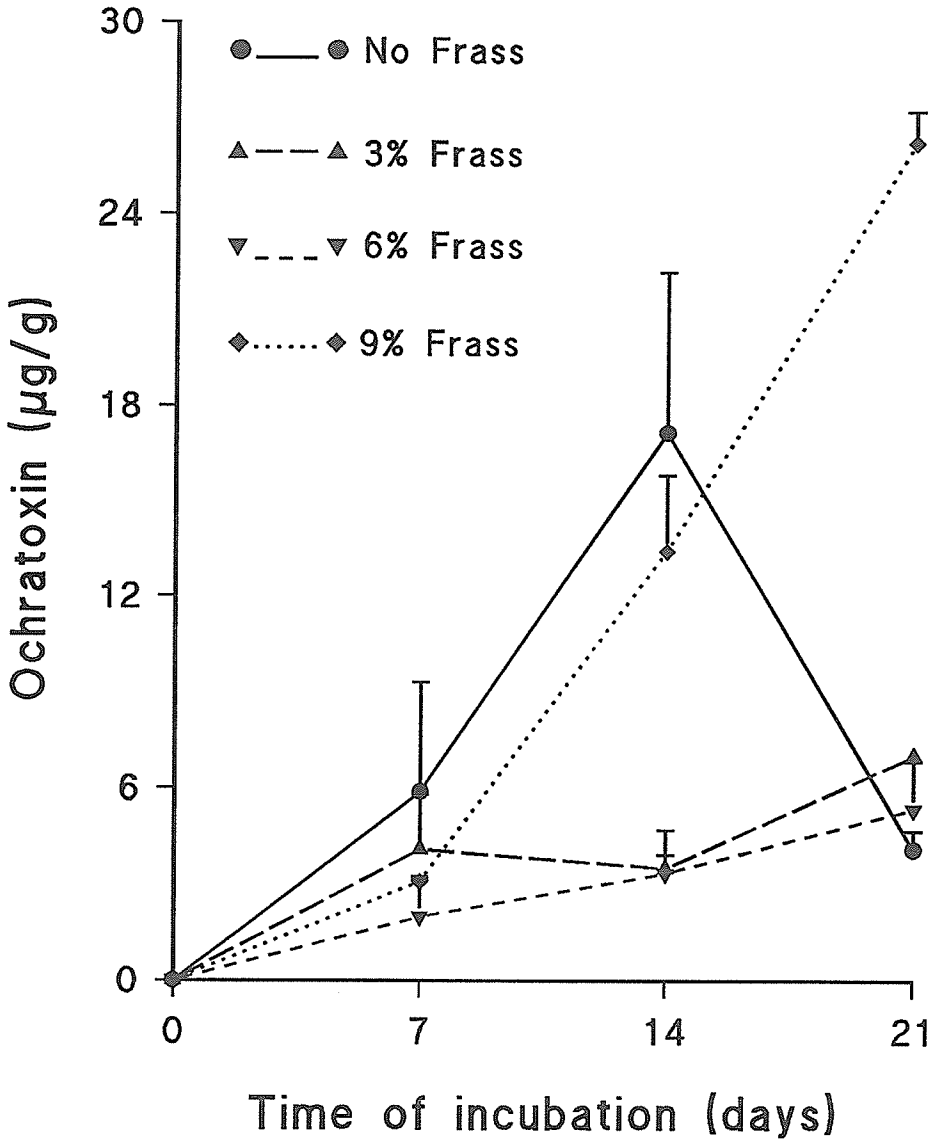

30
24
18
12
6
0
Ochratoxin (µg/g)
No Frass
3% Frass
6% Frass
9% Frass
0
7
14
21
Time of incubation (days)

Figure 4. Effect of 0, 3, 6, and 9% of frass on the growth (as determined by glucosamine concentration) of *A. ochraceus* in whole wheat. Bars represent standard deviation of the means.

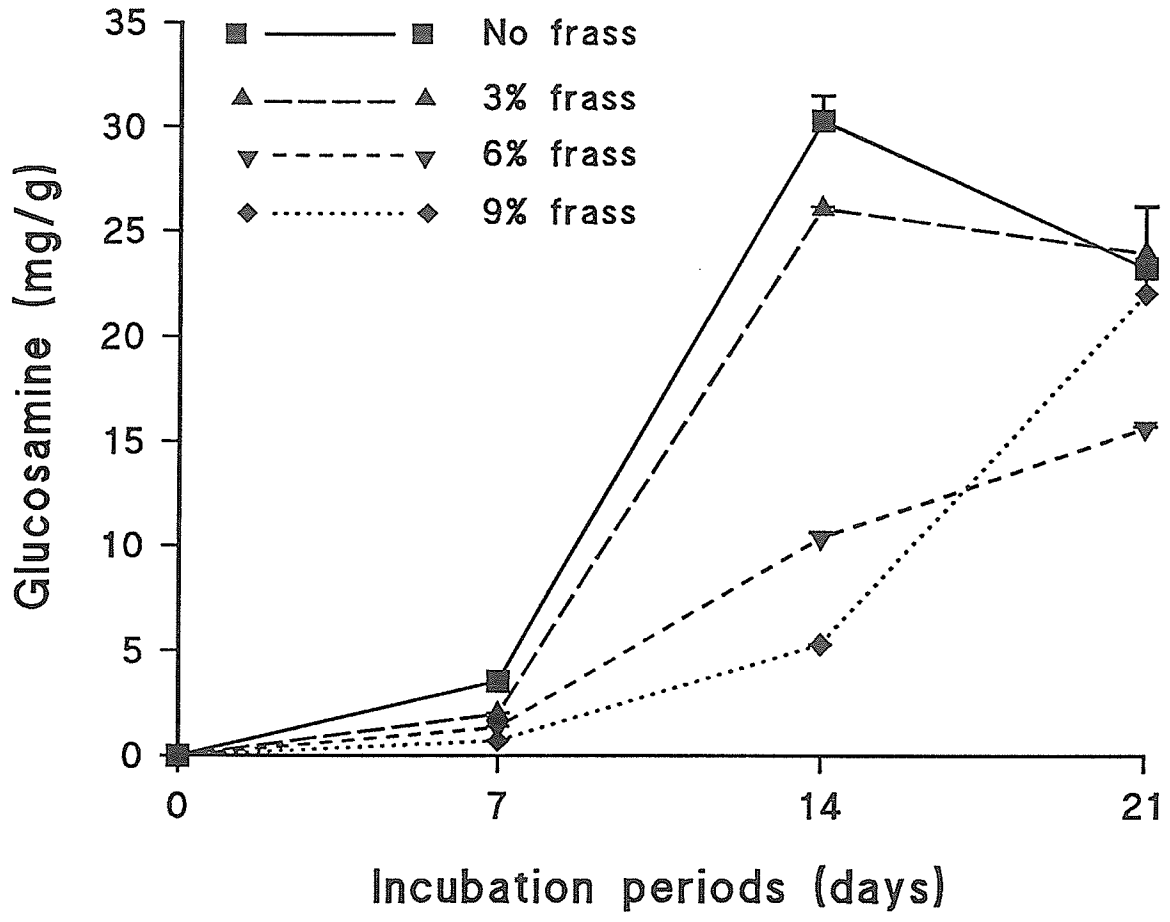

No frass
3% frass
6% frass
9% frass
Glucosamine (mg/g)
35
30
25
20
15
10
5
0
0
7
14
21
Incubation periods (days)

Figure 5. Effect of 0, 3, 6, and 9% of frass on the production of OA by *A. ochraceus* in ground wheat. Bars represent standard deviation of the means.

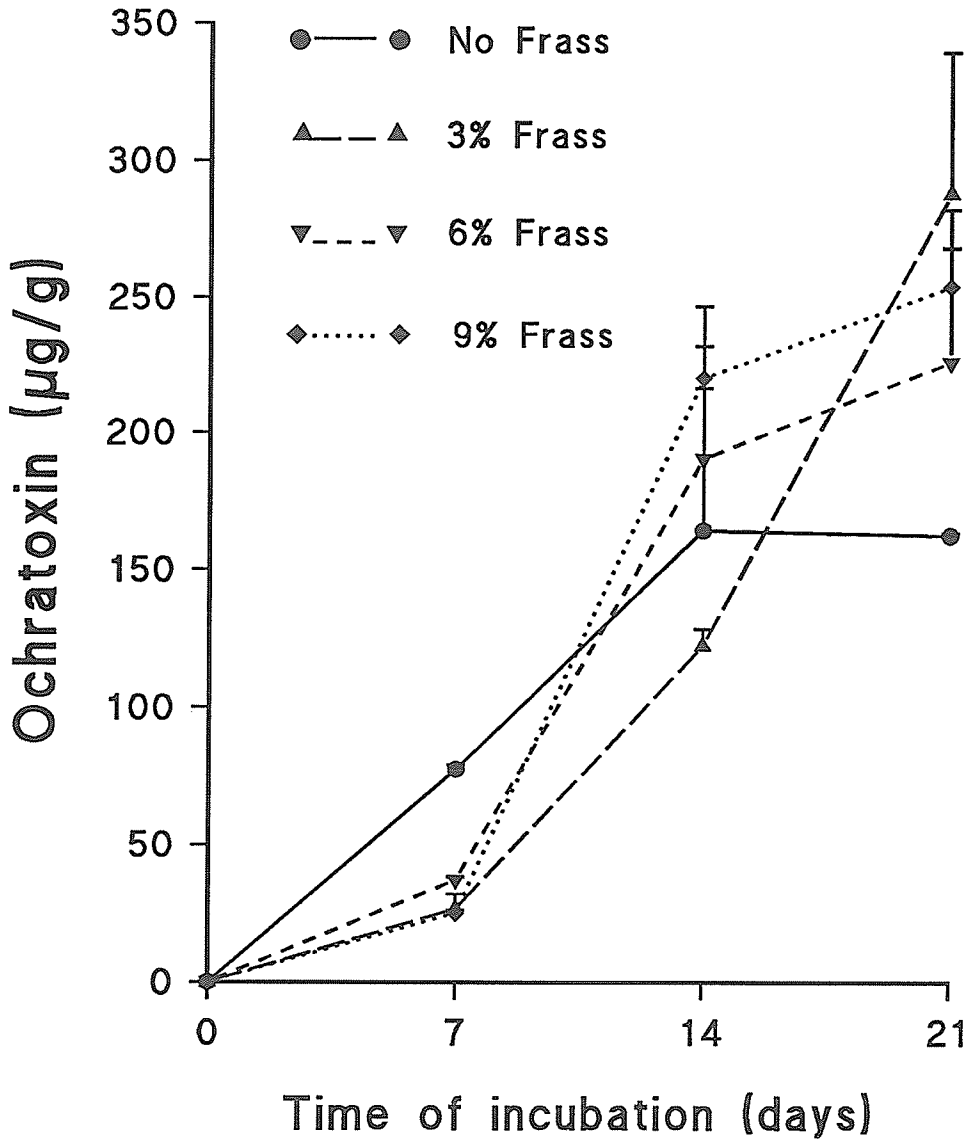
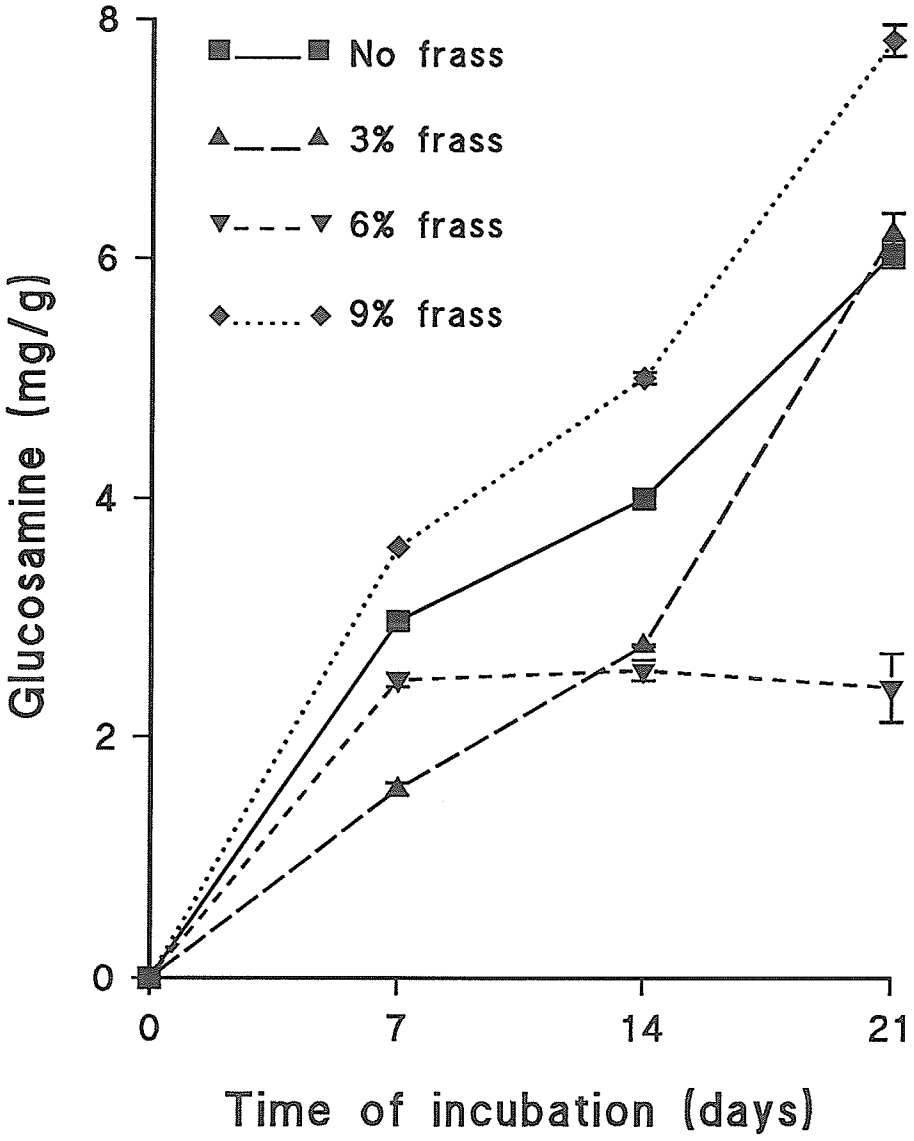

350
300
250
200
150
100
50
0
Ochratoxin (µg/g)
No Frass
3% Frass
6% Frass
9% Frass
0
7
14
21
Time of incubation (days)

Figure 6. Effect of 0, 3, 6, and 9% of frass on growth (as determined by glucosamine concentration) of *A. ochraceus* on ground wheat. Bars represent standard deviation of the means.


No frass
3% frass
6% frass
9% frass
Glucosamine (mg/g)
0
2
4
6
8
0
7
14
21
Time of incubation (days)

76.8, 153.5 and 230.4 mg uric acid (UA)/g ground wheat equivalent to the UA content n 0, 0.9, 1.8, 2.7 g frass/g ground wheat respectively) the overall production of toxin declined. Maximum toxin accumulation was observed in ground wheat at 14 d containing 230.4 mg UA/g; levels decreased thereafter. No significant difference was observed in the toxin levels among the remaining substrates ($P > 0.05$) (Appendix Table 6). At 21 d, toxin accumulation levels for all substrates were not significantly different (ca.9-11 μg/g). Biomass accumulation by *A. ochraceus* appeared to increase with time in all substrates (Fig. 9). Maximum accumulation (14.6 mg/g) occurred in wheat which contained the highest levels of UA at 21 d. Biomass accumulation at this time appeared lowest in the control. The substrate pH ranged from 6.05 (control) to 5.6 (ground wheat with 230.4 mg uric acid).

The production of OA in non sterile ground wheat is shown in Fig. 9. Substrates with or without frass containing either 10^5 or 10^6 CFU/g exhibited no significant difference ($P > 0.05$) (Appendix Table 8) in ochratoxin accumulation at 14 d. Substrates inoculated with 10^4 CFU/g yielded no toxin accumulation within the incubation period.

The accumulation of toxin in sterile ground wheat either with or without frass is shown in Fig. 10 and 11. Overall, toxin accumulation appeared markedly higher in substrates which were sterilized prior to inoculation. Substrates inoculated with either 10^5 or 10^6 CFU/g with or without frass contained no significant difference in toxin accumulation ($P > 0.05$). Toxin accumulation in the absence of frass at the 10^4 CFU/g inoculum level was, however, significantly ($P < 0.05$) higher than in the presence of frass. In both cases, substrates inoculated at the highest level exhibited the lowest

Figure 7. Effect of uric acid on OA production by *A. ochraceus* in ground wheat. Bars represent standard deviation of the means.

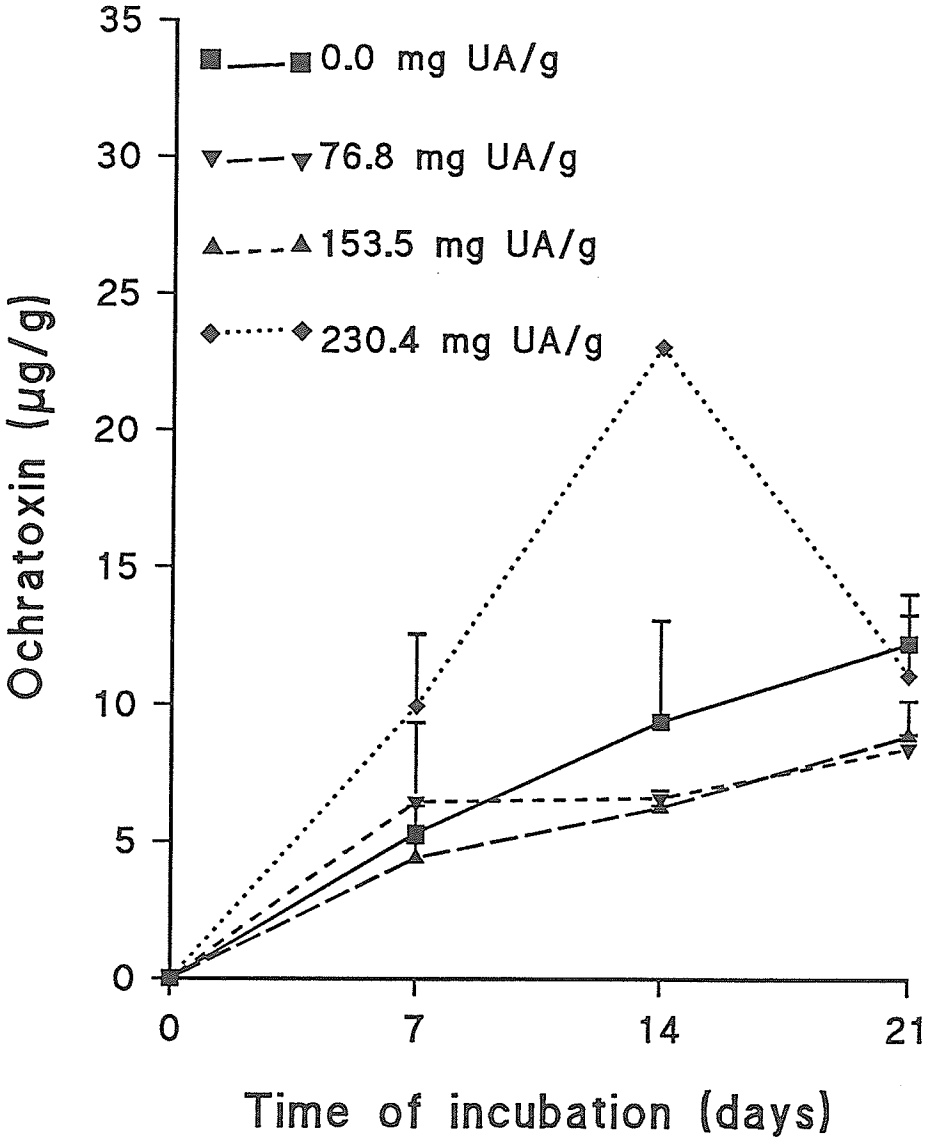

35
30
25
20
15
10
5
0
0.0 mg UA/g
76.8 mg UA/g
153.5 mg UA/g
230.4 mg UA/g
Ochratoxin (µg/g)
0
7
14
21
Time of incubation (days)

Figure 8. Effect of uric acid on growth (as determined by glucosamine concentration) of *A. ochraceus* on ground wheat. Bars represent standard deviation of the means.

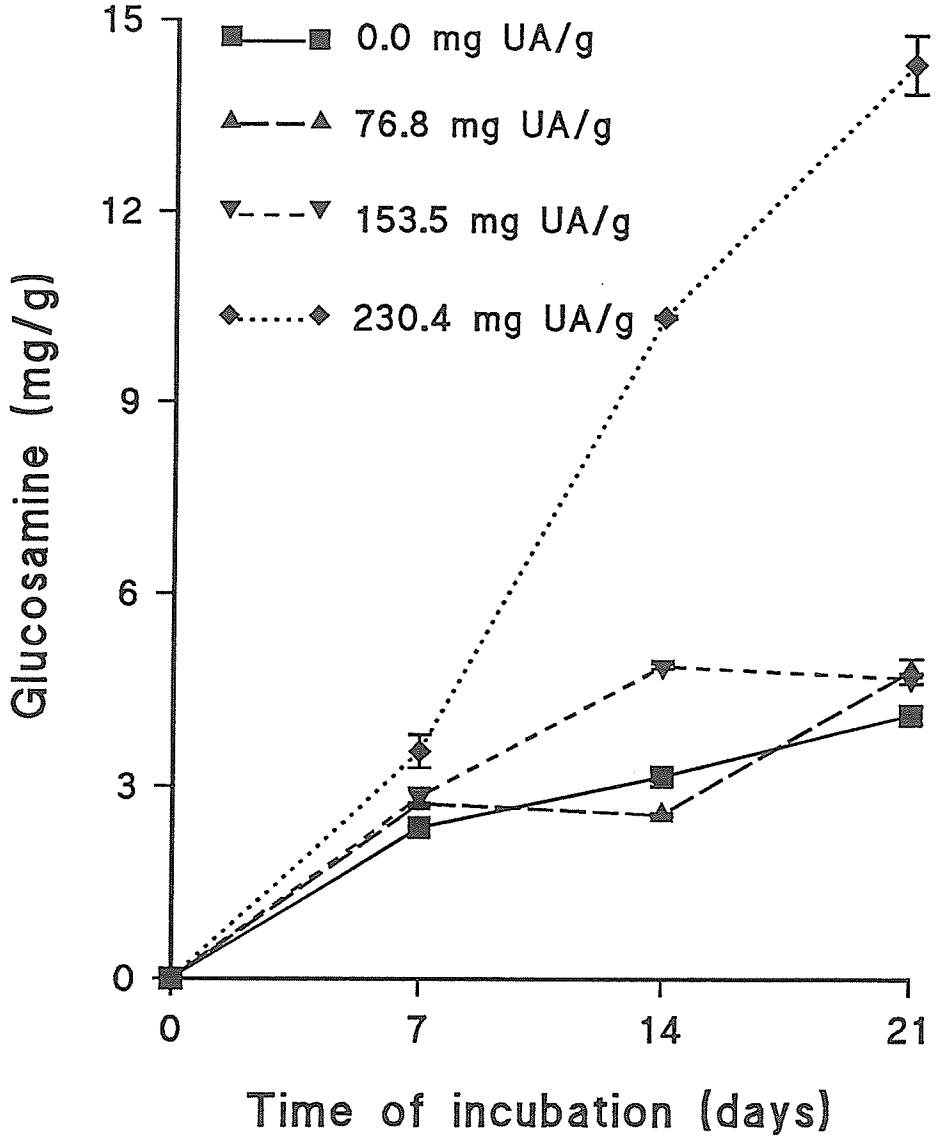

15
12
9
6
3
0
0
7
14
21
Glucosamine (mg/g)
Time of incubation (days)
0.0 mg UA/g
76.8 mg UA/g
153.5 mg UA/g
230.4 mg UA/g

Figure 9. Effect of inoculum size on OA production by *A. ochraceus* in non sterile ground wheat with and without frass. Bars represent standard deviation of the means.

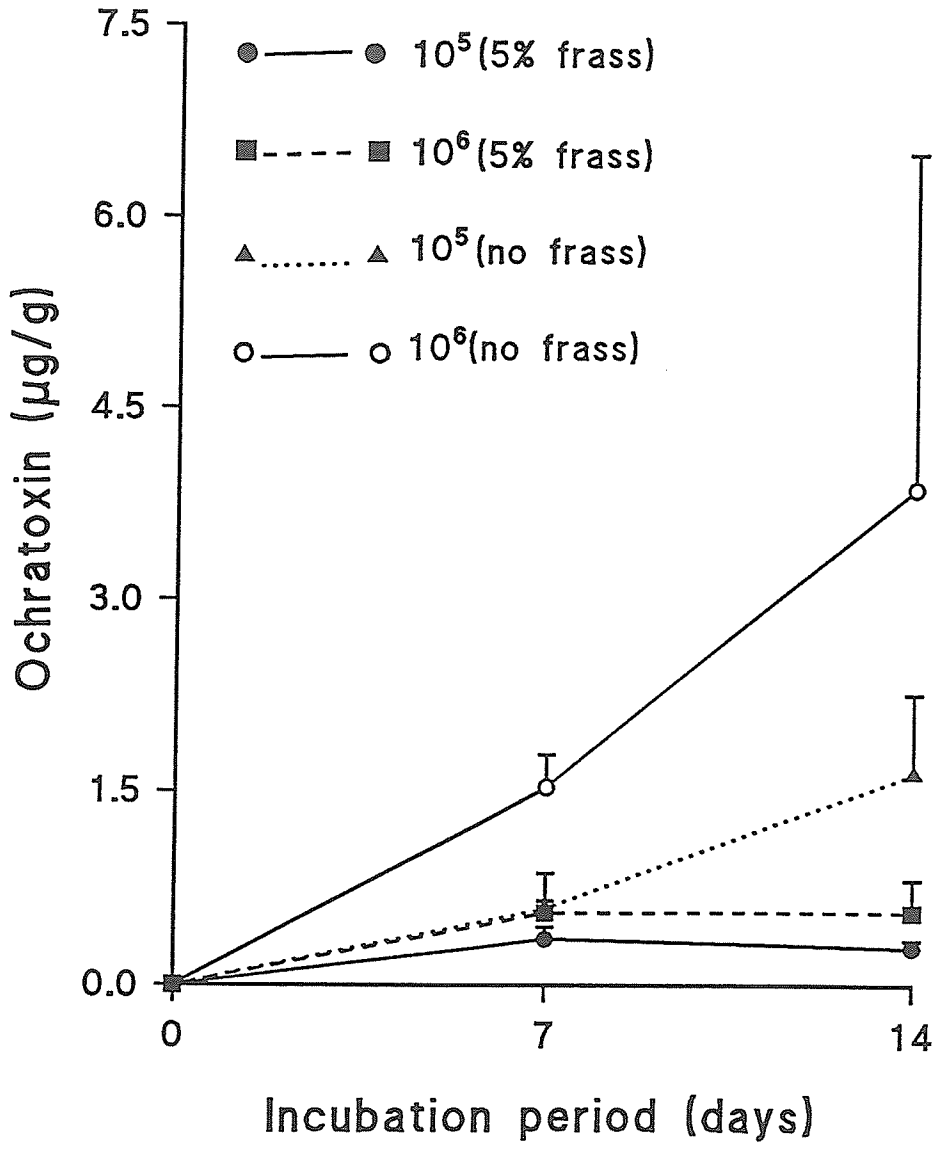

7.5
6.0
4.5
3.0
1.5
0.0
Ochratoxin (µg/g)
10^5(5% frass)
10^6(5% frass)
10^5(no frass)
10^6(no frass)
0
7
14
Incubation period (days)

Figure 10. Effect of inoculum size on OA production by *A. ochraceus* on sterile ground wheat without frass. Bars represent standard deviation of the means.

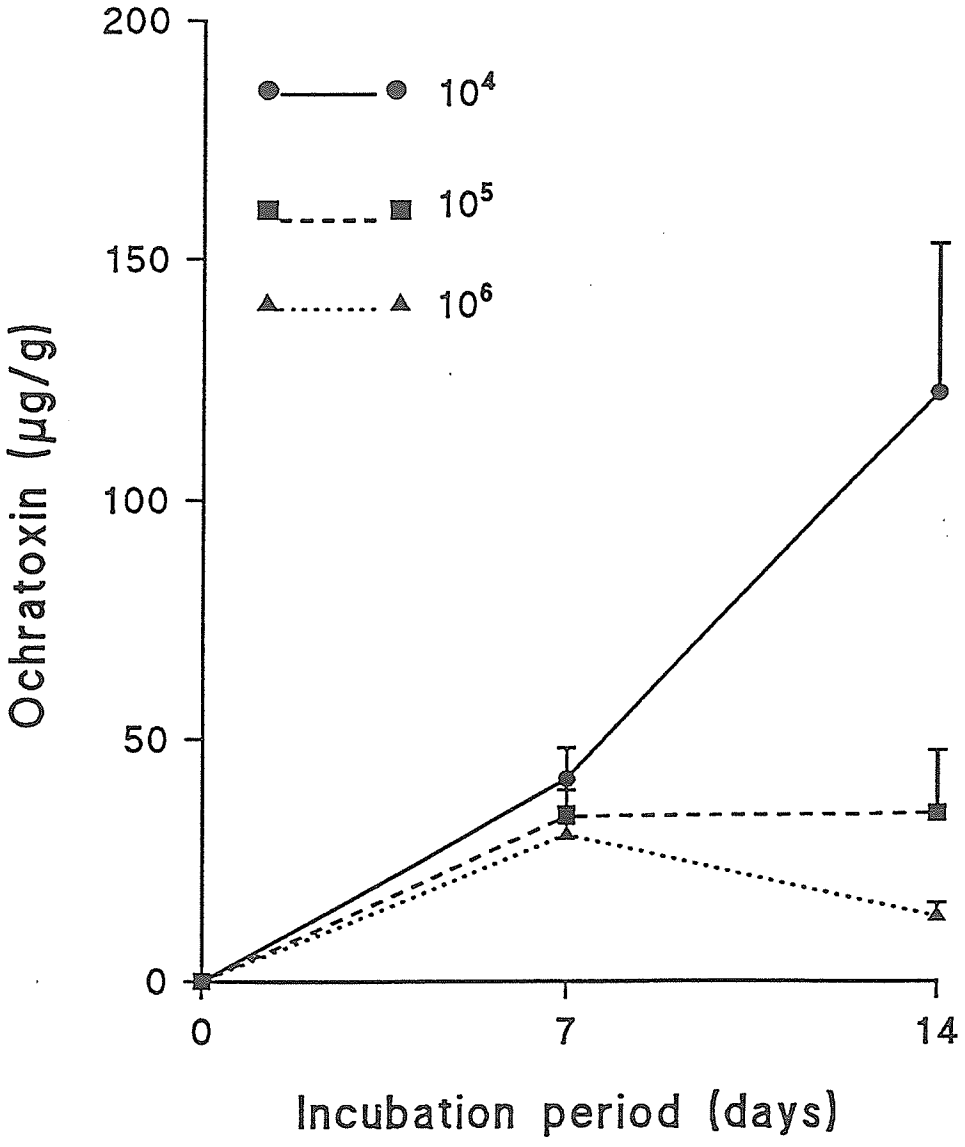
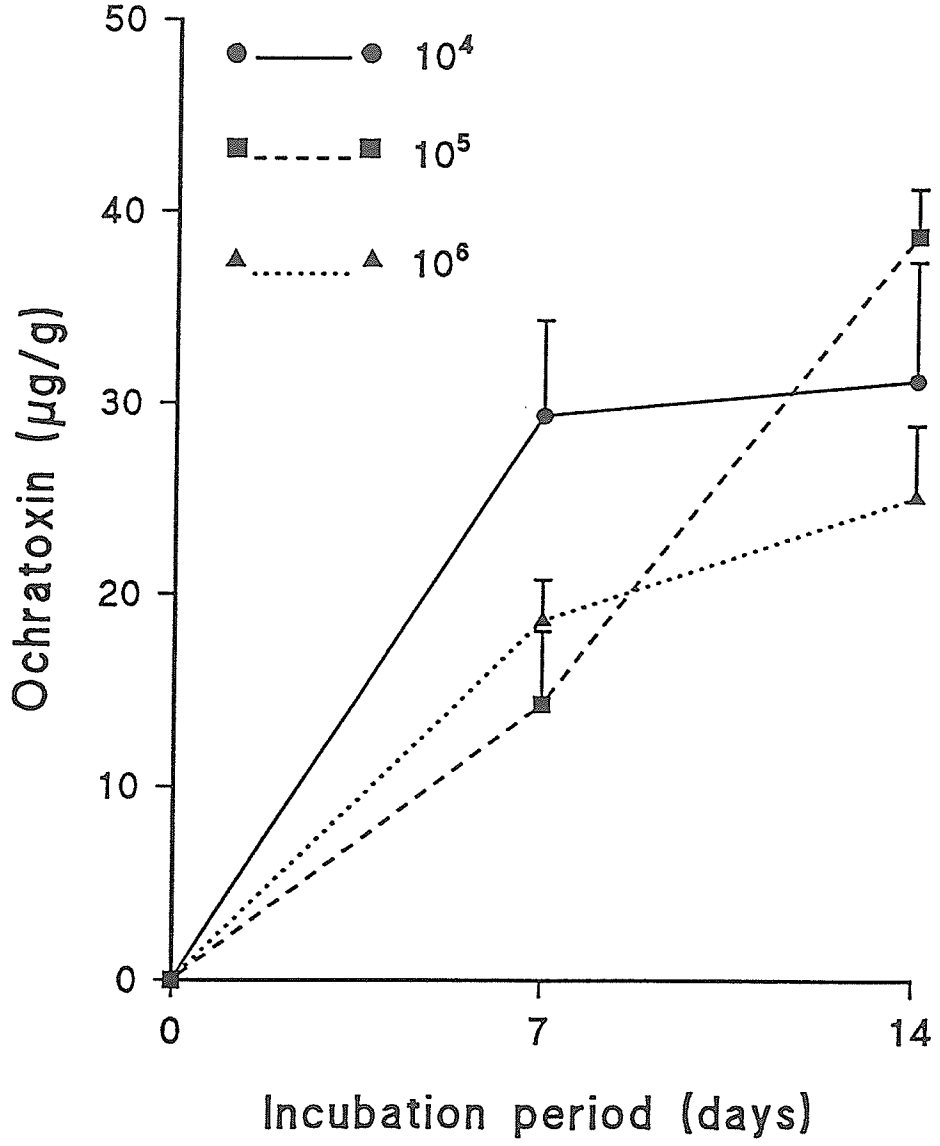

200
150
100
50
0
Ochratoxin (µg/g)
10^4
10^5
10^6
0
7
14
Incubation period (days)

Figure 11. Effect of inoculum size on OA production by *A. ochraceus* on sterile ground wheat containing 5% frass. Bars represent standard deviation of the means.


50
40
30
20
10
0
Ochratoxin (µg/g)
10^4
10^5
10^6
0
7
14
Incubation period (days)

level of toxin.

The microflora of the non sterile ground wheat included a yeast and mold count of 34×10^4 CFU/g and a standard plate count of 31×10^2 CFU/g. When *A. ochraceus* was cultured on potato dextrose medium (Difco) supplemented with 0, 1, 2 or 3% frass, at 22°C for 12 d the resulting colonies were shown to have diameters of 4.0, 4.5, 4.5 and 4.8 cm respectively (Table 3).

TABLE 3. Growth of *A. ochraceus* on potato dextrose agar at 22°C fortified with frass.

Frass (%)	Colony diameter (cm)
0	4.0 ± 0.0
1	4.5 ± 0.1
2	4.5 ± 0.0
3	4.8 ± 0.4

DISCUSSION

With their metabolic heat and water, insects can increase the water activity and temperature of grain to a level suitable for fungal growth (Dunkel, 1988). Insects can also serve as internal and external carriers of fungal spores. Through feeding they can destroy or damage large quantities of grain and provide environments suitable for spore germination. Fungal-insect interactions in stored cereals are intricate. The present study was initiated to assess an interaction which hitherto has not been fully investigated. Specifically, insects feeding in stored cereals excrete nitrogenous waste (frass) in the form of pellets. These excretory pellets are relatively high in nitrogen and have been reported to contain ca. from 10 to 22% uric acid (Gupta and Sinha, 1960). In addition, depending upon the insect species and the nature of their feed, starch and amino acids have also been detected in their frass (Gupta and Sinha, 1960). In view of the potential nutrient value of frass, the question arises as to wether this material can contribute to or influence the growth and or toxin production of grain infesting fungi. In the case of *Streptomyces*, for example, Wallace and Sinha, (1981) reported that the frass obtained from the cereal beetle could serve as a good substrate for growth. The frass used in this study contained ingredients or debris not of an excretory nature. This debris (insect and cereal material) could not be separated adequately from the frass pellets by sieving. Alternately manual separation with the aid of a stereoscope microscope could have been performed. However, based on the

anticipated levels required, this method was deemed unsuitable. The rate and extent of frass production by insects is highly variable and is known to depend on a multiplicity of factors many of which also affect insect feeding. The degree of infestation, the population pressure and the adequacy of the feed represent some of the more important factors (Farn and Smith, 1963). In order to established reference levels with respect to frass production and insect densities a preliminary study was undertaken. Following 14 d of feeding ca. 4.6% of frass was recovered from 100 g of grain infested with 50 *T. castaneum* adults. This level increased to 9 and 16% when the insect densities were increased to 100 and 250 respectively. Such levels of insect infestation would be categorized as "visible" (≥ 500-1000 insects/kg cereal) as opposed to "hidden" (< 1-10 insects/kg cereal) (White, personal commun.). As mentioned previously the production of frass is influenced in part by insect density which is extremely variable. Since "visible" levels of insect infestation are encountered in stored cereal crops including wheat (Storey, 1985) frass levels of 3, 6, and 9% were arbitrarily chosen. These levels of frass encompass the level produced in the preliminary study.

In this study no clear pattern on biomass accumulation was observed with regard to frass concentration. However, in the majority of trials, the rate and extent of biomass formation in grain containing frass was lower than that observed for the controls. The lack of a well defined growth pattern with *A. ochraceus* in frass may be due to an inherent problem which could not be adequately resolved. Specifically it was observed that during the incubation period, some of the frass would settle to the bottom of the culture flask as a result of standing and or periodic shaking. Either whole or ground wheat would often obscure the settled frass creating a heterogenous growth environment. Such a condition could have dramatic effects on mycelial mass

formation, viz, by limiting access of the fungus to part of the substrate. This situation was further aggravated when some of the settled frass become transformed into a sticky conglomerate, interwoven with mycelia. Acquiescence of moisture, possibly arising from respiration and or diffusion from the grain with the frass, most likely contributed to this situation. A similar scenario arose when uric acid was used in lieu of frass. In the majority of trials, shaking the flask contents often within 3-5 d of incubation did not result in an adequate redistribution of the substrate. At this time period, the substrate and resulting fungal growth primarily existed as one compact mass.

Although the addition of frass to grain created distribution problems, the situation may not be that different in a natural grain bulk. Grain dust, dockage and frass may also be distributed unevenly due to settling and or entrapment within the grain matrix especially in areas of high moisture content. The reduction in biomass observed when *A. ochraceus* was grown with frass may indicate possible inhibitory components. Uric acid, which markedly stimulated biomass formation was not likely involved. However, the protracted growth profiles exhibited by *A. ochraceus* may arise from a type of hinderance provided for by frass. For example, filamentous fungi when cultured on solid-substrate fermentations, natural materials such as straw, grass, woodchips and grain, grow at rate based primarily on the availability of nutrients and on the geometric configuration of the matrix (Moo-Young et al., 1983). In the case of cellulose degrading fungi growing on straw, the authors indicated that the growth rate was dependant on the dimensions of the interstitial space which provided for no nutrients and/or the compactness of the straw strands upon which the fungi could adhere to. Compactness of the substrate could also affect O_2 and CO_2 tensions within

the interstitional space, and the dissipation of heat which could lead to localized overheating. Fungal growth, enzyme production and product formation have been reported to be affected by substrate compactness (Silman, 1980).

One additional consideration that should be addressed when evaluating the growth of *A. ochraceus* is the carbon:nitrogen ratio of the substrate. This ratio, particularly in solid substrate fermentations, where vegetative growth and nutrient diffusion are somewhat reduced compared to conventional liquid fermentations, is critical not only in terms of biomass production but also in terms of product utilization and formation. Favorable C:N ratios for fungi vary (15:1 to 30:1) depending on the organisms and the fermentation (Ulmer et al., 1981). The source and availability of nitrogen have also been reported to exert a great influence over the direction of the fermentation (Moo-Young et al. 1983). Therefore the inclusion of frass with a relatively high nitrogen level, in the form of uric acid, may have had an influence on the growth pattern of the organism.

Ochratoxin production as influenced by frass was most apparent at the 9% level. The highest concentration of uric acid (230.4 mg/g) equivalent to that level in 9% frass also promoted peak toxin production. With the exception of trials employing uric acid, toxin production in frass containing substrates appeared protracted and in the majority of cases exhibited a steady increase, during the incubation period. A defined pattern of toxin production with respect to frass concentration was not observed. In part this may be due to problems previously discussed. Grinding wheat, in an effort to create a more homogeneous substrate resulted a marked increase in ochratoxin production. According to Diener and Davis (1969) shell and kernel damage (fragmentation) increases the opportunities for direct and rapid invasion of fungi which ultimately

increases the potential for toxin formation. In addition, the reduction in size of the substrate allows for increased surface area thereby facilitating nutrient acquisition. In agreement with previous investigators (Haggblom, 1982; Ciegler, 1971) ochratoxin accumulation did not appear to be positively correlated with biomass production.

The presence of competing indigenous microflora in non sterile wheat markedly reduced toxin production. When frass was added, toxin levels appeared to decrease further. It is probable that elevated nitrogen levels aided in the growth and reproduction of all organisms including those of a competitive nature. This increased competition would no doubt have a negative impact on toxin production and or stability. This interference and/ or competition with respect to mycotoxin production has also been reported by Chelack et al., (1991) and Northolt and Bullerman (1982). Increasing the inoculum level from 10^4 to 10^6 CFU/g appeared to result in increased toxin production presumably due to enhanced competition by *A. ochraceus*. In the case of sterilized wheat, increasing the inoculum level appeared to have the opposite effect on toxin production. In this instance it is possible that fungal germination and or growth was inhibited resulting in enhanced toxin production. Spores of many species of fungi fail to germinate when seeded at high densities in media which normally affords high germination. This self inhibition phenomenon has been traced to the production of inhibitors such as methyl ferulate (Allen, 1972) and the insufficient supply of CO_2 during germination (Trinci and Whittaker, 1968). Sharma et al. (1980) also reported that aflatoxin production by *Aspergillus parasiticus* was enhanced when the initial spore inoculum was decreased. The author proposed that decreasing the spore inoculum resulted in a prolonged lag phase which directly impacted on toxin synthesis. The addition of frass to sterile wheat markedly reduced toxin accumulation

but only in substrates inoculated at the lowest rate. It is possible that the presence of frass may have had a positive influence on the growth rate with respect to shortening the lag phase.

When incorporated into potato dextrose agar, frass was shown to have an enhanced effect on the growth of *A. ochraceus* when compared to the control. The enhanced nitrogen content contributed by uric acid undoubtedly contributed to this effect.

SECTION II

EFFECT OF AERATION ON INSECT MOVEMENT

REVIEW OF LITERATURE

VENTILATION

Under well ventilated conditions, grain can be stored for prolonged time periods without significant alterations in quality characteristics or losses in nutritional value. Ventilation is used widely throughout the world as a means of cooling or drying grain during storage and of removing metabolic CO_2 thereby protecting it against microbial (Hyde and Burrell, 1973; Lacey and Magan, 1991) and insect degradation (Williams, 1973). Ventilation can also be used to remove grain storage odors and to distribute fumigants throughout the grain mass (Brooker et al., 1974).

Types of Ventilation

Natural and forced ventilation systems are commonly used for stored grain products. In a natural ventilation system, the removal of moisture is accomplished by diffusion from the grain either by a ventilator or through a ventilated wall. In general, natural ventilation systems are ineffective for drying small sized grains (wheat, rye, sorghum, barley, millet, rice, oats) that are high in moisture except when

drying conditions are favourable (strong winds of low relative humidity air). In addition, these types of systems are capable of removing only 1 to 2% of the excess moisture (Barre, 1954). Bin capacities less than 25 tonnes, can be aerated by natural ventilation where wind current enter the bin through ventilators located either on the roof or in the eaves (Sinha and Watters, 1985).

In forced ventilation systems, a fan is fitted into one or more perforated ducts of various designs (depending on the quality of the grain and the dimensions of the silo). These systems help to move air through the intergranular spaces of the grain. Forced ventilation is more effective and is used more widely than natural systems (Sinha, and Watters, 1985). Two types of forced ventilation are commonly employed: air is either blown through the grain from a fan via ducting or air is drawn or sucked through grain to a duct and then exhausted. The ventilation system of a bin is ideal when the air flows through a perforated false floor (Burrell, 1974). Blowing is preferable for damp grain, where the air can be warmed slightly to reduce the relative humidity so that it can carry more moisture. The air must be removed from the top of the bin to prevent condensation on the roof, particularly of metal bins (Williams, 1991), but should be rejected if hot damp air from industrial processes would be drawn into the grain. Blowing should also be rejected if there is a possibility that dust laden air might increase insect or microbiological contamination (Burrell, 1974).

The success of ventilation using ambient air in a given climatic condition depends on sufficient airflow through the grain. Humidistats are used to shut off the fan when the relative humidity of the drying air exceeds a set point relative humidity. Time clocks also operate to switch off the fan during night hours, when the relative humidity of the ambient air is usually high. The airflow rates used are variable and

depend upon several important factors including the moisture content and temperature of the grain during harvest and storage. For drying corn, continuous ventilation with an airflow of 18.3 (L/sec) under an autumn shut off and spring start up procedure has been shown to be the most effective. The best airflow for drying wheat to near ambient drying varied from 37.3 to 57.7 (L/sec) with a moisture content of 20% and a bed depth of 2.5 m. (Bruce and Ryniecki, 1991). Metzger and Muir (1983) determined the effective airflow rates and fan control methods for intermittently operated aeration system, used for on-farm storage in Canadian Prairie regions based on historical weather data for 15 or more harvest years. For continuous aeration, the authors suggested that an airflow rate of 1.0 (L/sec)/m^3) was preferable with respect to minimizing overdrying, grain temperature and energy use.

Ventilation of Grain as a Method of Drying.

Prolonged periods of aeration can result in slow drying of grain particularly if the moisture content is greater than 17% (Hyde and Burrell, 1973). This action decreases the ability of storage fungi to grow during grain storage (Lacey and Magan, 1991). Methods of ventilation drying include the use of ambient temperature air or air which has been heated (40 to 100° C). In the latter method, the drying time is greatly reduced. After drying, the grain is cooled using ambient air which can serve to remove additional moisture (Bruce and Ryniecki, 1991).

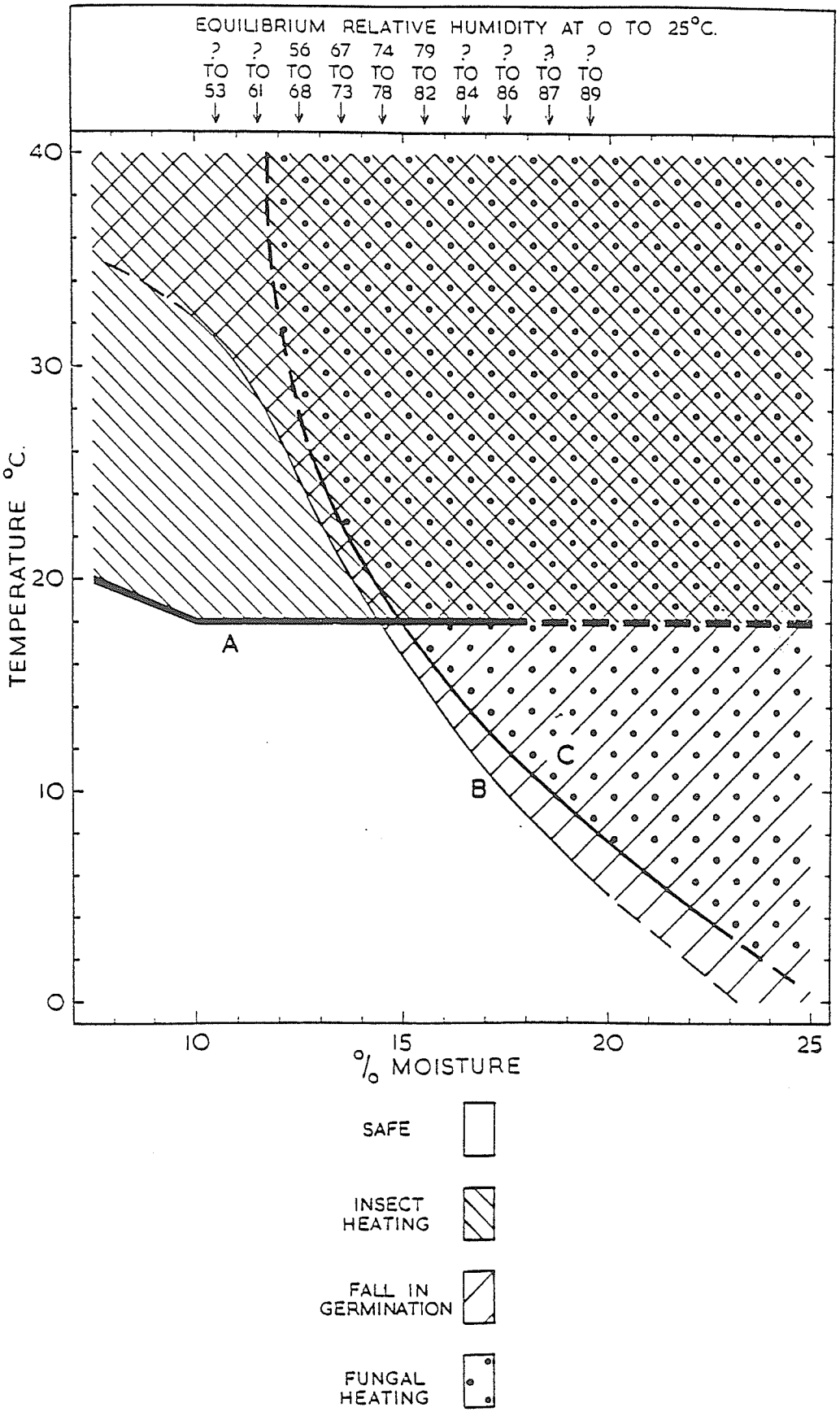
Conditions Necessary for Safe Storage.

The ability of insects and fungi to cause damage in stored grain is dependent on two major factors: temperature and moisture content (affected by relative humidity). In general, dry conditions favor long term storage of grain. Burges and Burrell (1964) demonstrated the combined effects of temperature and moisture content on the keeping quality of stored grain (Fig. 12). Aeration helps to reduce and or maintain a uniform temperature throughout the grain bulk. With uniform temperatures, moisture transfer throughout the grain also becomes uniform. This prevents the development of local and often hidden or unexpected hot spots which directly affect fungal and insect activity. Except for the granary weevil, *Sitophilus granarius* (Linnaeus) which can complete its development at 15°C, grain held at 20°C will stop the development of most stored-product insects (Fields, 1992). In the case of fungi, the lowest temperature of growth is normally influenced by the moisture activity of the substrate. For example, *Penicillium* species can often grow at temperatures of -5°C but only if the moisture content is greater than 17% (Hyde and Burrell, 1973).

Climatic Effects During Aeration.

Changes in temperature and moisture content of stored grain depend mainly on the relative humidity of the air used during aeration. Since the relative humidity can differ from day to night and month to month, it is important to recognize these differences and act accordingly (Burrell, 1974). Warm, humid air when used for ventilation will enhance mold growth; cool, dry air is ideal for successful aeration (Bruce and Ryniecki, 1991).

Figure 12. Relationship of storage temperature and grain moisture content to insect heating, reduction in germination and damp grain heating (from Burges and Burrell,1964).


EQUILIBRIUM RELATIVE HUMIDITY AT 0 TO 25°C.
? TO 53
? TO 61
56 TO 68
67 TO 73
74 TO 78
79 TO 82
? TO 84
? TO 86
? TO 87
? TO 89
TEMPERATURE °C.
40
30
20
10
0
A
B
C
10
15
20
25
% MOISTURE
SAFE
INSECT HEATING
FALL IN GERMINATION
FUNGAL HEATING

INSECT MOVEMENT WITHIN GRAIN

Insect movement within grain is influenced by several factors. Among stored product insects, moths such as the Angoumois grain moth, *Sitotroga cerealella* (Oliv.) are very fragile and weak and therefore confine their activities such as egg deposition to exposed grain surfaces. In contrast the distribution of invasive insects including beetles such as the confused flour beetle, *Tribolium confusum* (Duv.) is influenced by the temperature, moisture and chaff accumulation (dockage) within the grain. The majority of beetle species move freely in the grain (Cotton and Wilbur, 1974).

Effect of Temperature and Moisture

The temperature of stored grain, which varies with season and geographical location is known to affect the distribution of insects. The type or nature of the storage bin or silo can also influence the temperature and moisture content of the grain. For example, the temperature differences between small and large bins, wooden, steel or concrete bins and temperature differences in spring, autumn, summer and winter are known to affect the distribution of insects and their locomotory activity in bulk grain (Barre, 1954; Cotton and Wilbur, 1974; Muir, 1973). Since insects are mostly of subtropical origin they do not develop resistance to low temperatures (Cotton, 1954).

As with temperature, the moisture content of grain can also influence insect distribution (Howe, 1951; Lochiavo, 1983). Moisture content within grain is rarely uniform and is changeable from season to season and from one climatic zone to another (Sinha, 1973a). Damp or higher moisture content grain (greater than 13%)

attracts insects particularly fungus feeders (fungivors) or scavengers like the foreign grain beetle (Wallace and Sinha, 1962; Cotton and Wilbur, 1974; Lochiavo, 1983). For example, the adult saw toothed grain beetle, *Oryzaephilus surinamesis* avoids regions of high humidity (100%) yet responds hygropositivly to alterations in relative humidity (20-60%) (Arbogast and Carthon, 1972).

Effect of Packing and Dockage.

During storage, grain has the tendency to settle or pack. This packing action differs according to the nature of the grain. For example, light and heavy weight grain like oats or wheat pack differently. Grain also tends to separate into heavier and lighter components when poured or drawn from a bin. Smaller or broken kernels generally remain between larger kernels, while chaff and dust accumulate towards the bin walls (Bailey, 1974). Pressure changes or packing of grain can influence the distribution or free movement of insects (Howe, 1951). Naturally occurring dockage which includes broken or fractured grain, dust, weed seeds and other foreign materials has also been shown to influence insect movement (Lustig et al., 1977). Broken kernels and excessive amounts of foreign material can attract insects, especially as they provide favourable conditions for the development of the non boring types which do not develop on clean grain but rather on grain dust and broken kernels (Barre, 1954; Cotton and Wilbur, 1974; Li Li and Arbogast, 1991). As a result their movement will depend upon the amount of dockage present in the grain. Dockage is not only attractive, but essential for these type of insects. For example, McGregor (1964) and Li Li and Arbogast (1991) observed that the fecundity of the red flour beetle, *Tribolium castaneum* Herbst, increased rapidly with an increase in dockage; the

movement or distribution of the insect was also shown to be dependent on the amount of dockage present in the grain.

Positive Geotaxism.

Howe (1951) reported that the granary weevil, *Sitophilus granarius* (L.) exhibited a tendency to move downward (positive geotaxism) in a grain column. The authors also reported that some of the weevils tend to move toward the column edges and remain there. Positive geotaxism was also observed in red flour beetle, *T. castaneum* (Herbst) by Sharangapani and Pingale (1956). Watters (1969) also demonstrated that the rusty grain beetle, *C. ferrugineus* (Stephens) was positively geotactic in sound wheat, but not in previously infested wheat and was often found in large numbers near the floor. This insect species is generally present in large numbers in grain infested with fungi
(Wallace and Sinha, 1962).

MATERIALS AND METHODS

Insects and Rearing

Red flour beetles (*Tribolium castaneum* Herbst) obtained from Agriculture Canada (Winnipeg, Mb) were raised on a diet consisting of: whole wheat, 90%; broken wheat, 5%, and wheat germ 5%. The insects were reared in wide-mouthed glass jars (insectary) maintained in a controlled relative humidity chamber (54-60%) at 30 ± 1°C in the dark.

Effect of Aeration on Insect Movement

Ten sections of polyvinylchloride (PVC) pipe (5.0 cm high; 15.0 cm i.d.) were stacked vertically to form a single column or tower 50 cm in height. Adhesive tape was used to seal and join the section joints and provide column stability. The bottom section of the column was fastened to a wooden base in which a circular opening was made; the opening was of the same diameter as the column and was covered by a nylon mesh. A fan housed below the opening, located in the wooden base, was used to provide aeration (1.7 L/sec). The column was filled with red spring wheat (*Triticum aestivum)* which was previously sieved (8-mesh sieve, aperature 2.36 mm); each section contained 1.0 to 1.1 g of loosely packed wheat. A similar column without a fan was constructed and served as the control. Red flour beetles (100) of mixed age and sex were released in the center of the uppermost section (section one). The density of

insects used in this investigation ensured recovery of sufficient numbers from each section to permit analysis, while keeping intragroup disturbance low.

Prior to their release, the insects were sifted using a 20-mesh sieve (aperature ca. 1 mm) and starved for 24 h. The moisture content of the wheat was 13-15%, as determined by a moisture meter (Moister Master 101-A). The instrumental moisture values were slightly lower (0.9 to 1%) than those obtained by oven-drying (10-g samples of wheat dried for 19 h at 130° C; American Society of Agricultural Engineers, 1975.) In order to prevent insects from climbing and escaping the column, an additional section was placed on the top of the column. The interior of this section was painted with fluon and contained no grain (Navarro et al., 1981). All experimentation was conducted in the dark using an environmental chamber at 30° C with 54-60% relative humidity.

At 1, 3, 5, 7, and 12 days, the columns were dismantled by removing the adhesive tape from the section joints. Each section, starting with the top was carefully moved onto a semicircular metal receptacle. The receptacle was constructed to fit closely against the wall of the column, and as a result, facilitated sample collection from individual sections. The grain was collected in plastic bags, sifted and the number of beetles in each section was counted and recorded. The experiment was repeated six times.

In order to determine the influence of sex with respect to insect dispersion, the following study was performed. Males and females were differentiated at the pupal stage (Halstead, 1986) and unisex populations were maintained on whole wheat flour (95%) and wheat germ (5%). Either adult male (100) or female insects (100) were released on top of the either aerated or non aerated columns (section one) after an

initial 24 h starvation period. The insect distribution was evaluated at 7 days. The experiment was performed in triplicate. Column construction, operating conditions, and collection of samples was as described previously.

A similar protocol was repeated by releasing either adult males (100) or females (100) or a mixture of adult males (100) and females (100) in the middle section of the columns (between sections 5 and 6). The insect distribution was evaluated at 7 days. The experiment was repeated in duplicate.

Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS Institute Inc., Cary, N.C). Analysis of variance and Duncun's multiple range test (1955) was used to determine two way interaction between: aeration and storage time; column section and aeration; column section and storage time; male and female-top release; male and female-middle release.

RESULTS

The effects of aeration on beetle movement within a simulated granary column are shown in Figures 13 to 17. The insect population in the top section (section one) of both the aerated and non aerated columns (controls) was significantly higher ($P < 0.05$) than in the remaining column sections (Appendix Tables 9 and 10). The release of beetles in to section one was accompanied by a gradual dispersion to all sections. At the termination of the study, on day 12 , the insect population in section one decreased from 100% to 46% and 38% in the aerated and control columns respectively. Throughout the study it was observed that the insect density was ca. 10-15% higher in the uppermost section under aeration as compared to the control. During storage a downward movement of insects was observed by virtue of their appearance in the lower column sections. By the fifth day, (Fig. 15) insects could be recovered from all column sections; the bottom section (section ten) contained ca. 10% of the original insect population in both the aerated and control columns. At seven days of storage the insect population in section one decreased to approximately 60% and 40% in the aerated and control columns respectively (Fig. 16). Insect levels throughout the remaining sections also increased, most noticeably in the bottom section of the aerated column. The pattern of migration at 12 days (Fig. 17) of storage was almost similar to that at 7 days. The upper 5-10 cm of the column contained ca. 40-50% of the initial insect population; the bottom 40-50 cm contained ca.20- 30% of the insect population.

Figure 13. Dispersion behaviour of a mixed population of male and female, *T. castaneum* (Hbst.) in a grain column after one day with and without aeration (100 insects released at the top of the column).

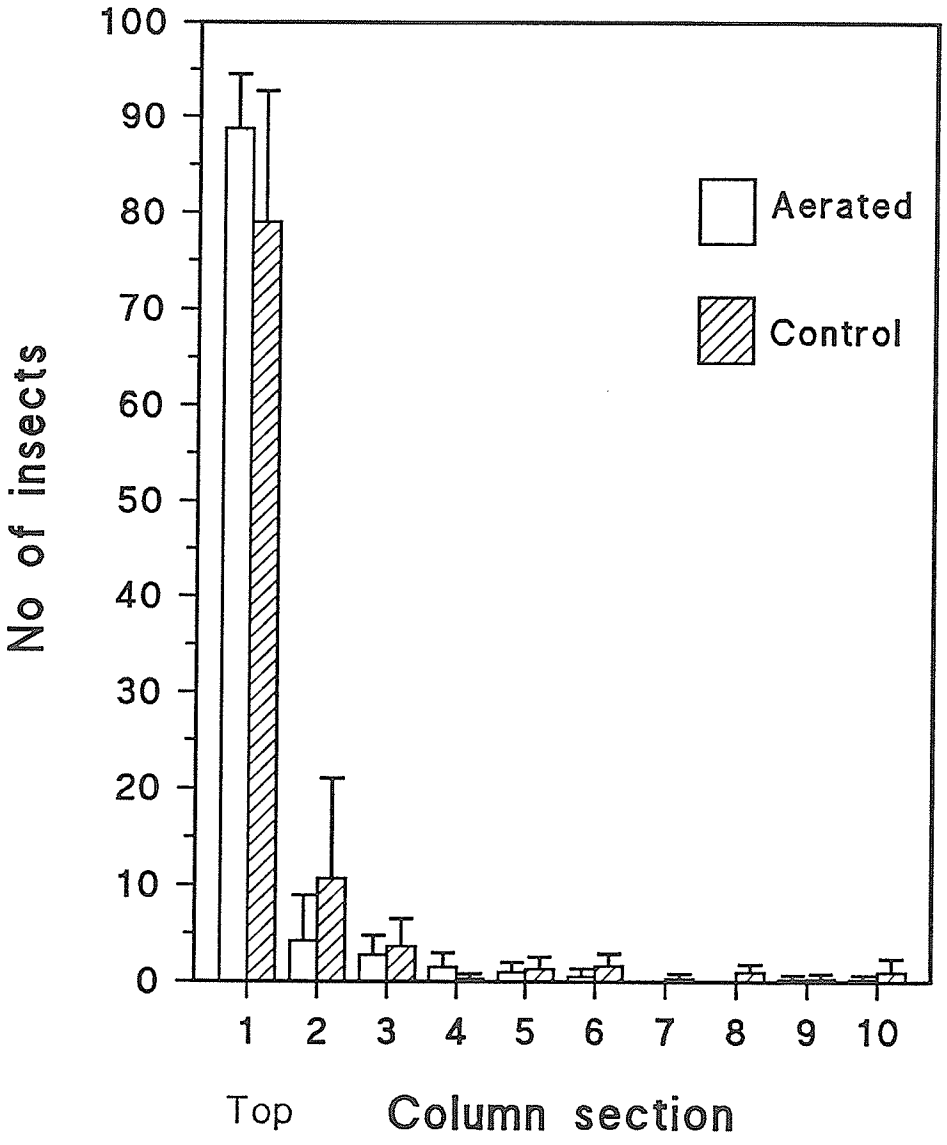

100
90
80
70
60
50
40
30
20
10
0
No of insects
Aerated
Control
1
2
3
4
5
6
7
8
9
10
Top
Column section

Figure 14. Dispersion behaviour of a mixed population of male and female, *T. castaneum* (Hbst) in a grain column after three days with and without aeration (100 insects released at the top of the column).

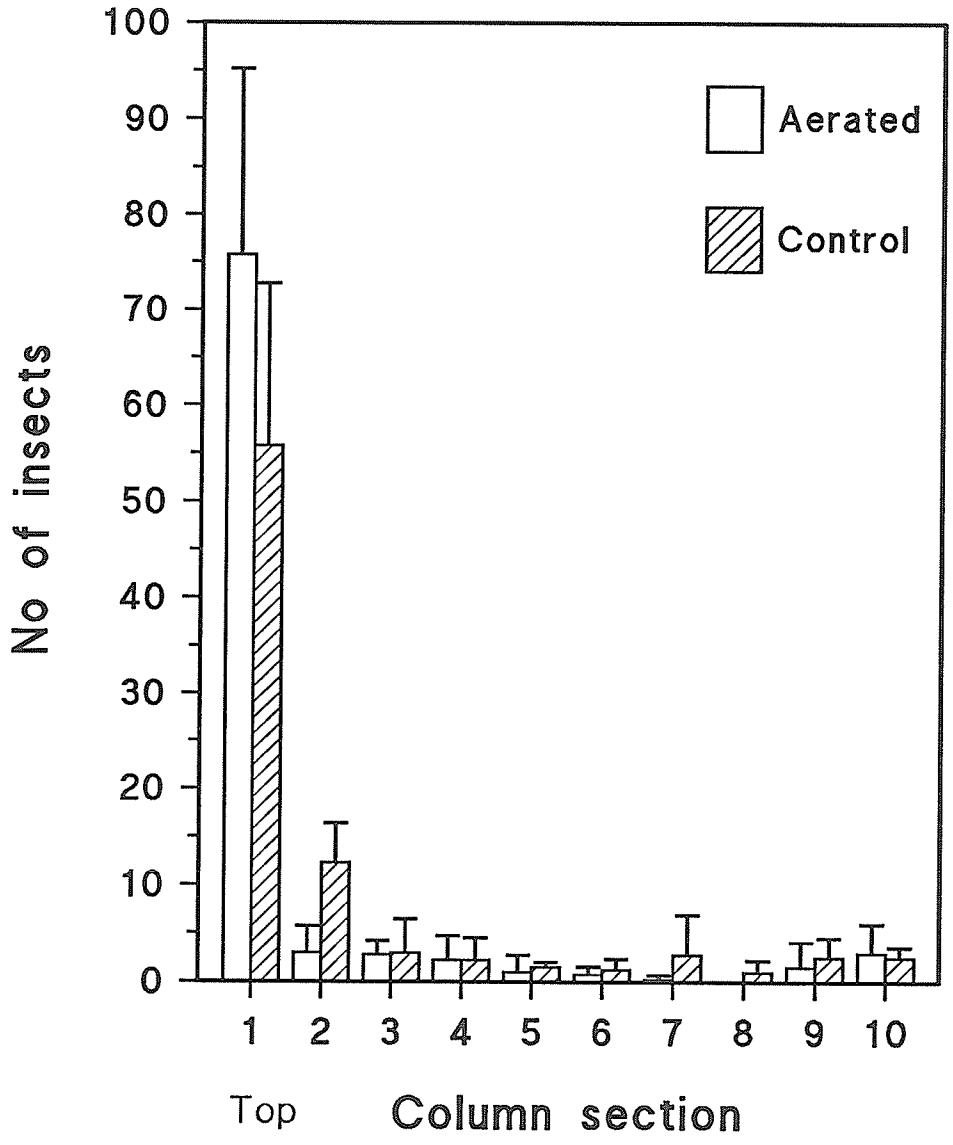

100
90
80
70
60
50
40
30
20
10
0
No of insects
Aerated
Control
1
2
3
4
5
6
7
8
9
10
Top
Column section

Figure 15. Dispersion behaviour of a mixed population of male and female, *T. castaneum* (Hbst) in a grain column after five days with and without aeration (100 insects released at the top of the column).

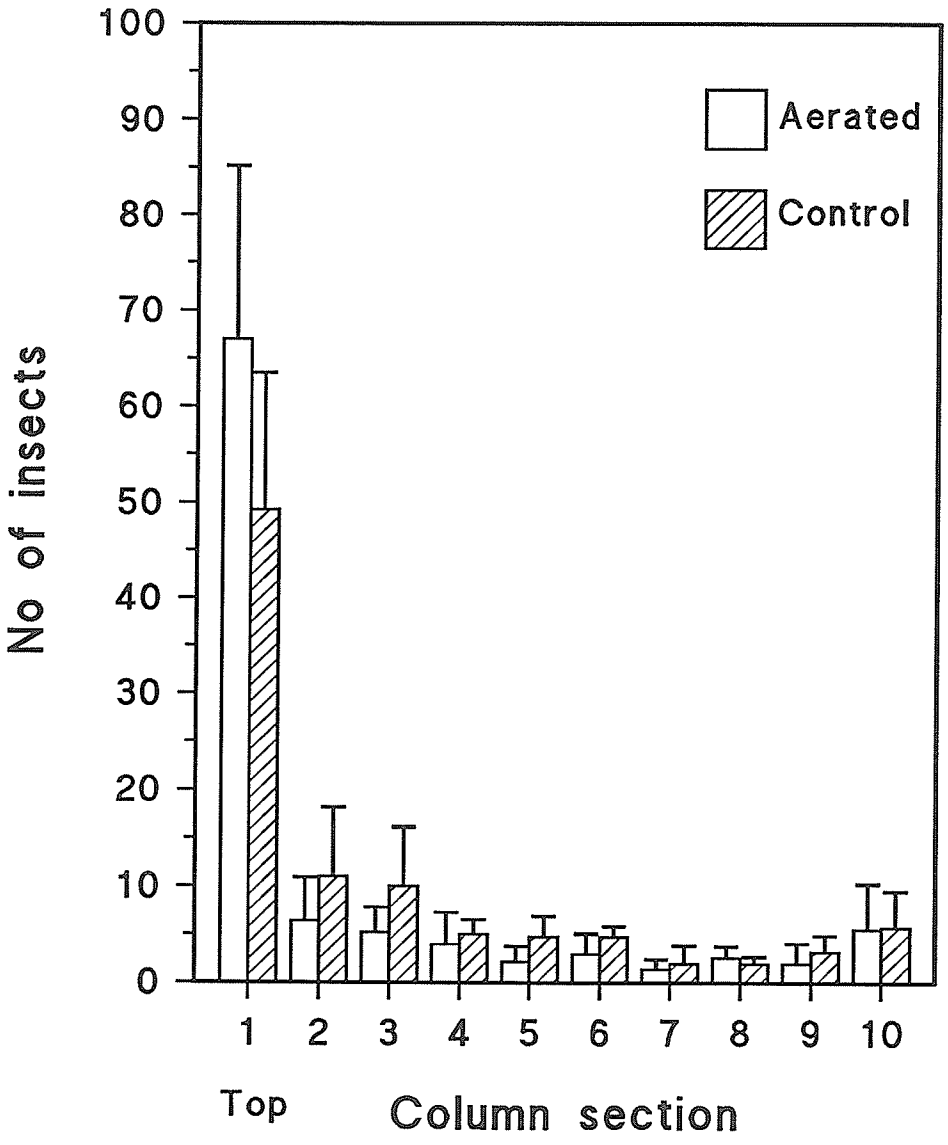

100
90
80
70
60
50
40
30
20
10
0
No of insects
Aerated
Control
1
2
3
4
5
6
7
8
9
10
Top
Column section

Figure 16. Dispersion behaviour of a mixed population of male and female, *T. castaneum* (Hbst) in a grain column after seven days with and without aeration (100 insects released at the top of the column).

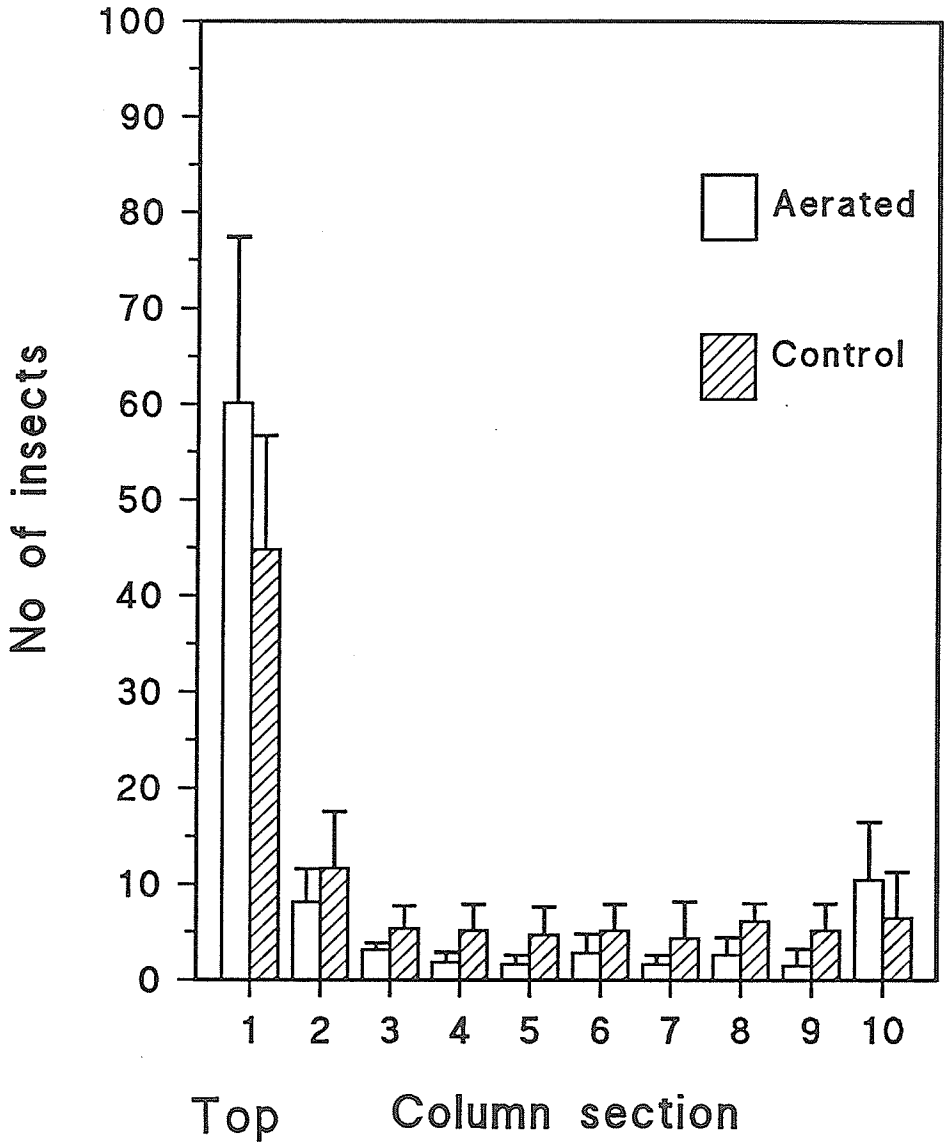
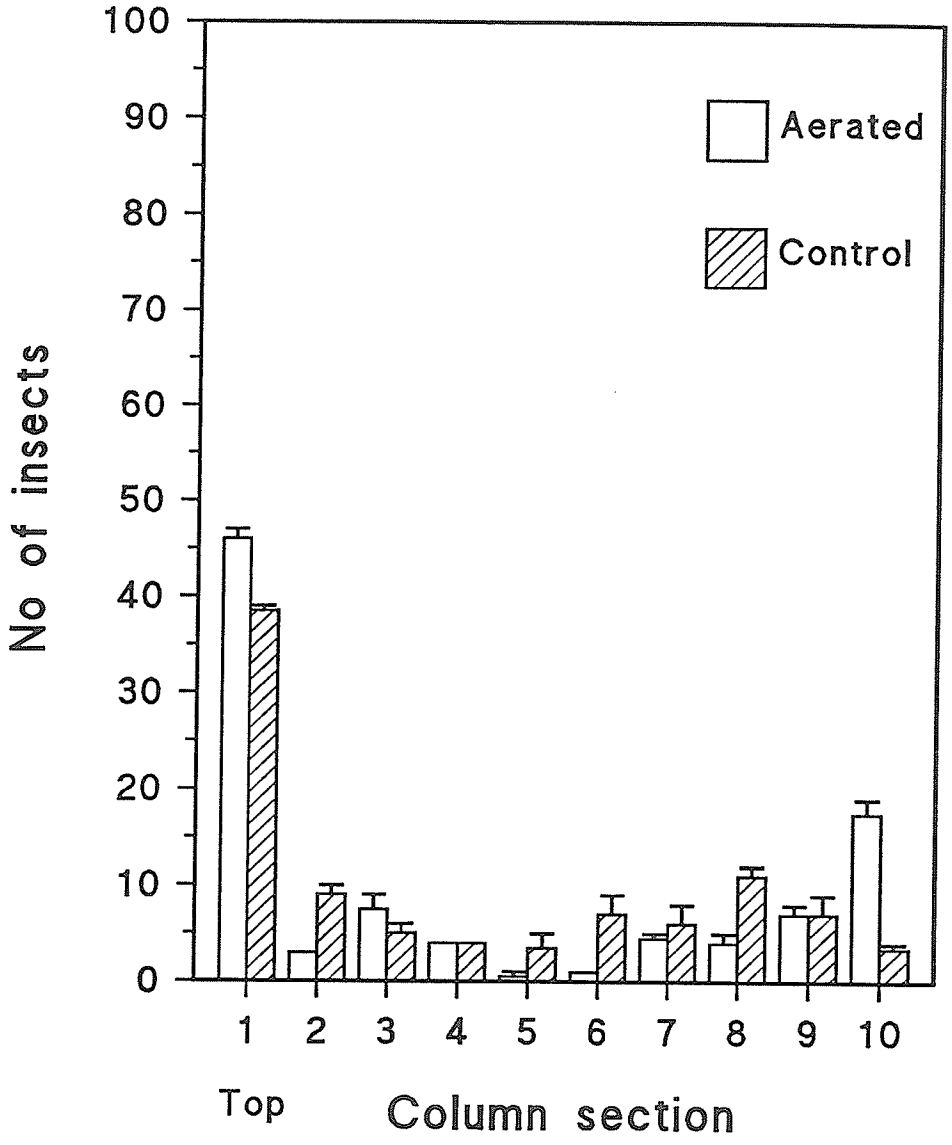

100
90
80
70
60
50
40
30
20
10
0
No of insects
Aerated
Control
1
2
3
4
5
6
7
8
9
10
Top
Column section

Figure 17. Dispersion behaviour of a mixed population of male and female, *T. castaneum* (Hbst) in a grain column after twelve days with and without aeration (100 insects released at the top of the column).


100
90
80
70
60
50
40
30
20
10
0
No of insects
Aerated
Control
1
2
3
4
5
6
7
8
9
10
Top
Column section

Insect populations in sections one and ten were significantly different from the remaining sections ($P < 0.05$) but only in aerated columns. Column sections 1, 7, 8, 9 and 10 were significantly different from the remaining sections in aerated columns at the 1, 10, 5, 10, 1% level; in the control column, sections 1, 4, 6, 8, 9 were significantly different from the remaining sections at the 5, 5, 5, 1 and 10% level respectively. (Appendix Table 11 and 12).

In aerated columns at 7 days, ca. 60% of the males released into section one remained within the top section of the column; 20% had penetrated the column to a depth of 45-50 cm, while ca. 20% were distributed throughout the remaining sections (Fig. 18). In the control columns, ca. 40-50% of the insects were retrieved in the top section while the residual population was distributed throughout the remaining sections; no clear distribution pattern was observed.

The downward movement of female beetles released into section one (Fig. 19) appeared greater than their counter parts. In the aerated columns, 50% of the female population was isolated in section one at 7 days, whereas 20% was isolated in section ten. In the control columns, ca. 30% of the female population was retrieved from section one at 7 days (Fig. 19) while ca. 15-20% was retrieved from section ten (Appendix Table 13).

When 100 male beetles were released in the middle of the aerated column (between section five and six), ca. 30-35% of the population became concentrated in section six at 7 days. Section ten contained the next highest population (25-30%). In the control column, the beetle distribution at seven days was largely confined to section five (30-35%) and six (15-20%). The bottom section contained the third

Figure 18. Dispersion behaviour of male *T. castaneum* (Hbst) in a grain column after seven days with and without aeration (100 insects released at the top of the column).

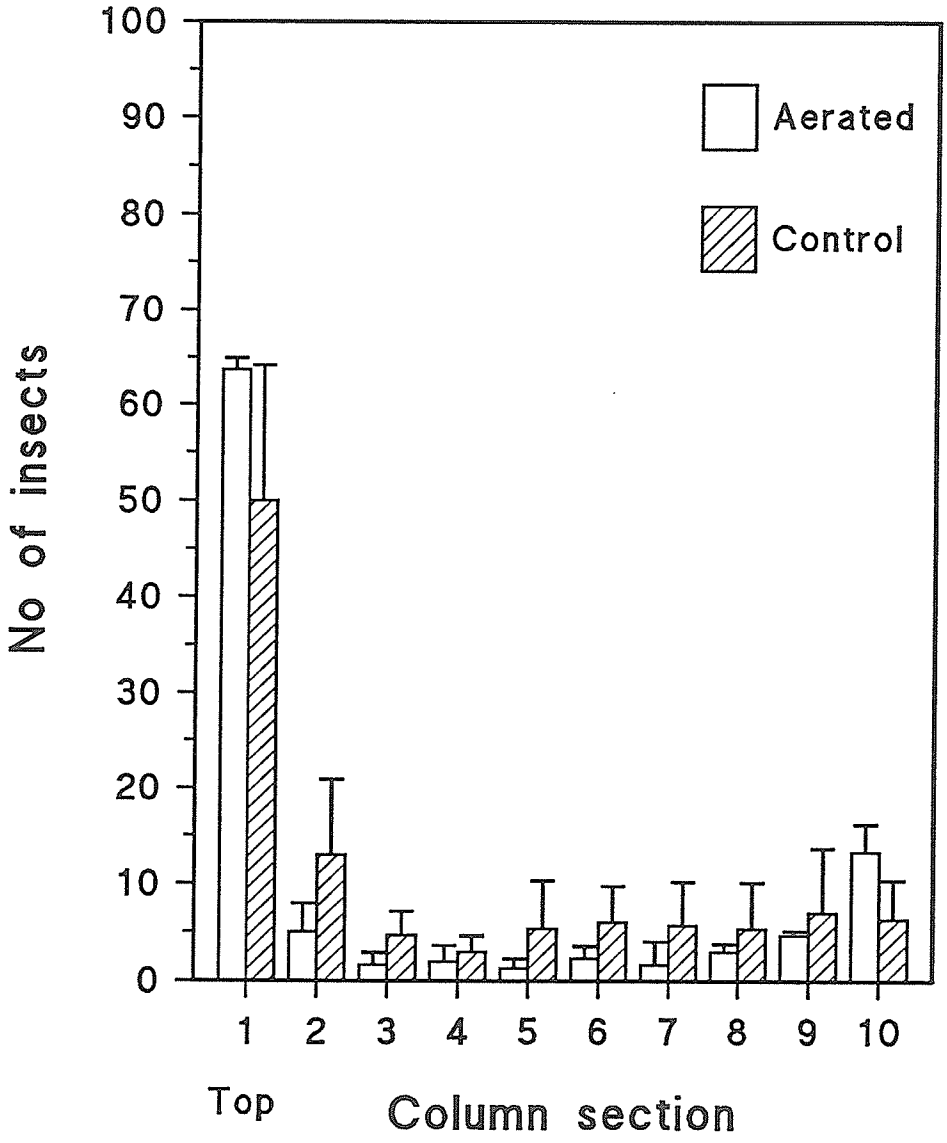

100
90
80
70
60
50
40
30
20
10
0
No of insects
Aerated
Control
1
2
3
4
5
6
7
8
9
10
Top
Column section

Figure 19. Dispersion behaviour of female *T. castaneum* (Hbst) in a grain column after seven days with and without aeration (100 insects released at the top of the column).

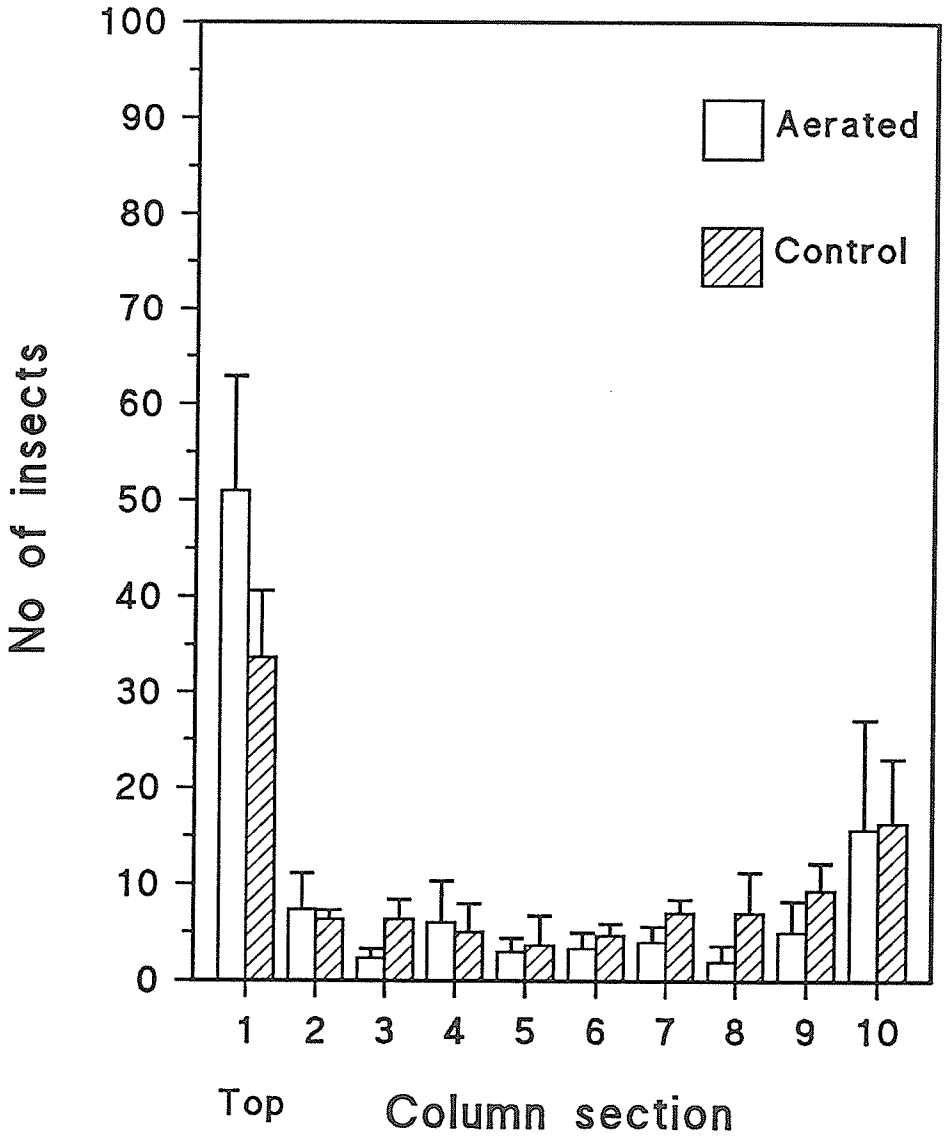
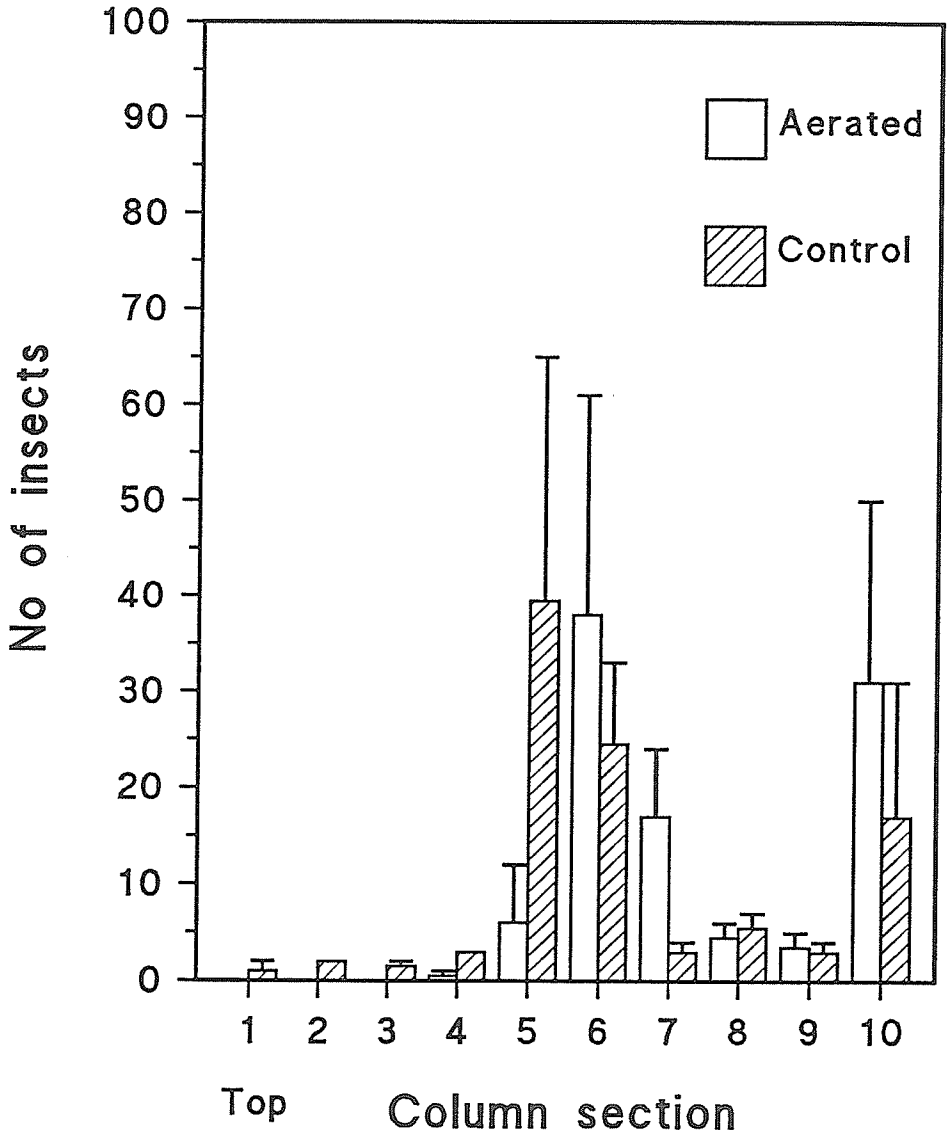

100
90
80
70
60
50
40
30
20
10
0
No of insects
Aerated
Control
1
2
3
4
5
6
7
8
9
10
Top
Column section

Figure 20. Dispersion behaviour of male *T. castaneum* (Hbst) in a grain column after seven days with and without aeration (100 insects released in the middle of the column).


100
90
80
70
60
50
40
30
20
10
0
No of insects
Aerated
Control
1
2
3
4
5
6
7
8
9
10
Top
Column section

highest population (ca. 15-20%). When this protocol was repeated with females, the highest population at 7 days was found in section six (40-50%) under non aerated conditions (Fig 21). Under aerated conditions the highest insect population was found in section ten (25-30%). No significant difference in distribution was observed between male and female populations ($P=0.05$)

When a mixed population consisting of 100 male and 100 female beetles was released in the middle of either control or aerated columns (between section five and six) over 90% of the insects dispersed to lower (6-10) sections (Fig.22). At seven days, major insect populations appeared in section six and ten in control and aerated columns, respectively. No significant difference was observed between either the dispersion behaviour of females and males or the column type (Appendix Table 14).

Figure 21. Dispersion behaviour of female *T. castaneum* (Hbst) in a grain column after seven days with and without aeration (100 insects released in the middle of the column).

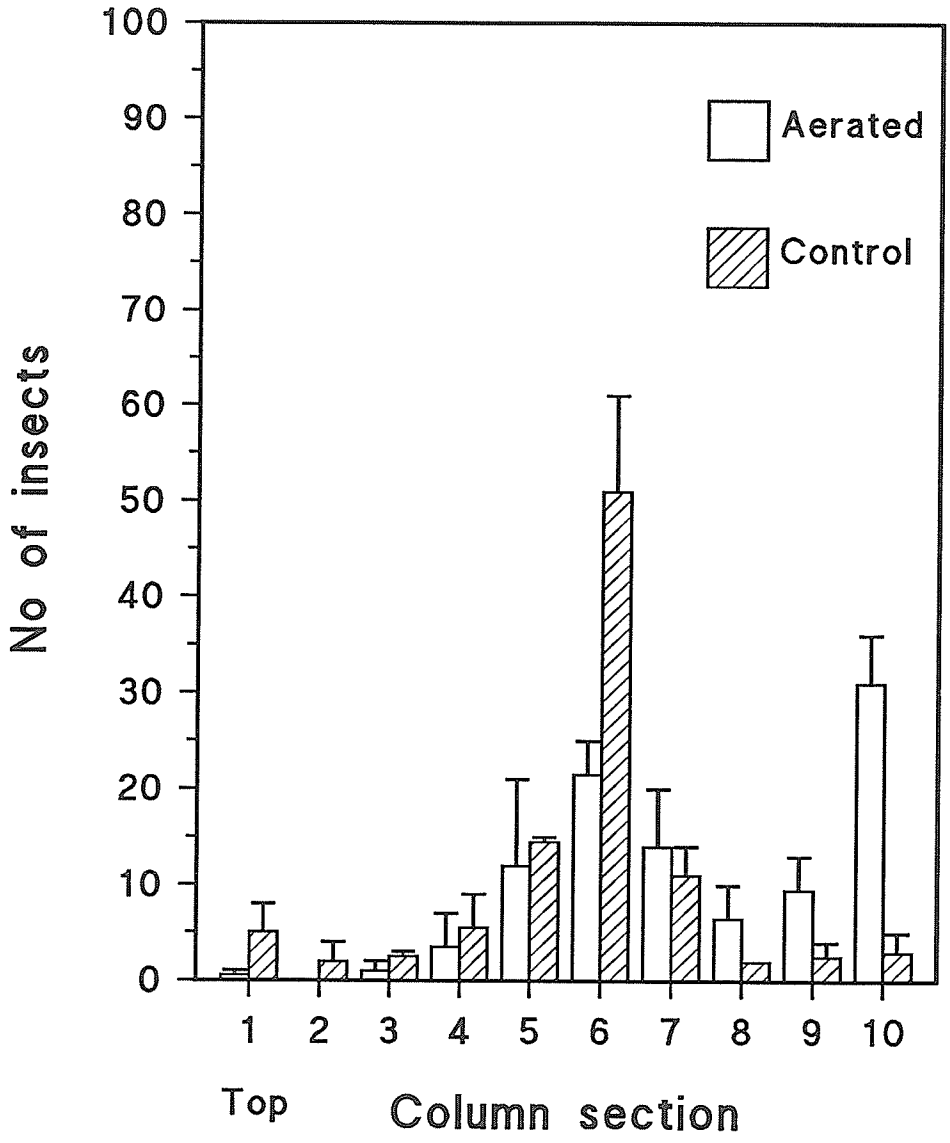
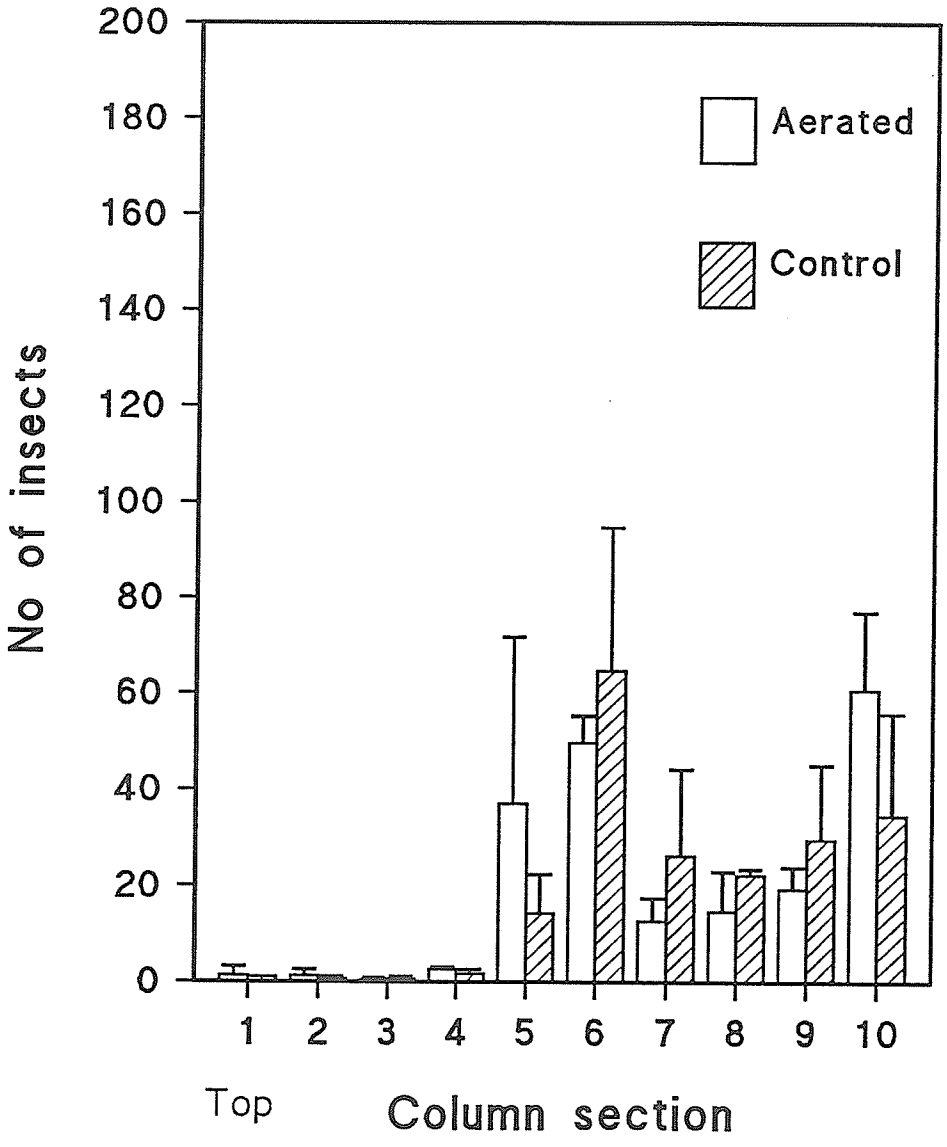

100
90
80
70
60
50
40
30
20
10
0
No of insects
Aerated
Control
1
2
3
4
5
6
7
8
9
10
Top
Column section

Figure 22. Dispersion behaviour of mixed (100 males and 100 females) *T. castaneum* (Hbst) in a grain column after seven days with and without aeration (insects released in the middle of the column).


200
180
160
140
120
100
80
60
40
20
0
No of insects
Aerated
Control
1
2
3
4
5
6
7
8
9
10
Top
Column section

DISCUSSION

The dispersion of grain infesting insects and or species is often random but can be influenced by a variety of factors including locomotory activity, boundary response and intragroup stimulation (Surtees, 1964).

In the present study the release of *T. castaneum* (Herbst) in the uppermost layer of grain resulted in a nonuniform vertical distribution. The highest insect density was observed 5 to 10 cm from the point of release. Dispersion occured to all sections of the column, often in one day. This distribution profile was observed in both aerated and non aerated columns and remained unchanged throughout the period of experimentation. White and Lochiavo (1986) and Surtees (1963) also reported highest retrieval of *T. castaneum* from the uppermost sections (5-10 cm) of non aerated grain columns. However, these authors reported that recoveries decreased from top to bottom and in this respect differ from the present results. Recoveries from the uppermost (5-10 cm) and lowermost sections (45-50 cm) were invariably higher in aerated columns.

The higher number of insects obtained from the top portion of the grain mass may be attributed to several factors of which grain packing is probably the most important. As suggested by Howe (1951) and Jones (1943) packing increases with depth. Tight packing of grain may therefore limit insect movement. In the case of weevils, for example, Howe (1951) suggested that size difference among species

greatly influenced their freedom of movement in bulk grain.

The uppermost sections in both aerated and control columns contained grain with the highest moisture content. The difference in the moisture content between the top and bottom sections (section one and ten) was ca. 1.0%. This may also explain the increased density of insects at the top since higher moisture grain is known to attract insects (Lochiavo, 1983) and stimulate oviposition (Smith, 1962). Increasing the moisture content of grain increases the intergranular space which also increases the ease of insect movement.

Despite packing effects and lower moisture grain ca. 10 to 20% of the population was able to penetrate the grain bulk to reach the lowest section of the column. The results of this downward movement are somewhat contradictory and may indicate an overriding attraction of the insects to reach the bottom of the column. Such positive geotaxism has also been observed by Sharangapani and Pingale (1956) with *T. castaneum* (Herbst). Surtees (1964) reported that insects also tend to aggregate in regions where locomotory activity becomes minimal. It is possible therefore that attraction to the column ends was geotactic in nature and that accumulation was enhanced by their slow redispersion into the grain bulk. Such an effect could result from reduced kinesis mitigated by a reduction in the moisture content of the grain in this section.

Temperature and moisture gradients in the grain, created by aeration may also account for the differences in insect dispersion between column types. For example, Surtees (1963) reported that both temperature and moisture had a profound effect on *Tribolium* movement. Although temperatures within the grain bulk were not monitored, it is not unreasonable to consider that aeration could create both

temperature and moisture gradients different from those in non-aerated columns despite common incubation in a temperature controlled environment. Aeration would also have the effect of decreasing grain packing, particularly at the upper levels of the column thereby creating a more conducive environment for movement and feeding.

The accumulation of intergranular CO_2 resulting from biological respiration would also be expected to decrease as a result of aeration. This diminution in CO_2 concentration may also affect insect movement. For example, Navarro et al. (1969) reported that insect aggregation was observed to increase in areas of high O_2 tension. Further studies by Navarro et al. (1981) with the sawtoothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus) indicated that dispersion was markedly curtailed by the presence of low O_2 concentration within a grain column.

The presence and distribution of volatile compounds in stored grain produced as end products of fungal or bacterial metabolism is influenced by aeration (Sinha et al., 1988; Tuma et al., 1989). Insects are known to respond to various volatile chemicals some of which, called pheromones, act as sexual attractants. Volatiles produced during grain storage may influence insect movement or aggregation by acting as either attractant or repellants. Aeration could therefore influence insect movement by its ability to remove, disperse or redistribute volatiles. For example, Kamiński and Wasowicz (1991) reported that the volatile alcohol concentration in aerated grain columns decreased from top to bottom and that the opposite pattern was observed in non-aerated columns. The authors reported that differences in volatile carbonyl concentration were also observed between aerated and non-aerated columns.

The separate release of females and males in the uppermost portion of either aerated or control columns resulted in distribution profiles somewhat similar to those

obtained using mixed populations. Highest insect densities were obtained in aerated columns, 5.0 cm from their point of release. Based on the migration profiles, it would appear that the females were more active than the males since fewer were retrieved in the uppermost section and more were retrieved from the bottom sections.

According to Howe (1951) neither age nor sex appeared to influence the locomotory activity of granary weevils. However, differences in the dispersion pattern were observed by Surtees (1964) between mated and unmated adults. In addition, Surtees (1964) observed that males of *T. castaneum* (Hbst) were more active compared to females. Since the respiratory activity of females is ca. 40% higher than the males, Surtees (1964) concluded that reduced kinesis in females would act to conserve moisture and weight. In this study, however, females appeared more active than males regardless of column type. Enhanced female activity was further demonstrated by their upward movement when released from the middle column sections. The release of *T. castaneum* (Hbst) from the middle sections resulted in some upward movement; this movement appeared more pronounced with females particularly in control columns. Downward movement was more pronounced in aerated columns; insect densities between sexes appeared minimal when the population was doubled. Movement was primarily downward and resulted in a more uniform distribution. The dispersion can be attributed to overcrowding and intragroup stimulation (Surtees 1963). The retrieval of insects in the bottom of the column reinforces the observation that *T. castaneum* (Hbst) is positively geotactic.

GENERAL CONCLUSION

Insects in their own right are important agents with regard to contamination and deterioration of stored cereal grains. However, insects also interact with fungi. Through feeding, insects can act as vectors for the dissemination of fungal spores. In addition, they may provide ideal environments for spore germination and development. Their detection and estimation in stored grain is of paramount importance. In this investigation, two studies related to the presence of insects in stored grain were examined. The first study (Section I) focused on an insect-fungus interaction which hitherto has not been fully investigated, viz. the impact of insect frass on fungal growth and toxin production. The second study (Section II) focused on the distribution of insect in bulk grain as affected by ventilation. If ventilation affects the locomotory activity of insects then it may also have a dramatic effect on the distribution of fungi within a grain bulk. When sampling to detect or estimate insects the distribution component must be considered. Accurate assessment of infestation must take into account the uneven distribution of insects with a grain bulk. Therefore, factors which contribute to the population dynamics of insects must be examined carefully.

In summary, the following points are presented:

1. The growth (glucosamine content) pattern of *A. ochraceus* in wheat (control) appeared curvilinear. Maximum growth occurred at 14 d thereafter it decreased rapidly. With the addition of frass, the growth pattern appeared to become linear.

Within the 21 d incubation period, maximum growth occurred in the controls.

2. The pattern of OA production on wheat (control) appeared curvilinear. Maximum levels occurred at 14 d thereafter decreasing. In substrates containing frass, *A. ochraceus* exhibited enhanced OA production particularly at 21 d. The pattern of OA production in substrates with frass appeared linear. The effect was also observed when barley was used as substrate.

3. The increased nitrogen content, supplied by the uric acid component of the frass is likely responsible for the altered patterns of growth and OA production by *A. ochraceus*.

4. The effect of frass concentration on growth and or OA production was not clear. Uneven distribution of frass within the substrate matrix may have contributed to this problem.

5. The inclusion of frass in non sterile grain contributed to lower production of OA. An increase in the overall growth of the microflora may have contributed to increased competition resulting in decreased OA production.

6. The locomotory activity of the red flour beetle, *T. castaneum* was observed in a grain column after 1, 3, 5, 7, and 12 days with and without aeration. After 12 d insects aggregated mostly at the top and bottom of the aerated column, whereas, in the control column no definite pattern was observed. The dispersion behaviour of the insects in the aerated column was believed to be due to the changing pattern of moisture, temperature, O_2 , and volatile compounds. Probably positive geotaxism is another factor. High levels of frass will be found in the grain bulk where insect aggregation is high. Higher insect aggregation may lead to increased fungal growth and or toxin production.

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Appendix Table 1

Effect of frass on OA production by *A. ochraceus* in barley with 24% moisture.

Frass (%)	Ochratoxin (μg/g)	
	Incubation Period (d)	
	7	14
0	$5.9^{a} \pm 0.4$	$21.9^{a} \pm 4.6$
3	$5.3^{a} \pm 0.7$	$14.9^{b} \pm 3.5$
6	$3.2^{b} \pm 0.8$	$20.0^{a} \pm 3.4$
9	$1.4^{c} \pm 0.2$	$12.4^{b} \pm 1.6$

Means $\pm$ SD followed by the same superscript within a column are not significantly different (P=0.05)
n=6

Appendix Table 2

Effect of frass on OA production by *A. ochraceus* in whole wheat with 24% moisture.

Frass (%)	Ochratoxin (μg/g)		
	Incubation period (d)		
	7	14	21
0	$5.9^{a} \pm 2.6$	$17.2^{a} \pm 4.5$	$4.1^{b} \pm 0.4$
3	$2.9^{b} \pm 0.0$	$3.2^{c} \pm 0.5$	$7.0^{b} \pm 0.4$
6	$2.0^{b} \pm 0.8$	$3.4^{c} \pm 0.9$	$5.3^{b} \pm 1.2$
9	$3.2^{b} \pm 0.3$	$13.3^{b} \pm 2.0$	$26.1^{a} \pm 5.5$

Means $\pm$ SD followed by the same superscript within a column are not significantly different ($P = 0.05$)
n=6

Appendix Table 3

Effect of frass on the growth (glucosamine content) of *A. ochraceus* in whole wheat with 24% moisture.

Frass (%)	Glucosamine (mg/g)		
	Incubation period (d)		
	7	14	21
0	3.55[a] ± 0.07	30.29 ± 1.71	23.73 ± 1.01
3	1.99 ± 0.07	26.06 ± 0.13	23.94 ± 3.17
6	1.35 ± 0.06	10.37 ± 0.36	15.65 ± 0.10
9	0.71 ± 0.04	5.29 ± 0.0	22.04 ± 1.95

[a]= values represent the average of duplicate determination ± SD.

Appendix Table 4

Effect of frass on OA production by *A. ochraceus* in ground wheat with 24% moisture.

Frass (%)	Ochratoxin (μg/g)		
	Incubation period (d)		
	7	14	21
0	$72.6^{b} \pm 13.7$	$164.2^{bc} \pm 58.3$	$162.4^{a} \pm 18.1$
3	$26.6^{c} \pm 4.3$	$122.2^{c} \pm 32.9$	$287.9^{a} \pm 58.9$
6	$37.0^{c} \pm 3.1$	$190.5^{ab} \pm 30.5$	$227.2^{a} \pm 53.2$
9	$250.7^{a} \pm 19.5$	$222.0^{a} \pm 27.0$	$253.8^{a} \pm 54.1$

Means $\pm$ SD flllowed by the same superscript within a column are not significantly different (P=0.05)
n=6

Appendix Table 5

Effect of frass on the growth (glucosamine content) of *A. ochraceus* in ground wheat with 24% moisture.

Frass (%)	Glucosamine (mg/g)		
	Incubation period (d)		
	7	14	21
0	2.96[a] ± 0.04	10.90 ± 0.48	6.04 ± 0.11
3	1.57 ± 0.04	2.75 ± 0.0	6.21 ± 0.16
6	2.47 ± 0.05	2.55 ± 0.08	2.41 ± 0.28
9	3.59 ± 0.0	5.00 ± 0.04	7.82 ± 0.13

[a]= values represent the average of duplicate determination ± SD

Appendix Table 6

Effect of Uric acid on OA production by *A. ochraceus* in ground wheat with 24% moisture.

Uric Acid (g)	Ochratoxin (μg/g)		
	Incubation period (d)		
	7	14	21
0.0	$6.1^{a} \pm 0.2$	$9.4^{b} \pm 2.8$	$10.8^{a} \pm 2.0$
0.0768	$4.4^{a} \pm 0.6$	$6.1^{b} \pm 0.4$	$8.9^{a} \pm 1.2$
0.1535	$6.4^{a} \pm 2.3$	$6.9^{b} \pm 1.2$	$9.1^{a} \pm 1.0$
0.2304	$5.6^{a} \pm 2.8$	$23.1^{a} \pm 10.5$	$11.1^{a} \pm 2.3$

Means $\pm$ SD followed by the same superscript within a column are not significantly different (P=0.05)
n=6

Appendix Table 7

Effect of uric acid on the growth (glucosamine content) of *A. ochraceus* in ground wheat with 24% moisture.

Uric acid (g)	Glucosamine (mg/g)		
	Incubation period (d)		
	7	14	21
0.0	2.36 ± 0.14	3.16 ± 0.13	4.13 ± 0.13
0.0768	2.74 ± 0.01	2.57 ± 0.02	4.81 ± 0.19
0.1535	2.84 ± 0.14	4.88 ± 0.04	4.70 ± 0.07
0.2304	3.55 ± 0.25	10.18 ± 0.19	14.65 ± 0.0

[a]= values represent the average of duplicate determination ± SD.

Appendix Table 8

Effect of inoculum size on OA production by *A. ochraceus* in sterile and nonsterile ground wheat with and without frass.

Frass(%)	Ochratoxin (μg/g) Inoculum size(CFU/ml)					
Sterile	10^4		10^5		10^6	
	7d	14d	7d	14d	7d	14d
0	$38.1^a \pm 24.0$	$122.2^a \pm 35.5$	$33.9^a \pm 6.2$	$34.6^a \pm 14.9$	$30.1^a \pm 6.6$	$13.4^b \pm 3.2$
5	$30.4^b \pm 5.4$	$31.2^b \pm 6.0$	$14.2^b \pm 3.9$	$38.8^a \pm 2.7$	$18.6^b \pm 2.3$	$25.0^a \pm 4.2$
Non sterile						
0	N D	N D	$0.5^a \pm 0.3$	$1.6^a \pm 0.7$	$1.5^a \pm 0.3$	$3.8^a \pm 3.2$
5	N D[1]	N D	$0.4^a \pm 0.1$	$0.3^a \pm 0.0$	$0.6^b \pm 0.1$	$0.6^a \pm 0.3$

Means $\pm$ SD followed by the same superscript within a column are not significantly different (P=0.05)
N D Not detected

Appendix Table 9

Dispersion behaviour of *T. castaneum* in a grain column with aeration.

Column section[1]	Column height(cm)	No of insects[a]				
		Day 1	Day 3	Day 5	Day 7	Day 12
1	5	$88.8^{a} \pm 6.7$	$75.8^{a} \pm 22.4$	$67.0^{a} \pm 20.3$	$60.2^{a} \pm 18.9$	$46.0^{a} \pm 1.4$
2	10	$4.3^{b} \pm 5.4$	$3.0^{b} \pm 3.2$	$6.4^{b} \pm 5.0$	$8.2^{bc} \pm 3.8$	$3.0^{efg} \pm 0.0$
3	15	$2.8^{b} \pm 2.4$	$2.8^{b} \pm 1.7$	$7.2^{b} \pm 5.3$	$3.2^{bc} \pm 0.8$	$7.5^{c} \pm 2.1$
4	20	$1.5^{b} \pm 1.7$	$2.3^{b} \pm 2.9$	$4.0^{b} \pm 3.7$	$1.8^{c} \pm 1.2$	$4.0^{def} \pm 0.0$
5	25	$1.0^{b} \pm 1.2$	$1.0^{b} \pm 2.0$	$2.2^{b} \pm 1.8$	$1.7^{c} \pm 1.0$	$0.5^{g} \pm 0.7$
6	30	$0.5^{b} \pm 1.0$	$0.8^{b} \pm 1.0$	$3.0^{b} \pm 2.3$	$2.8^{bc} \pm 2.1$	$1.0^{fg} \pm 0.0$
7	35	$0.0^{b} \pm 0.0$	$0.3^{b} \pm 0.5$	$1.4^{b} \pm 1.1$	$1.7^{c} \pm 1.0$	$4.5^{cde} \pm 0.7$
8	40	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$2.6^{b} \pm 1.3$	$2.7^{bc} \pm 2.0$	$4.0^{def} \pm 0.4$
9	45	$0.3^{b} \pm 0.5$	$1.5^{b} \pm 3.0$	$2.0^{b} \pm 2.3$	$1.5^{c} \pm 2.0$	$7.0^{cd} \pm 1.4$
10	50	$0.3^{b} \pm 0.5$	$3.0^{b} \pm 3.5$	$5.6^{b} \pm 5.2$	$11.0^{b} \pm 5.8$	$17.5^{b} \pm 2.1$

Means ± SD followed by the same superscript within a column are not significantly different (P=0.05).
[1] 100 insects released in section 1.
n=6

Appendix Table 10

Dispersion behaviour of *T. castaneum* in a grain column without aeration.

Column section[1]	Column height(cm)	No of insects[a]				
		Day 1	Day 3	Day 5	Day 7	Day 12
1	5	$79.0^{a} \pm 16.8$	$55.8^{a} \pm 19.6$	$49.3^{a} \pm 16.5$	$41.8^{a} \pm 12.0$	$38.5^{a} \pm 0.7$
2	10	$10.7^{b} \pm 12.7$	$12.3^{b} \pm 4.7$	$11.0^{b} \pm 8.2$	$10.6^{b} \pm 6.6$	$9.0^{bc} \pm 1.4$
3	15	$3.7^{b} \pm 3.5$	$3.0^{bc} \pm 4.0$	$10.0^{b} \pm 7.1$	$5.6^{b} \pm 2.9$	$5.0^{cd} \pm 1.4$
4	20	$0.3^{b} \pm 0.6$	$2.3^{bc} \pm 2.6$	$5.0^{b} \pm 1.8$	$5.8^{b} \pm 2.9$	$4.0^{d} \pm 0.0$
5	25	$1.3^{b} \pm 1.5$	$1.5^{bc} \pm 0.6$	$4.8^{b} \pm 2.5$	$5.0^{b} \pm 3.5$	$3.5^{d} \pm 2.1$
6	30	$1.7^{b} \pm 1.5$	$1.3^{bc} \pm 1.3$	$4.8^{b} \pm 1.3$	$5.6^{b} \pm 3.1$	$7.0^{cd} \pm 2.8$
7	35	$0.3^{b} \pm 0.6$	$2.8^{bc} \pm 4.9$	$2.0^{b} \pm 2.2$	$5.2^{b} \pm 4.1$	$6.0^{cd} \pm 2.8$
8	40	$1.0^{b} \pm 1.0$	$1.0^{c} \pm 1.4$	$2.0^{b} \pm 0.8$	$6.6^{b} \pm 1.9$	$11.0^{b} \pm 1.4$
9	45	$0.3^{b} \pm 0.6$	$2.5^{bc} \pm 2.0$	$3.3^{b} \pm 1.9$	$5.6^{b} \pm 3.2$	$7.0^{bcd} \pm 2.8$
10	50	$1.0^{b} \pm 1.7$	$2.5^{bc} \pm 1.3$	$5.8^{b} \pm 4.3$	$7.6^{b} \pm 5.1$	$3.5^{d} \pm 0.7$

[1] 100 insects released in section 1.
Means ± SD followed by the same superscript within a column are not significantly different (P=0.05)
n=6

Appendix Table 11

Dispersion behaviour of *T. castaneum* in a grain column with aeration.

Day	No of insects									
	C 1**	C 2 NS	C 3NS	C 4NS	C 5NS	C 6NS	C 7***	C 8**	C 9*	C10***
1	$88.8^{a}\pm 6.7$	$4.3^{a}\pm5.4$	$2.8^{a}\pm2.4$	$1.5^{a}\pm1.7$	$1.0^{a}\pm1.2$	$0.5^{a}\pm1.0$	$0.0^{c}\pm0.0$	$0.0^{b}\pm0.0$	$0.3^{b}\pm0.5$	$0.3^{c}\pm0.5$
3	$75.8^{ab}\pm22.4$	$3.0^{a}\pm3.2$	$2.8^{a}\pm1.7$	$2.3^{a}\pm2.9$	$1.0^{a}\pm2.0$	$0.8^{a}\pm1.0$	$0.3^{c}\pm0.5$	$0.0^{b}\pm0.0$	$1.5^{b}\pm3.0$	$3.0^{c}\pm3.5$
5	$67.0^{abc}\pm20.3$	$6.4^{a}\pm5.0$	$7.2^{a}\pm5.3$	$4.0^{a}\pm3.7$	$2.2^{a}\pm1.8$	$3.0^{a}\pm2.3$	$1.4^{b}\pm1.1$	$2.6^{a}\pm1.3$	$2.0^{b}\pm2.3$	$5.6^{bc}\pm5.2$
7	$60.2^{bc}\pm18.9$	$8.2^{a}\pm3.8$	$3.2^{a}\pm0.8$	$1.8^{a}\pm1.2$	$1.7^{a}\pm1.0$	$2.8^{a}\pm2.1$	$1.7^{b}\pm1.0$	$2.7^{a}\pm2.0$	$1.5^{b}\pm2.0$	$11.0^{ab}\pm5.8$
12	$46.0^{c}\pm 1.4$	$3.0^{a}\pm0.0$	$7.5^{a}\pm2.1$	$4.0^{a}\pm0.0$	$0.5^{a}\pm0.7$	$1.0^{a}\pm0.0$	$4.5^{a}\pm0.7$	$4.0^{ab}\pm0.4$	$7.0^{a}\pm1.4$	$17.5^{a}\pm2.1$

C Column section
* significant at 10% level
** significant at 5% level
*** significant at 1% level
NS Non significant
Means ± SD followed by the same superscript within a column are not significantly diferent (P=0.05)
n=6

Appendix Table 12

Dispersion behaviour of *T. castaneum* in a grain column without aeration.

Day	No of insects									
	C 1**	C 2 NS	C 3 NS	C 4**	C 5 NS	C 6**	C 7NS	C 8***	C 9**	C 10 NS
1	$79.0^{a}\pm16.8$	$10.7^{a}\pm12.7$	$3.7^{a}\pm3.5$	$0.3^{a}\pm0.6$	$1.3^{a}\pm1.5$	$1.7^{b}\pm1.5$	$0.3^{a}\pm0.6$	$1.0^{a}\pm1.0$	$0.3^{b}\pm0.6$	$1.0^{a}\pm1.7$
3	$55.8^{ab}\pm19.6$	$12.3^{a}\pm4.7$	$3.0^{a}\pm4.0$	$2.3^{ab}\pm2.6$	$1.5^{a}\pm0.6$	$1.3^{b}\pm1.3$	$2.8^{a}\pm4.9$	$1.0^{c}\pm1.4$	$2.5^{ab}\pm2.0$	$2.5^{a}\pm1.3$
5	$49.3^{b}\pm16.5$	$11.0^{a}\pm8.2$	$10.0^{a}\pm7.1$	$5.0^{a}\pm1.8$	$4.8^{a}\pm2.5$	$4.8^{ab}\pm1.3$	$2.0^{a}\pm2.2$	$2.0^{c}\pm0.8$	$3.3^{ab}\pm1.9$	$5.8^{a}\pm4.3$
7	$41.8^{b}\pm12.0$	$10.6^{a}\pm6.6$	$5.6^{a}\pm2.9$	$5.8^{a}\pm2.9$	$5.0^{a}\pm3.5$	$5.6^{a}\pm3.1$	$5.2^{a}\pm4.1$	$6.6^{b}\pm1.9$	$5.6^{a}\pm3.2$	$7.6^{a}\pm5.1$
12	$38.5^{b}\pm0.7$	$9.0^{a}\pm1.4$	$5.0^{a}\pm1.4$	$4.0^{ab}\pm0.0$	$3.5^{a}\pm2.1$	$7.0^{a}\pm2.8$	$6.0^{a}\pm2.8$	$11.0^{a}\pm1.4$	$7.0^{a}\pm2.8$	$3.5^{a}\pm0.7$

C Column section
* significant at 10% level
** significant at 5% level
*** significant at 1% level
NS Non significant
Means ± SD folowed by the same superscript within a column are not significantly different (P=0.05).
n=6

Appendix Table 13

Dispersion behaviour of male and female *T. castaneum* in a grain column after seven days (sexes released separately at the top of the column)

Column	No of insects									
	S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8	S 9	S 10
Aerated										
M	$63.6^{a}\pm 1.5$	$5.0^{a}\pm 3.6$	$1.6^{a}\pm 1.5$	$2.0^{a}\pm 2.0$	$1.4^{a}\pm 1.1$	$2.3^{a}\pm 1.5$	$1.6^{a}\pm 2.8$	$3.0^{a}\pm 1.0$	$4.6^{a}\pm 0.5$	$13.4^{a}\pm 3.5$
F	$51.0^{a}\pm 14.5$	$7.3^{a}\pm 4.6$	$2.3^{a}\pm 1.1$	$6.0^{a}\pm 5.2$	$3.0^{a}\pm 1.7$	$3.3^{a}\pm 2.0$	$4.0^{a}\pm 2.0$	$2.0^{a}\pm 2.0$	$5.0^{a}\pm 4.0$	$15.6^{a}\pm 14.0$
Control										
M	$46.0^{a}\pm 23.0$	$13.0^{a}\pm 9.6$	$4.6^{a}\pm 3.0$	$2.6^{a}\pm 1.5$	$5.3^{a}\pm 6.1$	$5.0^{a}\pm 4.5$	$5.6^{a}\pm 5.5$	$4.3^{a}\pm 4.7$	$6.6^{a}\pm 7.6$	$7.0^{a}\pm 7.2$
F	$33.6^{a}\pm 8.5$	$6.3^{a}\pm 1.1$	$6.3^{a}\pm 2.5$	$5.0^{a}\pm 3.6$	$3.6^{a}\pm 3.7$	$3.0^{a}\pm 1.5$	$7.0^{a}\pm 1.7$	$7.0^{a}\pm 5.1$	$9.3^{a}\pm 3.5$	$16.3^{a}\pm 8.1$

M male
F female
S column section
Means $\pm$ sd followed by the same superscript within a column are not significantly different (P=0.05)
n=3

Appendix Table 14

Dispersion behaviour of male and female *T. castaneum* in a grain column after seven days (sexes released separately between column 5 and 6)

Column	No of insects									
	S1	S 2	S 3	S 4	S 5	S 6	S 7	S 8	S 9	S 10
Aerated										
M	$0.0^{a}\pm0.0$	$0.0^{a}\pm0.0$	$0.0^{a}\pm0.0$	$0.5^{a}\pm0.7$	$6.0^{a}\pm8.4$	$38.0^{a}\pm32.5$	$17.0^{a}\pm9.8$	$4.5^{a}\pm2.1$	$3.5^{a}\pm2.1$	$30.5^{a}\pm26.8$
F	$0.5^{a}\pm0.7$	$0.0^{a}\pm0.0$	$1.0^{a}\pm1.4$	$3.5^{a}\pm4.9$	$12.0^{a}\pm12.7$	$21.5^{a}\pm 4.9$	$14.0^{a}\pm8.4$	$6.5^{a}\pm4.9$	$9.5^{a}\pm4.9$	$31.0^{a}\pm 7.0$
Control										
M	$1.0^{a}\pm1.4$	$2.0^{a}\pm2.8$	$1.5^{a}\pm0.7$	$3.0^{a}\pm0.0$	$39.5^{a}\pm36.0$	$24.5^{a}\pm12.0$	$3.0^{a}\pm1.4$	$5.5^{a}\pm2.1$	$3.0^{a}\pm1.4$	$17.0^{a}\pm19.7$
F	$5.0^{a}\pm4.2$	$2.0^{a}\pm2.8$	$2.5^{a}\pm0.7$	$5.5^{a}\pm4.9$	$14.5^{a}\pm0.7$	$51.0^{a}\pm14.1$	$11.0^{a}\pm4.2$	$2.0^{a}\pm0.0$	$2.5^{a}\pm2.1$	$3.0^{a}\pm 2.8$

M male
F female
S column section
Means $\pm$ sd followed by the same superscript within a columnn are not significantly different ($P = 0.05$)
n=3

Appendix Table 15

Dispersion behaviour of 200 (100 males and 100 females) *T. castaneum* in a grain column after seven days (insects released between column 5 and 6)

Column section	Column height (cm)	Aeration	Control
1	5	1.3 ± 2.3[a]	1.0 ± 0.0
2	10	1.3 ± 1.5	0.6 ± 0.5
3	15	0.3 ± 0.5	0.6 ± 0.5
4	20	2.6 ± 0.5	1.6 ± 1.1
5	25	37.0 ± 4.2	13.3 ± 9.0
6	30	49.6 ± 6.8	64.6 ± 36.6
7	35	12.6 ± 5.8	26.3 ± 21.7
8	40	14.6 ± 10.2	22.3 ± 1.5
9	45	19.3 ± 5.6	29.6 ± 18.8
10	50	60.6 ± 20.0	34.6 ± 25.8

[a] Mean ± sd
n=2