

Tritrophic Interactions in Maize Storage Systems

A dissertation submitted to the University of Neuchatel
for the degree of Doctor of Natural Sciences

Presented by

Anita Savidan

Institute of Zoology
Laboratory of Animal Ecology and Entomology,

Neuchatel, 2002

CONTENTS

Summary.....	3
Résumé.....	6
General Introduction.....	9
Outline.....	24
Chapter 1 : Maize resistance to storage pests.....	27
Chapter 2 : Direct Benefits from parasitic wasps for maize seed.....	66
Chapter 3 : Effect of maize varieties on the performance of parasitoids of storage pests.....	90
Chapter 4 : Olfactory attraction of parasitic wasps to stored maize.....	130
Chapter 5 : Effect of storage method on maize storage pests and their natural enemies.....	157
Chapter 6 : Interaction of maize weevils and parasitoids with different storage methods in the field.....	185
General Discussion.....	210
Outlook.....	216
References.....	218
Curriculum vitae.....	224

SUMMARY

The interaction and compatibility of natural enemies with two currently used strategies of integrated pest management to lower post-harvest losses in maize were investigated, namely insect resistance, and storage practice.

In a first study, insect resistance of a large number of maize genotypes, including Mexican landraces and tropical hybrids, was tested against five common storage pests in tropical maize stores. A large range of resistance was found to the maize weevil, *Sitophilus zeamais*, the larger grain borer, *Prostephanus truncatus* and the coffee beetle, *Araecerus fasciculatus*. The most resistant maize genotypes were a Mexican landrace and a population from the Caribbean. These genotypes were resistant to all storage beetle. However, they were susceptible to the Angoumois grain moth, *Sitotroga cerealella*, indicating that different mechanisms were responsible for resistance to this pest. The genotype most resistant to the moth larvae was a white tropical hybrid (CML264xCML254). None of the maize genotypes was resistant to the Indian meal moth, *Plodia interpunctella*. The wild relative of maize, teosinte, protected by its fruitcase was completely resistant to attack by the maize weevil and the Angoumois grain moth. No resistance factor inherent to this wild grain that could be of use to increase resistance in domesticated maize was found. These studies on the interactions of the pest insects with different maize varieties were conducted to use the results for the design of further studies that also included the natural enemies of the pests.

The chance that maize seed germinate after being attacked by the maize weevil or the Angoumois grain moth is reduced but not lost, and varies among maize genotypes. If the immature of the Angoumois grain moth is successfully attacked by a parasitoid; *Pteromalus cerealellae*, the rate of germination is significantly higher indicating that seeds may obtain a direct benefit from attracting parasitoids.

There was no difference in effectiveness of the maize weevil parasitoid, *Anisopteromalus calandrae*, in eliminating its host in maize kernels of resistant or susceptible maize genotypes, despite the high level of kernel hardness in some varieties, indicating that maize resistance can work in synergy with this natural enemy. There was a small but consistent difference in the effectiveness of *Pteromalus cerealellae* to parasitize the Angoumois grain moth in kernels of a susceptible and a resistant genotype. This difference was found in three distinct experiments and was thought to be due to a higher detectability of the host in the resistant maize genotype. Therefore, for the control of this insect pest, maize resistance is also synergistic with the effectiveness of the natural enemy.

Using a six-arm olfactometer, the maize weevil parasitoid, *A. calandrae* was shown to be attracted to odors from infested and uninfested maize when experienced, but showed no attraction to intact maize kernels when they lacked preemergence or oviposition experience with this seed.

The female parasitoids showed no preference for the odors of any of six maize genotypes, neither infested nor intact. The odors of the kernels of the six maize varieties were collected, but detectable volatile compounds only emanated from infested maize. There was no difference in quantity and quality of the volatile blends among the varieties. Two of the four consistently detected compounds were identified as pentadecane and tridecane and we suspect that the other two are (Z) -7-pentadecene and (Z) -6-pentadecene which have been previously reported to be associated with granary weevil and mite infestation in wheat.

Storing maize as shelled grain or on the cob was shown to have an impact on the storage pests as well as on their natural enemies in laboratory experiments. The increase of the maize weevil population was greatly reduced when maize was stored on the cob, but the efficiency of the parasitoid, *A. calandrae*, was also negatively affected in this case. In contrast, the larger grain borer had a higher rate of increase on maize stored on the cob, and

the performance of its predator, *Teretriosoma nigrescens*, was not affected by the storage method. The Angoumois grain moth showed a slightly higher rate of increase on maize stored as shelled grain. Its parasitoid, *P. cerealellae*, was highly efficient on this storage method but had almost no impact on pest increase when maize was stored on the cob.

In a field experiment, maize was exposed to a natural insect infestation in the humid tropics. Fewer maize weevils emerged from maize stored on the cob than from shelled maize and even fewer emerged from cobs stored with the husk on. Two parasitoids, *Anisopteromalus calandrae* and *Lariophagus distinguendus* had a significant impact on maize weevil density only in maize stored as shelled grain, where they parasitized up to 67% of suitable hosts. Nevertheless, 100 % of the shelled grain was damaged after only 11 weeks in storage, whereas 50% of grains were still undamaged when they were stored with the husk on after four months in storage. Hence, in this case storage method was considerably more important than the impact of the parasitoids.

In conclusion, it was found that grain resistance is compatible with parasitoids. However, no maize variety showed cross resistance to all storage pests. All maize varieties examined were equally attractive to the parasitoid *Anisopteromalus calandrae*. The best storage method to reduce insect infestation is not always beneficial to the pests' parasitoids and the evaluation of importance of the impact of each of these control measures under realistic conditions should be decisive for their application.

RESUME

Pour limiter les pertes post-récoltes du maïs, deux stratégies sont couramment utilisées dans la lutte intégrée contre les ravageurs : la résistance variétale du grain contre les insectes ravageurs, et la méthode de stockage du grain. L'interaction et la compatibilité, de ces deux stratégies avec des ennemis naturels ont fait l'objet de cette recherche.

Dans une première étude, la résistance d'un grand nombre de génotypes de maïs, dont des races mexicaines et des hybrides tropicaux, a été testée contre cinq ravageurs fréquents dans les greniers de maïs des régions tropicales. Certaines de ces variétés sont très résistantes contre le charançon du maïs, *Sitophilus zeamais*, le grand capucin du maïs, *Prostephanus truncatus*, et la bruche des graines du café, *Araecerus fasciculatus*. Les génotypes les plus résistants sont une race Mexicaine et une population des Caraïbes. Les variétés montrent des degrés de résistance similaires contre chacun des trois ravageurs. Par contre les variétés les plus résistantes sont susceptibles aux attaques de la mite Angoumois des graines, *Sitotroga cerealella*, indiquant que d'autres facteurs sont responsables de la résistance contre ce ravageur. La variété la plus résistante est un hybride tropical. Aucun génotypes de maïs n'est résistant contre la mite Indienne de la farine, *Plodia interpunctella*.

Les graines du maïs sauvage, teosinte, protégées par des glumes lignifiées sont complètement résistantes à l'attaque du charançon du maïs et de la mite Angoumois. Aucun facteur de résistance inhérent à la graine de teosinte, qui puisse être utilisé pour améliorer la résistance du maïs domestiqué n'a été mis en évidence. Ces études sur l'interaction des insectes ravageurs avec les variétés du maïs ont servi de base pour concevoir les études suivantes, qui incluent les ennemis naturels des ravageurs.

La capacité des graines de maïs de germer suite à une attaque par *S. zeamais* ou *S. cerealella* est réduite mais non nulle et le degré de germination varie suivant le génotype du

maïs. Si la larve de *S. cerealella* est attaquée et tuée par un parasitoïde, *Pteromalus cerealellae*, le degré de germination est nettement plus élevé, suggérant que les graines peuvent obtenir un bénéfice direct si elles sont attractives pour les parasitoïdes.

L'efficacité du parasitoïde *Anisopteromalus calandrae* pour réduire *S. zeamais* est la même quelle que soit la variété de maïs sur laquelle son hôte se nourrit, indiquant qu'il y a une synergie de la résistance du maïs avec l'action des parasitoïdes. Il y a une différence dans l'efficacité du parasitoïde *Pteromalus cerealellae* à éliminer son hôte, *S. cerealella* dans des graines de maïs susceptible ou résistant : cette différence s'est maintenue à travers trois expériences différentes et semble être due à une plus grande facilité pour trouver l'hôte dans la variété résistante. Il y a donc à nouveau une synergie entre la résistance contre les ravageurs et l'interaction des parasitoïdes.

Utilisant un olfactomètre à six bras nous avons pu montrer que les parasitoïdes du charançon du maïs, *A. calandrae*, sont attirés par les odeurs provenant de graines de maïs infestées ou non infestées, s'ils ont eu une expérience antérieure avec du maïs. Sans expérience, ils ne sont attirés que par les odeurs du maïs infesté. Les parasitoïdes femelles ne démontrent de préférence pour aucune de six variétés de maïs, infestée ou non. Les composés volatiles de ces variétés ont été collectés, mais des quantités significatives ont émanées uniquement des graines de maïs infestées. Il n'y a pas de différence dans la qualité ou quantité des composés volatiles. Deux des quatre composés rencontrés ont été identifiés comme pentadécane et tridécanes. Les autres sont probablement (Z) -7-pentadécène et (Z) -6-pentadécène. Ces composés ont tous déjà été trouvés associés à des infestations par le charançon du blé et des acariens dans du blé.

Par expérimentation en laboratoire il a été démontré que la manière de stocker le maïs, égrené ou sur l'épi, a un impact sur le taux de croissance des ravageurs et sur l'efficacité de leurs ennemis naturels. La croissance de la population de *S. zeamais* a été beaucoup moins

importante quand le maïs était stocké sur l'épi, mais l'efficacité du parasitoïde *A. calandrae* est également réduite dans ce cas là. Le grand capucin du maïs, au contraire, a une croissance supérieure dans le maïs stocké sur l'épi, et la performance de son principal prédateur, *Teretriosoma nigrescens*, n'est pas affectée par la méthode de stockage. La mite Angoumois du grain a un taux de reproduction un peu plus élevé dans le maïs égrené. Son parasitoïde, *P. cerealellae* est très efficace pour réduire ce ravageur dans le maïs égrené, mais n'a pas d'impact si le maïs est stocké sur l'épi.

Finalement, une quantité de maïs a été déposée dans un grenier expérimental et exposée à l'infestation naturelle dans une région tropicale humide. Le taux d'infestation par *S. zeamais* a été bien plus élevé pour le maïs égrené que pour les épis, mais les épis stockés avec les spathes ont montré un taux encore inférieur. Deux parasitoïdes, *Anisopteromalus calandrae* et *Lariophagus distinguendus* ont un impact significatif sur ces ravageurs, mais seulement quand le maïs était stocké égrené, où ils parasitent jusqu'à 67% des hôtes. Malgré cela 100 % des graines étaient endommagées après seulement onze semaines, alors que le maïs stocké avec spathes présent seulement 50% des graines endommagées au bout de quatre mois.

En conclusion nous avons trouvé que la résistance variétale du grain contre les insectes ravageurs est compatible avec leurs parasitoïdes. Par contre, aucune variété n'est résistante contre tous les insectes ravageurs. Il n'y a pas de différence dans l'attractivité des variétés pour le parasitoïde *Anisopteromalus calandrae*. La meilleure méthode de stockage du grain peut être néfaste pour le parasitoïde. L'évaluation de l'impact de chaque stratégie pour contrôler les ravageurs dans des conditions réalistes est fondamentale et doit intervenir avant la mise en application.

GENERAL INTRODUCTION

The storage environment

Stored maize is not a natural habitat, but one created artificially by man. Many insect species have adapted to the peculiar physical conditions of a storage environment and to the changes in nutritional quality of their food through the domestication of wild plants (Buckland, 1981). In some cases, the seed predators can be found on the wild species as well as on their cultivated form in storage, indicating a relatively simple adaptation to the man-made conditions. This is the case for example in beans where bruchid beetles that occur in storage and their parasitoids may be found on the wild parent plants. Through domestication and selection for increased yield, nutritional quality, and specific plant resistance factors of seed may have been lost (Benrey et al., 1998). In the case of stored cereals however, the major insect pests have probably adapted from other, completely unrelated, but native host plants, like seeds of trees or even from wood (Schulten, 1976; Buckland, 1981; Delobel and Grenier, 1993), and specific resistance to today's storage pests may never have existed in wild relatives or early domesticated races. The adaptation of these insects from their original host plant to the relatively poor nutritional quality of cereals, may in some cases have been facilitated by symbiotic bacteria, which provide vitamins and amino acids to their hosts (Delobel and Grenier, 1993).

The synanthropic fauna has considerably changed over time through dispersal by trade and military campaigns (Buckland, 1981) and adaptation to adverse physical factors and pesticides. These changes are ongoing and present a big challenge for the protection of stored products in the future.

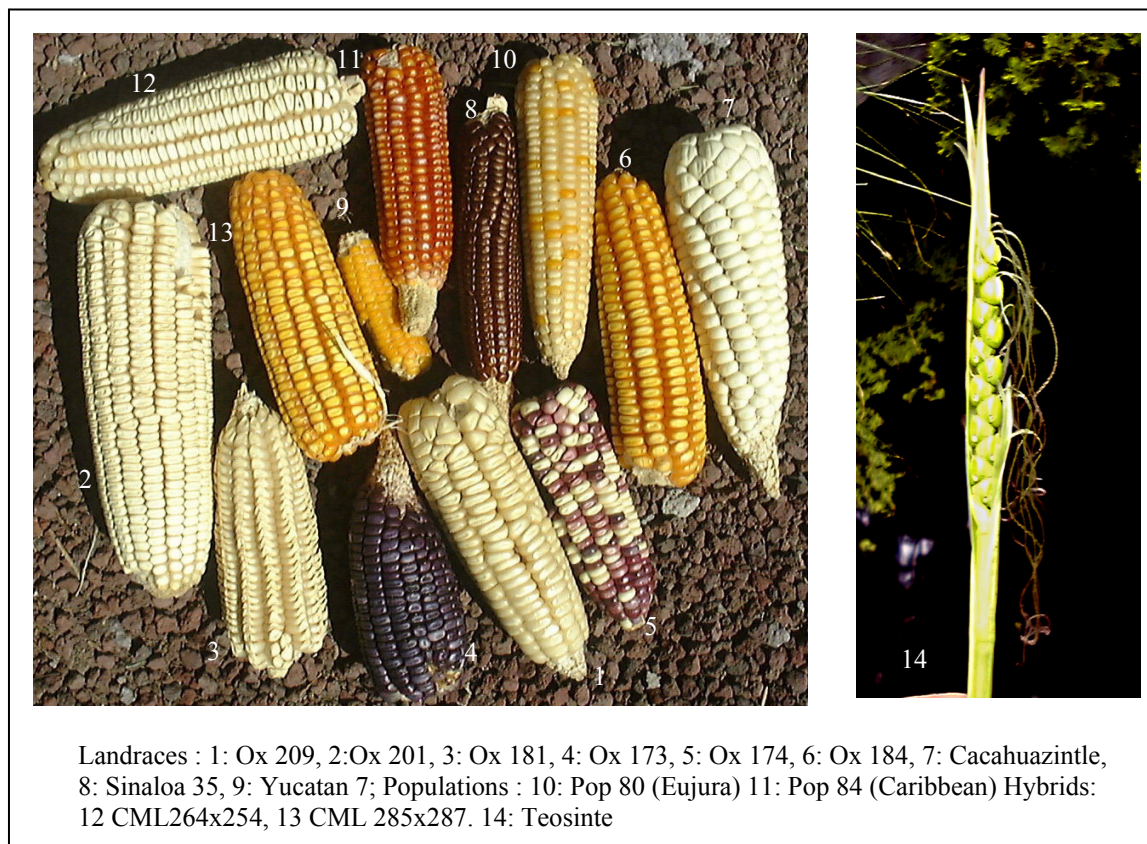
Compared to many natural environments the storage habitat is unstable, as it will eventually be consumed by man or life stock. The strategy of insect storage pests as well as their predators and parasitoids, is to build up a large population within a very short time. This will eventually lead to a high concentration of insects in a limited space. The intra- and inter-specific interactions that derive from such situations are still poorly understood. While most studies concern the autoecology of a single species in relation to its environment the synecology of storage insects that live in the same environment has received relatively little attention (Dobie et al, 1991).

The abiotic factors in a storage facility, such as temperature and humidity are relatively stable if compared to the natural environment, and the effects of climatic changes will be attenuated. Free water is not available and moisture content of stored commodities is very low. Storage insects are adapted to dry conditions, and ambient humidity will have a decisive influence on which species is present.

The physical form of food (milled, whole grain) and the storage structure itself will also influence the population dynamics of each insect species, their rate of migration and their ability to survive between storage seasons. Moreover, some insect species have conserved their ability to survive and reproduce in natural habitats, while others are virtually tied to crops cultivated and stored by man.

Maize

The origin of maize has been a controversial subject for a long time. Today the idea that maize has been domesticated by man from a previous form of the Mexican annual teosinte is widely accepted, and supported by cytological and molecular evidence (McClintock et al., 1981; Doebley, 1990). One of the most important differences between teosinte and maize, is the fact that maize is unable to reproduce itself without the help of man. Unlike in other



plants, the

maturing maize ear never emerges from its husk (Harlan, 1992). The seeds stay confined together on the cob, enclosed by the husk and can only be dispersed by man. The artificial selective pressure for longer and tighter husk leaves has been applied for thousands of years to protect the seed from damage by birds and insects, and has somewhat compensated the loss of the lignified fruitcase that protects the seed of teosinte (Galinat, 1977).

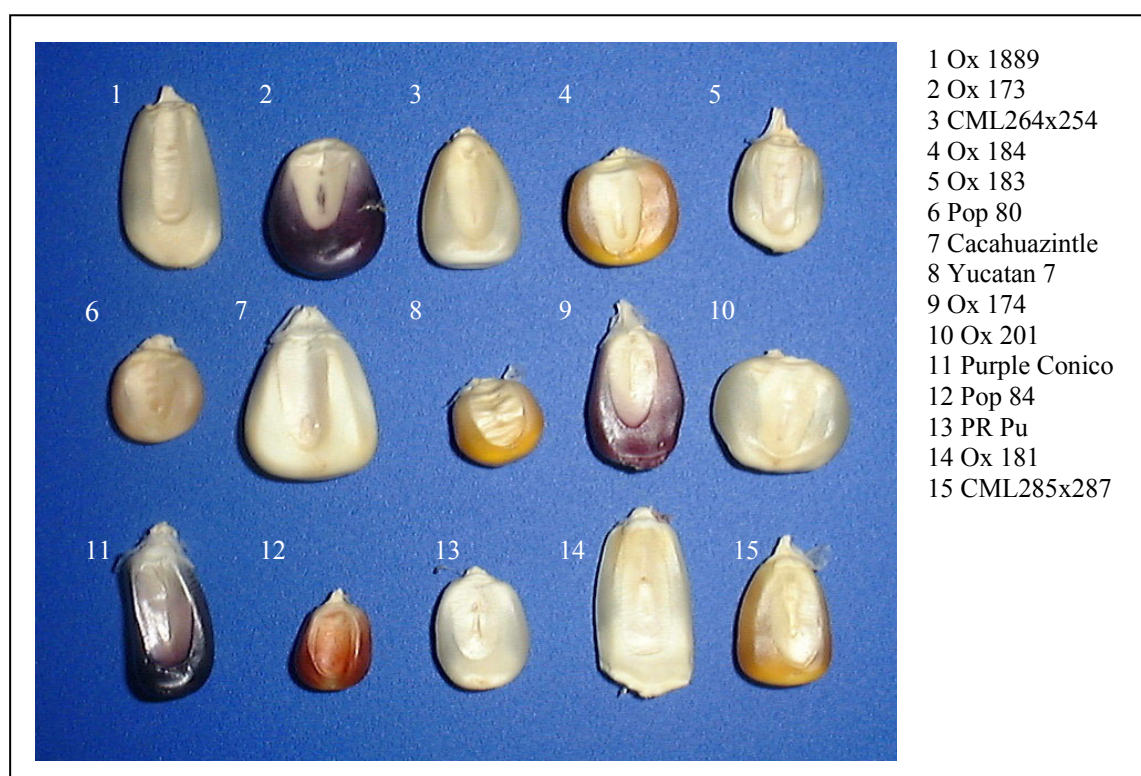
The first classification of maize was based on kernel characteristics. The content of sugar and the proportion of hard (vitreous) and soft (floury) endosperm conducted to the division into *sweet corn*, *popcorn* (only hard endosperm), *flint* (mostly hard endosperm), *dent* (hard and soft endosperm) and *floury* (only soft endosperm). Later Wellhausen et al. (1951, 1957) elaborated a classification of Latin American land races based on morphological and physiological characteristics of the maize cob and the plant, that reflected the ecology and



evolution of maize. More recently Doebley et al. (1985) proposed a classification that contrasts sharply with the division of Wellhausen as there seems to be a limited degree of gene flow between varieties of maize adapted to different elevations (Doebley, 1990). From its origin maize spread and is now cultivated all over the world and has become one of the most important food crops for the third world.

Breeding of maize - from its first domestication until the last century involved mass selection (Sprague and Eberhart, 1977), which resulted in large varietal and genetic diversity that is still conserved in many parts of Central America and Mexico (Smale et al., 1998). These landrace

populations are mixtures of genotypes adapted to a particular region and to cultural practices in vogue (Harlan, 1992). It has been suggested that varietal hybridization by the pre-columbian farmers was one of the important factors in the diversity now found in Mexico (Wellhausen et al., 1952). Modern breeding through inbred lines and their single cross hybrids greatly increased the rate of progress in selection for desirable agronomic traits, but resulted in erosion of genetic variability. The improvement of parental breeding populations by gradually increasing the frequency of the favourable genes is the foundation of a more effective selection system. A new tool for incorporating desirable traits rapidly into elite lines is the use of a new DNA-based crop selection technique, known as marker assisted selection, that can in some cases replace conventional screening (Bergvinson, 2001).



Although there are modern maize varieties and hybrids that possess improved agronomic performance adapted to regions in the third world, they are not readily accepted by subsistence farmer because of lack of resistance to storage pests (Giles and Ashman, 1971; Kossou et al 1993.; Arnason, 1994).

Insects

Storage Pests

The first insects to colonize stored grain are called primary pests. They are able to attack undamaged grain and the larval development usually takes place inside the seed. Populations of secondary pests will build up only once the grain is damaged. They feed mostly on the germ and have a much larger food range than primary pests. The term ‘secondary pest’ is no indication of their economic importance; they may be the most damaging pests in many stored commodities (Dobie et al., 1991), but on stored maize the primary pests such as the maize weevil, *Sitophilus zeamais*, the larger grain borer, *Prostephanus truncatus* and the Angoumois grain moth, *Sitotroga cerealella* are by far the most destructive (Schulten, 1976 and Markham, 1994).

Maize weevil, *Sitophilus zeamais* Motschulsky

(Curculionidae : Coleoptera)

This insect is a cosmopolitan pest of stored cereal, and the most important pest of stored maize. Infestation of maize cobs may start before harvest (Dix and Al, 1985).

The female lay their eggs under the pericarp, and the larvae complete their development entirely within a single grain. Adults continue to feed on the grain. They live for several months and lay an average of 350 eggs during their lifetime.

Maize weevils will leave maize damaged with exit holes of irregular shape, and tunnelling under the pericarp may be visible.



Larger Grain Borer, *Prostephanus truncatus* Horn

(Coleoptera: Bostrichidae)

The larger grain borer originated in Meso America and was accidentally introduced to East Africa where it increased losses of stored maize dramatically. They may infest maize before harvest and are thought to have adapted to storage products only recently. Their natural habitats are forest areas where they feed and reproduce on decayed wood.

Adults bore into the grain where they lay their eggs. Larvae will develop within the powder produced by the adults and are not confined to a single grain.

Damage is visible soon after infestation because of an important powder production, which goes far beyond their need for food.



Angoumois Grain Moth, *Sitotroga cerealella* (Olivier)

Lepidoptera: Gelechiidae

The Angoumois grain moth is an cosmopolitan pest of cereal grain, and may infest maize before harvest (Weston et al., 1997). It probably originated in Africa and has been observed to reproduce on wild cereals (Joubert, 1966)

Females lay their eggs on the grain or in small interstices nearby. The emerging larvae bore in the grain and complete their development entirely within a single seed. Before pupation the larvae excavates its chamber beyond the pericarp, forming a characteristic 'window'.

Adults do not feed and live only for a few days during which some 150 eggs are laid.

Damage to maize is recognisable through circular exit holes, usually with a small piece of seed coat attached.



Indian Meal Moth, *Plodia interpunctella* (Huebner)

Lepidoptera: Pyralidae

This moth is a cosmopolitan pest of a wide variety of stored commodities, including cereal grain.



Adult moths live only a few days and lay about 400 eggs on, or close to the food. Larvae will feed on the embryo of the seed and are not confined to one kernel. When fully grown, the larvae are very active and may be seen migrating from the grain in search for a pupation site.

Silk webs and faeces attached to them are the main characteristics of damage.

Coffee Beetle *Araecerus fasciculatus* (Degeer)

(Coleoptera: Anthribidae)

The coffee beetle is a well-known pest of coffee beans and dried cassava and an occasional pest on stored maize in the humid tropics.



The larvae develop most quickly at high moisture contents and are severely affected by low humidity.

Larvae grow inside the kernel or within the powder produced by the adults. A female lays about 50 eggs during her lifetime.

Irregular holes and large amounts of powder are the characteristics of damage

Natural enemies of storage pests

Some storage beetles like *Tribolium castaneum* have a dual role as grain pest and as predator (Rees et al., 1990). Other generalist predators such as bugs of the Anthricidae family feed mainly on free living larvae of secondary pests (Brower and Press, 1992). The larvae of the primary pests are mainly attacked by several species of parasitic wasps of the family of the Pteromalidae (Dobie et al., 1991). A histeroid beetle, *Teretriosoma nigrescens*, preys on larvae of the larger grain borer (Rees, 1985, 1987; Rees et al., 1990).

Anisopteromalus calandrae
(Howard)

This parasitoid has a world wide distribution and attacks a large number of internally feeding storage Coleoptera, such as the maize weevil, in stored seed.



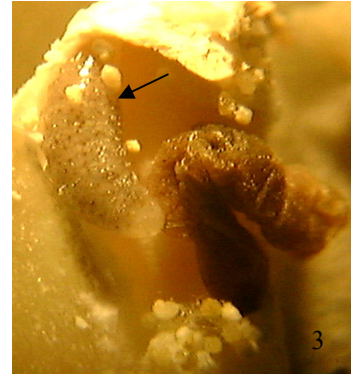
Pteromalus cerealellae
(Walker)

This wasp is a parasitoid of *Sitotroga cerealella*. Although it has been found to parasitize a large number of other hosts in stored grain it is usually found in association with the Angoumois grain moth



Reproductive behaviour:

These pteromalid parasitoids, including *Lariophagus distinguendus*, another generalist, are solitary ectoparasitoid. After detecting an internally feeding host they insert their ovipositor through the pericarp of the grain and paralyze their host(1). Then the ovipositor is inserted again and a single egg is laid in the cavity(2). The parasitoid larvae feeds on its host, which dies when the parasitoid is fully grown (3). The parasitoid pupates still inside the cavity in the seed (4) and emerges from it as an adult wasp by chewing its way out, leaving a small circular hole(5). Adult wasps feed on host hemolymph through a tube they make through the shell of the grain (6)





Teretriosoma nigrescens, a predator of the larger grain borer, *Prostephanus truncatus*

Pest management in stored maize

For a long time the efficacy of a particular control measure was the only consideration in pest control, which led to the wide use of insecticides in maize stores around the world. The application of fumigants and pesticides has not been satisfactory to many small scale farmers in the developing countries, as they are expensive and may be applied in inappropriate doses by the farmers (Markham et al., 1994). Moreover, storage insects are increasingly becoming resistant to many chemical compounds (Golob et al., 1990; Guedes et al., 1994) and fewer new insecticides are available because of increasing costs for their development and stricter regulations for their use (Schoeller et al., 1997). The growing concerns about the effect of pesticides on users, consumers, beneficial organisms in the agro-ecosystem or the environment have led to the concept of integrated pest management (IPM). Physical, chemical, biotechnical, biological, technical and hygienic measures should be applied in a manner to assure protection to human health and the environment, and reduce pest infestation to an acceptable level. The foundations of this concept, and the key to its success lies in the

thorough knowledge of the biology of the pests and their natural enemies. Whereas in a field-crop ecosystem a certain number of pest insects may be tolerated, the threshold level is much lower in a stored-grain ecosystem, and the control measures have to be optimised. Moreover in the developing countries the costs of these measures have to be low and the application simple to be acceptable to small scale farmers.

Grain protectants

While the insecticidal activity of extracts of some plant species, such as the neem tree (*Azadirachata indica*) and *Ricinus communis* is well-known, much research has recently focused on the use of extracts of local plants as a cheap and available means to reduce post-harvest losses in maize (Niber, 1994; Peña et al., 1991). The application of ashes of wood and other materials that are locally available is widespread (Golob et al., 1982). The application of diatomaceous earth in grain stores reduces insect infestation by causing death through desiccation (Korunic, 1997; Fields, 2002)

Grain resistance

Resistance of stored grain to insect attack is another means of lowering post harvest losses. The resistance factors that lower insect infestations can be inherent to the grain itself (Dobie, 1974; Urello et al., 1989; Arnason et al. 1994), or due to a better protection of pre- and post-harvest infestation by a better husk cover (Eden, 1952; Kossou et al., 1993; Meikle et al., 1998).

Structural methods

The search for safe storage structures at low cost has had a varying degree of success. For example small metal silos have been a certain success in Honduras (Anonymous, 1989), but

have not been accepted in Africa (FAO, 1980). The difficulties of new technologies to be adopted can be attributed to socio-economic reasons and to technical factors due to local conditions (Markham et al., 1994).

Technological management practices

These methods include the altering of the time of harvest, to obtain optimal drying of the grain and low field infestation, methods for appropriately handling and drying grain after harvest. The selection of maize cobs after the harvest has been proposed to avoid insect infestation at the beginning of the storage time. Storage practices such as storage as shelled grain, on the cob or with the husk on may influence insect development, but also the efficiency of pesticide application.

Hygienic and sanitary measures

The proper cleaning of a store prior to the next harvest is of utmost importance. Many storage pests are able to survive on residues and debris of stored grain or within the storage structure and act as sources for new infestation.

Biological Control

The use of parasitic wasps has been proposed to reduce insect infestation in grain stores or to eliminate residual populations between storage seasons. Naturally occurring parasitoid populations have been found to follow the increase of storage parasitoids, but failed to prevent substantial damage (Arbogast and Mullen, 1990). A predator, *Teretriosoma nigrescens*, of the larger grain borer, *Prostephanus truncatus*, was released in Africa following the grain borers accidental introduction. Although some reports related an important reduction in post-harvest losses (Richter et al., 1997), there are strong grounds to

believe that this predator alone cannot achieve control of this pest (Markham et al. 1994; Holst and Meikle, 2002)

Punctual use of pesticides in case of outbreaks

The punctual use of pesticides has to be considered in cases where outbreaks of an insect pest that has a natural reservoir occur, and where other methods are not efficient enough to keep damage below an economically acceptable level. It requires the monitoring of insects in small farmer stores and close collaboration with the farmer.

Tritrophic interactions in storage systems

Among the natural enemies of storage pests there are several parasitoids that occur frequently in rural maize stores. Most of the studies about parasitoids concern the direct interaction with their hosts or abiotic factors such as temperature, humidity and pesticide resistance.

The concept of three trophic level interaction states that interactions between plant and host will affect enemy success (Price et al, 1980). For example, a smaller host size due to plant resistance will affect the size and vitality of the parasitoid as well, and lower host density or a longer development cycle will have an impact on parasitoids. Combining plant resistance and natural enemies may slow down the adaptation of insect pests to resistance factors of plants (Kennedy et al, 1984). In stored maize, a more resistant variety might have a synergistic or an antagonistic effect on parasitoid success through indirect interaction. But plant characteristics can also affect parasitoids directly, through physical properties or plant-mediated chemical attractants called synomones. Plants in their vegetative stage can benefit directly from parasitoid action and are under evolutionary pressure to develop

characteristics that are attractive for parasitoids (Turlings and Benrey, 1998) and increase their effectiveness (Botrell and Barbosa, 1998).

In stored maize, such a benefit is not obvious because a seed is in a relatively inert stage, unable to react to an insect attack and unable to grow back any lost tissue. A benefit would only exist if germination were markedly higher after the host of an attacked grain has been parasitized. As yet, this has not been investigated. In stored maize such interactions could also be affected by the storage form, shelled on the cob, or still protected by its husk.

Conclusion

It can be concluded that the impact of the above mentioned control measures to reduce post-harvest losses have been investigated extensively. However most studies concern only one control strategy and one or two insect species at a time. Hence, grain resistance has been the subject of many research programs, but it is not known if resistant genotypes are cross resistant to all major storage pests. If they are not, the successful introduction of resistant varieties could be jeopardized.

Integrated pest management to reduce post-harvest losses requires the use of several control measures at a time. Therefore synergism between these strategies is crucial for their success. The compatibility of insect resistant maize in the field with natural enemies has been the subject of several studies (Smith, 1994), but the interaction of parasitic wasps with grain resistance has not yet been investigated. An important factor determining the effectiveness of parasitoids is their successful use of semiochemicals to locate hosts. Ample evidence exists that parasitoids use plant- provided cues to locate herbivores, but for herbivores on seed there have been only a few studies (Cortesero et al., 1995, Steidle and Schoeller, 1997; Steidle and Ruther, 2000) and none of these studies has considered stored maize. Storage practice as

shelled grain, on the cob, or with husk on is known to have an effect on population dynamics of the major storage pests, but the synergism of these different storage methods with biological control agents remains to be studied.

OUTLINE

Interactions of maize seed with storage pests

Grain resistance is an important strategy to reduce post-harvest losses. We tested the resistance of 20 maize genotypes to five common storage pests in the tropics and aimed to answer the following questions:

- Do resistance factors in maize genotype affect all storage pests similarly?
- Is the wild ancestor of maize teosinte a source of resistant germplasm?
- How are parasitoids of storage pests affected by maize varietal differences?

Benefit for the seed through parasitoids

In this section it was tested if the germination rate of a maize seed is higher if the larval pest that is feeding inside it is attacked and killed by a parasitoid. The question asked is:

- Does the seed have a direct benefit from the parasitoid, so that natural selection would favor traits that increase the rate of parasitism?

Plant genotype and parasitoid effectiveness

In this section we investigate the direct effect of maize genotype on the performance of two parasitoids of internally feeding storage pests. The questions were:

- Are some maize kernels large enough so that the host can escape from the parasitoids ovipositor?
- Do parasitoids have the same performance in all maize varieties? Is there a synerchism of grain resistance and parasitoids?
- What are the possible reasons for a higher performance of a parasitoid on a resistant genotype in the case of the Angoumois grain moth?

Olfactory attraction

The olfactory attraction of parasitoids to different maize varieties, either infested or uninfested, were evaluated in a 6-arm olfactometer. Volatile compounds emanating from these maize varieties were trapped and analyzed. The questions to be answered were:

- Is a generalist storage parasitoid attracted to volatiles from infested and uninfested maize?
- Can host location cues be learned through an oviposition experience?
- Do some maize varieties attract more parasitoids than others , so that this trait could be used to increase biological control of storage pests?
- Are the volatile compounds emanating from different maize genotypes different in quality or quantity?

The effect of storage method

The two last sections examine differences in parasitoid performance when maize is stored as shelled grain, on the cob, or with the husk on. Experiments that were conducted in the laboratory determined the effect of maize that is shelled or stored on the cob on population dynamics of three storage pests and on the performance of their natural enemies, to answer the following questions:

- How are the pests and their natural enemies affected by the storage method?
- Which storage method should be recommended, in function of climate and insect species, to avoid serious damage?

Finally maize stored with the husk on, without husk or as shelled grain was exposed to natural insect infestation in the humid tropics. In this field experiment no insects were released. The

changes in damage and insect population over time were monitored by regular sampling to determine:

- How important is the impact of the local parasitoid population?
- Can results from laboratory experiments predict population dynamics of the pests and their natural enemies and grain damage in the field?

MAIZE RESISTANCE TO STORAGE PESTS

Abstract

A large number of maize genotypes, including Mexican landraces, tropical hybrids and populations, were tested for resistance to five storage pests of tropical maize. These insect pests included: the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae), the larger grain borer, *Prostephanus truncatus* (Coleoptera: Bostrichidae), the Angoumois grain moth, *Sitotroga cerealella* (Lepidoptera: Gelechiidae), the Indian meal moth, *Plodia interpunctella* (Lepidoptera : Pyralidae) and the coffee beetle, *Araecerus fasciculatus*, (Coleoptera : Anthribidae). The maize varieties tested showed a range of resistance to the coleopteran pests, and some resistance to the Angoumois grain moth. But while several of the same genotypes showed resistance to all coleopteran pests, they were not resistant to the lepidopteran species, indicating that these species are affected by other resistance mechanisms.

Wild maize, teosinte, protected by its fruitcase was completely resistant to attack by the maize weevil and the Angoumois grain moth. However, deprived of its protective shell it showed no more resistance to either insect pest than a moderately susceptible maize genotype. Resistance factors involved in storage pest resistance in maize are discussed.

Key words:

Maize-genotypes, storage resistance, *Prostephanus truncatus*, *Sitophilus zeamais*, *Sitotroga cerealella*, *Plodia interpunctella*, *Araecerus fasciculatus*.

Introduction

Maize originated from Mesoamerica where a large number of landraces are still cultivated, and its wild relative, teosinte, grows close to maize fields. In course of the domestication of maize dramatic changes have occurred. Yields have increased considerably, but modern maize is deprived of the fruitcase that protects its ancestors (Galinat, 1977). The added husk leaves that enclose the cob give developing seeds of cultivated maize a certain protection from early insect attack and birds (Eden, 1952 a,b; Schulten, 1976). Thousands of years of cultivation and selection by early farmers without the use of insecticides has resulted in some resistance traits, particularly in tropical regions with high insect pressure. This, in part explains, why most small scale farmers in Mexico still cultivate their own landraces. These landraces are a promising source of potent resistance genes that allow the plants to withstand insect and fungal pests. Screening of landraces has led to the discovery of resistance to corn earworm *Helicoverpa zea* (Waiss et al., 1979) and to the maize weevil, *Sitophilus zeamais* (Dobie, 1977; Fortier et al., 1982).

Post harvest losses are an ongoing problem in the tropics, especially for subsistence farmers. To reduce losses and improve food quality, the use of insect resistant varieties has been proposed (Markham et al., 1994), along with optimizing storage methods and quantifying the impact of natural enemies. The major pests of stored whole maize are the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae), the larger grain borer, *Prostephanus truncatus* (Coleoptera: Bostrichidae), and the Angoumois grain moth, *Sitotroga cerealella* (Lepidoptera: Gelechiidae). Most have adapted to stored cereals only after the onset of agriculture, because storage situations in nature are either rare or non-existent (Buckland, 1981; Levinson and Levinson, 1995). The evolutionary pathway of each of these pests is certainly different and adaptation to the storage habitat must have occurred at different times. Adaptation of the insects to the practices of early farmers is more likely than

co-evolution with wild progenitors of cereals (Buckland, 1981). Because of the distinct evolutionary backgrounds, and because of differences in their biology and phylogeny these insects may interact differently with various maize genotypes.

The cereal weevils of the genus *Sitophilus* adapted to cereals a long time ago (Buckland, 1981), and are the most important storage pest world-wide. All known species of this genus originate from Eurasia and the known wild species feed on tree seeds (Delobel and Grenier, 1993). The granary weevil, *S. granarius*, appears so well adapted to storage that it has lost its ability to fly. The two other grain weevils, *S. oryzae* and *S. zeamais* are closely related (Hidayat et al., 1996), but much more polyphagous than one would expect (Delobel and Grenier, 1993). Their food range includes fruits and seeds of wild trees as well as stored products, as documented by several authors (Holloway and Smith, 1985; Joubert, 1966). Delobel and Grenier (1993) found that their food spectrum is not controlled by the systematic position of the food plant but rather by the size of the seed, due to the strictly internal feeding and pupation of larvae.

Maize varieties have been screened for resistance to maize weevils a number of times (Dobie, 1974; Fortier et al., 1982; Arnason et al., 1994) and more resistance was encountered among genotypes from tropical areas, where insect pressure is high (Urrello and Wright, 1989). Resistance is expressed in a lower oviposition rate, less progeny and a longer development time. These parameters have been used to calculate an index of susceptibility (Howe, 1971; Dobie, 1974). The mechanisms of resistance are thought to be related to varietal differences in concentrations of phenolic compounds under the pericarp, which confer hardness to the kernels (Classen et al., 1990; Arnason et al., 1992). While there are some doubts about hardness alone as a resistance factor (Meikle et al, 1998), no soft genotype has yet been described as resistant.

The larger grain borer, *Prostephanus truncatus* (Coleoptera : Bostrichidae) is another very damaging pest whose adaptation to stored maize is thought to be fairly recent (Scholz et al., 1997). Its origin is in Central and North America where it can cause heavy damage in stored maize (Martinez and Velasco, 1982; Tigar et al., 1994). Twenty years ago they were accidentally introduced to Africa where they caused extensive damage, not only in maize but also in stored tubers of yucca and yam (Markham et al., 1994). It has since spread to 16 sub-Saharan countries (Farrell , 2000). In its wild habitat *P. truncatus* survives and reproduces on degraded wood (Ramirez-Martinez et al., 1991; Nang'ayo et al. 1993). In maize stores, adults bore into the grain where they lay their eggs. Larvae develop in the large amounts of powder produced by the adults and do not necessarily remain within the kernels. Because of the fairly recent appearance of *P. truncatus* as a world-wide pest, relatively little screening of maize varieties for resistance has been done (Conilh de Beyssac, 1990). Arnason et al. (1994b) found that the same phenolic compounds that seem to confer some resistance to the maize weevil also are responsible for resistance to *P. truncatus*, but most research has focused on chemical or biological control of this pest (Hodges and Meik, 1984; Markham et al., 1994).

The Angoumois grain moth, *Sitotroga cerealella* (Gelechiidae : Lepidoptera), is a very damaging pest of stored maize and other cereals in the tropics (Giga and Kateere, 1996). Like the maize weevil their larvae develop inside a single seed. Pupation may occur in, or between grains to overcome the small size of some cereal grain. When cereals are stored in bulk, *S. cerealella* larvae and adults cannot penetrate deeper than about 12 cm into the store (Muhihu, 1984), damage is then relatively limited. They are known to be a pest of cereals and, buckwheat (Candura, 1954), including wild cereals (Joubert, 1966). In regions with high humidity they compete with *S. zeamais* (Avertay, 1980), therefore they are more frequent in regions that are too dry for the maize weevil. The origin of *S. cerealella* is reportedly Africa from where it spread and became a cosmopolitan pest (Buckland, 1981).

The Indian meal moth, *Plodia interpunctella* (Pyralidae : Lepidoptera), is a cosmopolitan pest of a wide range of stored product, from peanuts to dried fruit and whole and milled cereals. In maize kernels the larvae feed mainly on the embryo of the seed and are not confined to one seed for development. Hockensmith et al. (1986) found that development of *P. interpunctella* on maize seed resistant to *S. zeamais* was slower and pupal weight lower. He also observed that larval mortality was high if the pericarp of maize is intact.

The coffee beetle, *Araecerus fasciculatus*, (Coleoptera : Anthribidae) is a well-known cosmopolitan pest of coffee beans in the field and in storage, but can attack stored tubers such as cassava and yam (Plumbley and Rees, 1983; Kumar et al., 1996) and severely damage maize stored in the humid tropics (Taylor et al, 1978). Its larvae develop within the maize kernel or in the powder produced by the adults. Because of their minor importance as pests of stored maize no screening of maize varieties for resistance to this insect has been done.

In the current study, we addressed the question of varietal resistance to the above storage pests. Based on the fact that they all have different backgrounds (Buckland, 1981), different phylogeny, biology and feeding habits we hypothesise that resistance to one insect pest does not necessarily implicate resistance to all storage pests. The range of resistance to the maize weevil and the Angoumois grain moth within maize genotypes was compared to the growth rate of these insects on other cereals. For many plant resistance factors for cultivated crops, the germplasm of wild relatives is a good source because they have been selected to survive under adverse conditions (Galinat, 1977). We tested if the mature seeds of tesosinte are fully protected by the fruitcase to the maize weevil and the Angoumois grain moth, or if there is any other resistance factor inherent to the grain that could be useful for improvement of grain resistance in maize. We also discuss the results in the context of possible impact of grain resistance on the efficiency of storage parasitoids.

Material and Methods

1. Plant material and insects

1.1. Grain

The seed from a wide range of maize accessions and populations were used (Table 1.1).

CIMMYT (International Maize and Wheat Improvement Center) provided tropical lowland hybrids grown at the experimental station in Poza Rica, a Caribbean accession being improved for grain resistance, an introduction from Africa with high tryptophan content, tropical and highland landraces, and two tropical synthetics. A large number of landraces, grown by small-scale farmers, were collected in the state of Oaxaca. Another highland landrace, purple Conico, called maize azul, was purchased at a local market in Texcoco (State of Mexico).

As susceptible controls we used Cacahuazintle, a floury highland landrace. Sinaloa 35 and Yucatan 7 served as resistant controls (Arnason et al., 1994). The quantities that were available ranged from 5 to 100 cobs per genotype. At least 5 cobs were shelled and mixed together for later use.

Teosinte seeds were collected in a maize field Southwest of Mexico City, near Chalco, wheat (*Triticum aestivum*) was purchased at a local market in Mexico, and little spelt (*Triticum monococcum*) in France.

Prior to use, all maize and wheat was frozen to kill any insects originating from natural infestation and were then incubated at the experimental conditions of 26.5° C and 75 % R.H., for at least three weeks prior to the experiments to equilibrate grain moisture. Moisture content of maize varieties was measured at the end of each series of experiments with a Dole Model 400B moisture tester (Agri-Tronix Corporation, 2001 North U.S. 31 Franklin, Indiana 46131).

Table 1.1: Maize varieties used

Variety	Abr.	type	Climate, Origin	from	color	endosperm
Cacahuazintle	Ca	landrace	highland	CIMMYT	white	floury
Purple Conico	Azul	landrace	highland	market	purple	floury
CML264xCML254	Hy w	hybrid	tropical	CIMMYT	white	dent
CML287xCML285	Hy y	hybrid	tropical	CIMMYT	yellow	dent
CML 244xCML349	Hy R	hybrid	highland	CIMMYT	white	dent
Population 84	Pop 84	population	tropical, Cuba	CIMMYT	orange	flint
Ejura	Pop 80	population	tropical, Africa	CIMMYT	white	flint
Sinaloa 35	Sin	landrace	tropical	CIMMYT	brown	flint
Yucatan 07	Yuc	landrace	tropical	CIMMYT	yellow	flint
Criollo Poza Rica	PR Pu	synthetic	tropical	farmer	white	dent
Criollo Poza Rica	PR Cri	synthetic	tropical	CIMMYT	white	dent
167 Mazaltepec	Ox 167	landrace	mid-altitude, Ox	farmer	white	dent
173 Roagua A.	Ox 173	landrace	mid-altitude, Ox	farmer	black	dent
174 Roagua A.	Ox 174	landrace	mid-altitude, Ox	farmer	several	dent
181 S.C. Albarrados	Ox 181	landrace	mid-altitude, Ox	farmer	white	dent
184 Valdeflores	Ox 184	landrace	mid-altitude, Ox	farmer	yellow	dent
190 Valdeflores	Ox 190	landrace	mid-altitude, Ox	farmer	white	dent
201 Sta Ana	Ox 201	landrace	mid-altitude, Ox	farmer	white	dent
209 Anatengo	Ox 209	landrace	mid-altitude, Ox	farmer	white	dent
Sitio 2 (Tuxpeño)	Sitio 2	landrace	mid-altitude, Ox	farmer	yellow	dent
Oaxaca Bolito	Ox B	landrace	mid-altitude, Ox	farmer	white	dent

Varieties were from Mexico, unless otherwise mentionned; Ox: originating from the state of Oaxaca (Mexico)

Kernel hardness was measured with a displacement force test station, Model 921A (Tricor Systems Inc, Elgin Illinois 60123). The equipment was fitted with a 20Kg load cell and a 0.8 mm dia. probe to determine the force needed to crack the grain. The cross head speed of the probe was 1cm/s. The force was measured on the flat side of the grain opposite the germ, approximately in the middle. The average weight of 100 kernels was measured for each genotype. Where only small quantities of maize were available not all physical measures could be taken or replicated.

1.2. *Insects*

All insects were reared at the experimental conditions of 26.5° C and 75 % R.H., unless otherwise specified.

Maize weevil (*Sitophilus zeamais*) colonies were established with specimens collected at CIMMYT's experimental station in Poza Rica (20.492N; 97.547W). Adults were retrieved from the culture jars containing a white highland hybrid maize CML244xCML349 (Hy R) after an oviposition period of two weeks and subsequently discarded. New jars were set up every three months. The colony was maintained for 6 to 8 months and then replaced.

Larger grain borer (*Prostephanus truncatus*) adults were collected at CIMMYT's experimental station in Tlaltizapan. In this case, the parent generation was left in the culture jars containing CML244xCML349 as diet, and new jars were set up every two to three months.

The Angoumois grain moth (*Sitotroga cerealella*) originated from maize bought at a market in Pahuatlan (Puebla). Adults were allowed to oviposit on folded wax paper stripes, and maize was infested with eggs only. The colony was renewed with new individuals after one year.

Indian meal moth (*Plodia interpunctella*) adults from Tlaltizapan (Morelos) were used to establish a colony. As culture medium we used wheat feed mixed with maize flour or broken maize grains. Adults were allowed to oviposit for two days in glass jars closed with a plastic mesh and placed upside down on a petri dish. Only eggs were used to infest the culture medium.

Coffee beetles (*Araecerus fasciculatus*) originating from Poza Rica, and collected on stored maize, were used to establish a colony on CML244xCML349 (Hy R). Adults were retrieved from the culture jars after an oviposition period of two weeks. They were reared at 30 ° C and 90 % R.H.

The granary weevil, *Sitophilus granarius*, originated from chick-peas bought at a market in Pahuatlan (Puebla). They were reared on wheat.

Culture jars were placed in trays with mineral oil to avoid mite infestation.

2. Experiments

2.1. *Varietal resistance to storage insects*

In the first experiment, 4 samples of each maize genotype were tested for resistance by exposing them to each insect species in a no choice situation. The experimental design was adapted to the biology of each of the insects, and the parameters used were those that can be measured and are most relevant for the performance of each insect, such as larval development time, total progeny, survival to adult and number of adults. The samples contained either an equal number of kernels or the same quantity in weight, depending on whether the larval development was strictly confined to one seed or not. For more details see the experimental procedures (3). A summary of the procedures is given in Table 1.2. The lepidopteran species were exposed to a multiple-choice test, as described in the procedures (3.3).

2.2 *Maize resistance compared to other cereals*

Three to five selected maize genotypes were tested alongside other cereal grains such as wild maize (teosinte), wheat, and little spelt (*Triticum monococcum*) in no-choice tests for the same parameters as described for *S. zeamais* and *S. cerealella*. Among the maize genotypes was a susceptible (Cacahuazintle), and a resistant control (Sinaloa 35 for *S. zeamais*, CML264xCML254 for *S. cerealella*). A white hybrid, CML244xCML349, was tested without germ (for the maize weevils only). Seeds of teosinte were tested whole with the fruitcase, with part of the fruitcase cut off (teos+) or without the fruitcase (teos-). Wheat was tested with two maize weevil strains, one reared on maize and the other reared on wheat for four generations. In the same experiment, the granary weevil (*S. granarius*) was also tested on wheat. Five females were used to infest the samples, otherwise the method described in procedures was used (3.1). For the Angoumois grain moth only one strain was used.

Samples of 40 kernels of maize and teosinte were tested, and 2.2 g (=minimum weight of 40 teosinte seed) samples of wheat and little spelt. The same method as described in procedures was used (3.3).

2.3 Effect on weight of adult *Sitophilus zeamais*, *Sitotroga cerealella* and *Plodia interpunctella*

S. zeamais

Several of the maize genotypes of experiment one, were infested with maize weevils for a seven day oviposition period and then discarded. Weevil were either from a culture that originated from Veracruz (first generation [C0] or after n cycles [Cn] in the laboratory) or from the state of Oaxaca (Ox). Samples of the three maize genotypes and the other cereal grains used in experiment two, were infested with weevils and another sample of wheat with a colony reared for 4 generations on wheat (T). Emerging weevils were killed and weighted.

S. cerealella

Several of the maize genotypes were infested with eggs of *S. cerealella*. Emerging adults were killed, sexed and weighed individually (15 females) . The experiment was repeated with six of the maize genotypes, teosinte with and without their fruitcase removed, and wheat.

P. interpunctella

Several of the maize genotypes were damaged and infested with eggs of *P. interpunctella*. Emerging adults were killed, sexed and weighed individually (15 females).

3. Experimental procedures for susceptibility of maize genotypes

3.1 *Sitophilus zeamais*

The procedures are based on those described by Dobie (1974) and used by many authors for screening for resistance (Urrello et al., 1989; Arnason et al., 1992). Samples of 50 kernels of a

Table 1.2: Procedures used to determine susceptibility of maize genotypes to several storage pests

Insect	Infestation			Maize	Parameters measured	Duration of experiment
	stage	number	duration			
<i>Sitophilus zeamais</i>	Adults	6+3	7 d	50 K	MDT ; F1	60 d
<i>Prostephanus truncatus</i>	Adults	20	42 d	20 g	NA; Pow.	42 d
<i>Sitotroga cerealella</i>	Eggs	40-50	3 d	40 K	MDT ; % Survival	55 d
<i>Plodia interpunctella</i>	Eggs	40	2 d	20g dam.	MDT; % Survival	55d
<i>Araecerus fasciculatus</i>	Adults	20	14 d	20 g	MDT; F1;	90 d

genotype were placed in 60 ml glass vials together with six females and three males that were between 10 and 20 days old. The weevils had been conditioned on the same genotype for 5 days prior to infestation. They were sexed under a stereo microscope using snout characteristics (Halstead, 1963). After 7 days, the adults were removed and the presence of dead adults was recorded. The vials were then returned to the incubation chamber. Emerging weevils were counted every other day, from 30 days until 65 days after the first day of infestation (F1), and the medium time of development (MDT) was calculated as the time from the middle of the oviposition period until half of the F1 generation had emerged. The susceptibility index was calculated as $100 \cdot \ln F1 / MDT$ (Dobie, 1974). The experiment was repeated four times.

3.2 *Prostephanus truncatus*

Twenty grams of each maize genotype were placed in 60ml glass vials and infested with 20 unsexed adult *P. truncatus*. Five glass balls (10mm diam.) were used to weigh down the grain

because the larger grain borer has difficulties to attack loose maize kernels (Cowley et al., 1980), and the vials were closed with a wire mesh screen. Four replicates were made. After 15 days, the kernels were sieved through a No 50 sieve and the powder produced was weighted. The powder was then added back to the kernels and the vials were again incubated. The adults were not retrieved because by doing so kernels and clutches of eggs would be destroyed. After a total of 42 days of incubation all samples were frozen and analyzed. The powder production was again measured, and all adult insects were counted. Total progeny was calculated by subtracting the parent generations. These numbers were square root transformed for analyses (Horber, 1987). No susceptibility index was calculated.

3.3 *Sitotroga cerealella*

Glass vials containing 40 kernels of maize were infested with pieces of wax paper strips containing 40 to 50 *S. cerealella* eggs laid during a three day period. The wax paper strips had been washed previously under running tap water, and rinsed with distilled water, to avoid infestation by mites. Eggs were counted under a binocular microscope. The vials were closed with wire mesh and tissue paper. The wax paper stripes were collected ten days later and hatched eggs were counted under a binocular microscope. Emerging adults were counted every other day from 29 days until 56 days after oviposition. Resistance was measured as percentage of survival from hatched egg to emerged adult, and as medium development time (MDT), being the time from the middle day of oviposition until half of the F1 generation had emerged. These methods are similar to those used by Cogburn et al. (1983) and Russel and Cogburn (1976) to evaluate resistance to Angoumois grain moth in rice.

Six genotypes were used for the multiple choice test. Fifty kernels of each genotypes were put together in a half-liter glass jar and infested with 100 eggs. Hatched eggs were

counted after 10 days and the percentage of emerging adults per genotype was calculated. This was replicated five times.

3.4 *Plodia interpunctella*

Similar methods were used for the Indian meal moth. In the first experiment, 100 *P. interpunctella* eggs were used to infest 100 undamaged kernels of each genotype in glass vials covered with tissue paper and metal mesh, and incubated. Emerging adults were counted from day 30 until day 60 after oviposition.

In a second experiment, kernels were artificially damaged by cutting off the pedicel of each kernel with small scissors. Four 20g samples of each genotype were prepared this way and infested each with 40 eggs. To determine the rate of hatching, a sample of 120 to 150 eggs was counted and incubated in a small dish surrounded by wheat germ. The percentage of hatched eggs was recorded 10 days later. Emerging adults were counted daily for the first ten days and then every other day from day 28 to 50 after oviposition. Percentage survival from egg to adult and medium development time were determined.

For the multiple-choice test, the infestation procedure was the same as for *S. cerealella*. Kernels were damaged using a needle to puncture the pericarp above the germ (Hockensmith et al., 1986). Kernels were separated and examined for infestation after 10 to 15 days. Percentage of infestation for each genotype was calculated. Infested kernels were incubated individually, and emerging adults were recorded. Percentage of survival per single kernel was calculated for each genotype.

3.5 *Araecerus fasciculatus*

Twenty unsexed adults, less than two weeks old were used to infest 20 g of each maize genotype. The adults were removed and discarded after 2 weeks. Emerging beetles were removed and counted

once a week until no more emerged. The experiment was carried out once at the conditions described above, and twice at 30° C and 90 % R.H. Four replicates were made in each case. Weight loss of the seeds was measured at the end of the experiment. For sex-ratio determination, two hundred adult beetles from the culture were randomly collected, killed and sexed.

Results and Discussion

1. Varietal resistance to storage insects

Sitophilus zeamais

The parameters evaluated showed clear differences for resistance to *S. zeamais* among varieties (Tables 1.3, 1.4 and Fig. 1.1). A one-way analysis of variance indicated significant differences for number of progeny (Fig. 1.1) and the Dobie index (Table 1.4). The differences for the (median) time of development (MDT) were much smaller, but differences in their non parametric values were significant (Table 1.4). Longest MDT was 37, the shortest 33 days. Number of progeny and MDT did not correlate. Adult mortality was low .

Up to five times more weevils emerged from susceptible than from the resistant varieties. The most susceptible genotype was Purple Conico (Azul), a floury highland landrace like the susceptible control, Cacahuazintle. Most other varieties were moderately or very susceptible, and differences were small. The most resistant genotypes were the resistant control, Sinaloa 35 and the Caribbean introduction Population 84. Some of the Oaxacan landraces were moderately resistant, as was the other resistant control Yucatan 7 (Fig. 1.1).

Prostephanus truncatus

A similar pattern was found for the larger grain borer *P. truncatus* (Tables 1.3 , 1.4 and Fig. 1.2). The total number of adults and powder production after 42 days were significantly different. Susceptible varieties contained up to five times as many adults as resistant ones. Even powder production after only 14 days showed already a clear pattern of susceptibility, but differences were not significant (Table 1.4). Powder production after 14 days was correlated with number of adults and powder production after 42 days (0.74 and 0.91, respectively).

The same varieties that showed resistance to *S. zeamais* proved to be resistant to *P. truncatus* as well, the correlation coefficient was 0.74 (progeny *S. zeamais* x adults *P. truncatus*), and 0.72 (adults *P. truncatus* x Dobie index *S. zeamais*).

Very high numbers of adults were found in the susceptible varieties, and the maize kernels were completely destroyed. Again, only a few varieties showed resistance. In all samples large numbers of immatures were found, indicating that there was no resource limitation.

Sitotroga cerealella

Varieties also differed significantly in rate of survival and index of susceptibility for the Angoumois grain moth, *S. cerealella*, but the range was much smaller (Table 1.3, 1.4 and Fig. 1.3). The survival rate ranged from almost 40 % to 71 %. The difference between the shortest and longest MDT was four days. Because few adults emerged in some samples, all samples for each genotype were pooled to calculate MDT.

The results from the multiple choice test confirmed those from the no-choice experiment, the susceptible varieties had a much higher emergence rate than the more resistant ones (Table 1.5 and Fig. 1.6). Although the floury Cacahuazintle was again among the very susceptible varieties there was no correlation with the index for susceptibility or any other parameter of the maize weevil and the larger grain borer (0.2 and 0.3, respectively). The weevil resistant flint varieties were all susceptible and a *S. zeamais* susceptible hybrid (Hy w) was most resistant against the Angoumois grain moth. In a long-term experiment reported in chapter 3 this was again the most resistant variety.

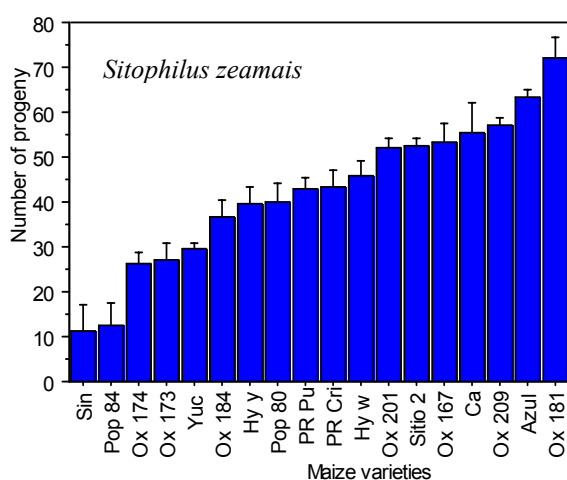


Fig. 1.1

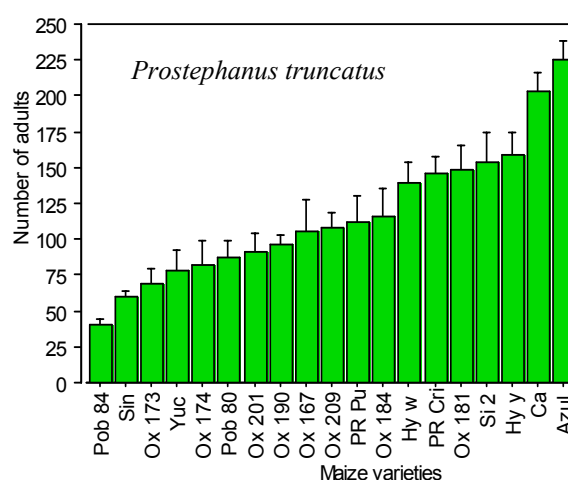


Fig 1.2

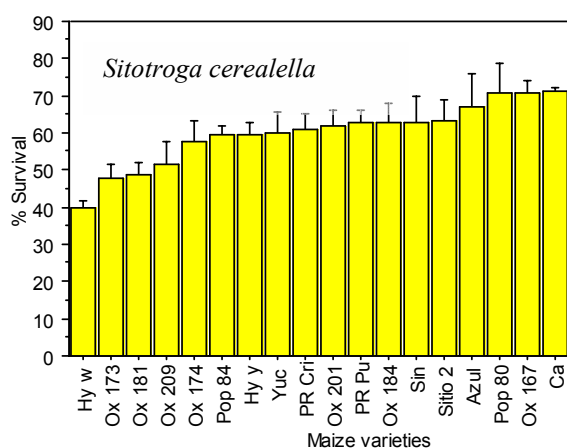


Fig. 1.3

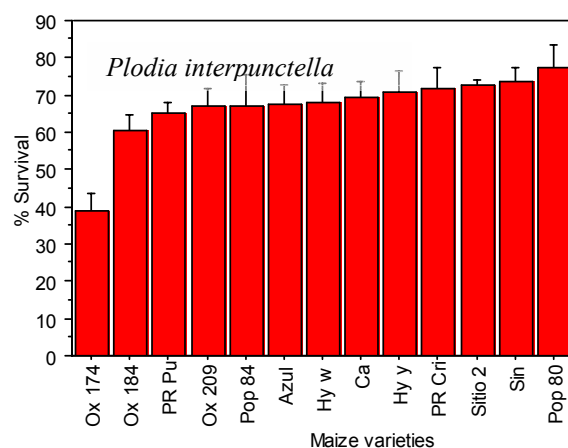


Fig. 1.4

Fig. 1.1: Mean progeny of *Sitophilus zeamais* in different maize varieties.

Numbers were analyzed with ANOVA and used to calculate an index of susceptibility. $df=17, 50$ $F=18.7$ $P<0.0001$

Fig. 1.2: Mean number of adult *Prostephanus truncatus* in different maize varieties.

Numbers were analyzed with ANOVA after square root transformation $df=18, 53$ $F=10.5$ $P<0.0001$

Fig. 1.3: Percent survival of *Sitotroga cerealella* to adult in different maize varieties.

Results were analyzed by ANOVA and means were used to calculate an index of susceptibility. $df=17, 54$ $F=2.9$ $P=0.002$

Fig. 1.4 : Percent survival from egg to adult of *Plodia interpunctella* on different maize varieties that were damaged. Results were analyzed by ANOVA and means were used to calculate an index of susceptibility . $df=13,38$ $F=8.34$ $P<0.0001$

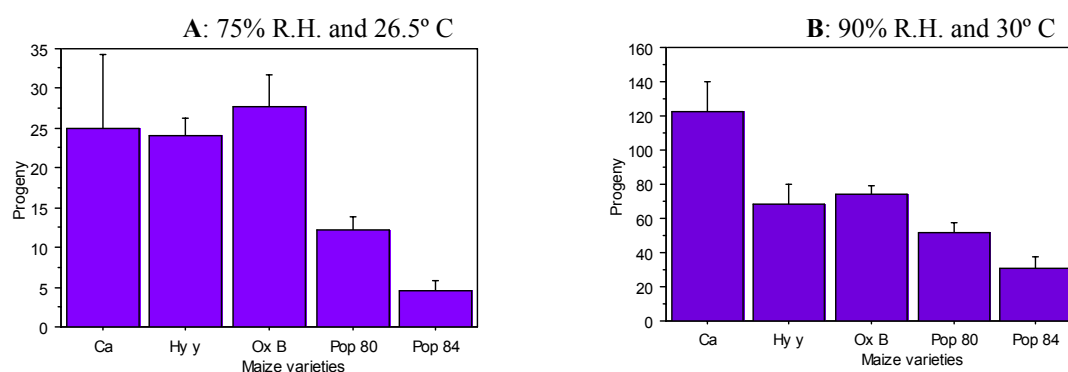


Fig. 1.5 : Progeny of *Araecerus fasciculatus* on different maize varieties at two climatic conditions

A: 75% R.H. and 26.5°C. **B:** 90 % R.H. and 30°C

Results were analyzed by ANOVA and means were compared with Fishers protected LSD (Table 1.6)

A: df=4, 15 F=4.49 P=0.014

B: df=4, 30 F=11.47 P<0.0001

Table 1.3 : Susceptibility data and index for four storage pests

Variety	<i>Sitophilus zeamais</i>		<i>Prostephanus truncatus</i>		<i>Sitotroga cerealella</i>		<i>Plodia interpunctella</i>	
	Index		Number of Adults		Index		Index	
Azul	12.4	a	22.5	a	12.2	be	13.7	a-c
Ca	11.2	a-d	20.3	a	12.9	a	13.7	a-c
Hy w	10.3	b-e	13.9	b-d	9.8	h	13.5	a-c
Hy y	9.7	c-f	16.7	b	11.1	ef	14.1	ab
Pop 84	5.5	g	4.1	f	11.6	c-e	13.4	bc
Pop 80	10.0	c-f	8.8	ef	12.2	cb	14.3	a
Sin 35	4.1	g	6.0	f	11.2	d-f	14.5	a
Yuc 07	9.1	ef	7.9	ef	12.0	bc		
PR Pu	10.6	b-e	11.2	d-f	11.0	ef	14.0	a-c
PR Cri	10.9	a-d	14.7	b-d	12.0	bc	14.3	a
Ox 167	11.3	a-c	10.6	d-f	12.9	a		
Ox 173	8.6	f	6.9	f	10.2	gh		
Ox 174	8.8	ef	8.2	ef	11.2	d-f	11.8	d
Ox 181	11.7	ab	14.8	b-d	10.9	fg		
Ox 184	9.7	d-f	11.6	c-e	12.5	ab	13.1	c
Ox 190			9.6	ef	1.2	d-f		
Ox 201	11.0	a-d	9.2	e-f	11.5	c-f		
Ox 209	10.7	a-e	10.8	d-f	11.1	d-f	13.5	a-c
Sitio 2	10.7	a-e	15.3	b	11.8	cd	14.4	a

Susceptibility index for *Sitophilus zeamais* was calculated as $\ln(\text{number of progeny}) / \text{MDT} \times 100$. MDT is the medium development time. For *Sitotroga cerealella* and *Plodia interpunctella* %survival was used instead of progeny. For *Prostephanus truncatus* no index was calculated the number given is the total number of adults/10 present at the end of the experiment.

Results were analyzed by ANOVA. Means followed by the same letter in a column are not significantly different for Fisher's protected LSD (P=0.05)

S. zeamais: df=17, 50 F=6.553, p<0.0001; *P. truncatus*: df=18, 53 F=10.522, P<0.0001;

S. cerealella: df=18, 57 F=11.266, P<0.0001; *P. interpunctella*: df=13, 38 F=15.947 P<0.0001

Plodia interpunctella

Undamaged kernels of all varieties were very resistant to attack by the Indian meal moth, *P. interpunctella*, only between 0 and 3 % survived, and development time was long, up to 52 days. For damaged kernels there were small differences among varieties (Table 1.3, 1.4, and Fig. 1.4). Only the Oaxacan landrace174 showed a lower rate of survival. MDT was shorter, ranging from 29 to 30 days.

The multiple choice experiment showed that first instar larvae prefer genotypes with larger kernels (Table 1.5 and Fig 1.7). Cacahuazintle, which was the largest grain tested, was able to

Table 1.4 : Detailed susceptibility data of maize genotypes to four storage pests

Variety	<i>Sitophilus zeamais</i>		<i>Prostephanus truncatus</i>		<i>Sitotroga cerealella</i>			<i>Plodia interpunctella</i>		
	Number of Progeny	Development time(days)	Powder produced after		% Survival	Development time(days)	Weight Female (g)	Development time (days)	% Survival	Weight Female (g)
Azul	63.5	33.3	2.2	12.0	67.0	34.3	0.058b	31.0	67.5	0.132
Ca	55.5	35.8	2.0	11.9	71.4	33.0	0.070a	31.0	69.4	0.127
Hy w	46.0	36.5	1.2	5.3	39.6	37.6	0.064ab	30.7	68.1	0.131
Hy y	40.0	37.9	1.3	6.6	59.6	36.8	0.063ab	31.0	70.6	0.141
Pop 84	12.5	37.1	1.0	3.9	59.3	35.1	0.059ab	32.0	66.9	0.109
Pop 80	40.0	36.5	1.0	4.3	70.7	34.8	0.060ab	31.0	77.5	0.139
Sin 35	11.3	36.7	1.1	4.2	63.0	37.0	0.058ab	29.7	73.8	
Yuc 07	29.5	36.3	1.2	4.4	59.0	33.9				
PR Pu	43.0	36.4	1.8	6.5	62.9	37.5		29.8	65.0	
PR Cri	43.3	34.5	1.5	6.9	61.1	34.2	0.062ab	29.9	71.9	0.131
Ox 167	53.5	34.9	1.4	5.9	70.8	33.0	0.062ab			
Ox 173	27.3	38.5	1.4	6.0	47.8	37.7				
Ox 174	26.3	36.5	1.4	5.7	57.8	36.1		30.9	37.5	0.125
Ox 181	72.0	36.1	1.4	7.4	48.6	35.5				
Ox 184	36.5	36.9	1.0	5.6	63.0	33.0	0.063ab	31.3	60.6	0.135
Ox 190			1.1	4.5	53.7	35.3				
Ox 201	52.3	36.5	1.3	5.5	61.8	35.8				
Ox 209	57.0	37.1	1.1	5.5	51.4	35.2	0.065ab	31.4	66.9	0.123
Sitio 2	53.0	37.0	1.4	7.5	63.2	35.0		29.6	72.5	

Sitophilus zeamais: F1: ANOVA df=17, 50 F= 18.7 P<0.0001; MDT: Kruskal-Wallis P=0.0027

Prostephanus truncatus: ANOVA: Powder production: P14 differences are not significant; P42: df=18, 54 F=12.98 P<0.0001

Sitotroga cerealella: ANOVA : %Sur : df=17, 54 F=2.9 P=0.0018, MDT was calculated by pooling samples, Weight: ANOVA: df=11, 176 F=1.9 p=0.04

Plodia interpunctella: ANOVA %Sur: df=13,38 F=8.34 P<0.0001, MDT: Kruskal-Wallis test t : P=0.0034, Weight : Anova : differences were not significant.

Means followed by the same letter in column Weight are not significantly different in the Tuckey-Kramer test (P=0.05)

support about one quarter of the larvae through to maturity. In the other genotypes the rate was lower, and none survived on the smallest grain, Sinaloa 35 (Table 1.5). The development time was very long in all cases, 45 days and more.

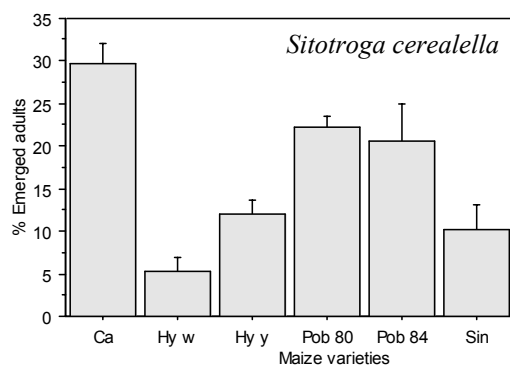


Fig. 1.6

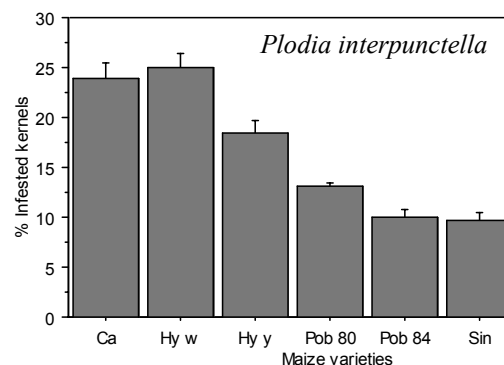


Fig. 1.7

Fig. 1.6 : Multiple choice for *Sitotroga cerealella*:

Percentage adults that emerged for each variety, when first instar larvae were allowed to choose among the six varieties. Results were analyzed with ANOVA, and means compared with Fishers protected LSD df=5, 18 F=12.01 P<0.0001 LSD=7.8

Fig. 1.7 : Multiple choice for *Plodia interpunctella*:

Percentage of infested grain of each variety, when first instar larvae were allowed to choose among the six varieties. Results were analyzed with ANOVA, and means compared with Fishers protected LSD df=5, 18 F=36.04 P<0.0001 LSD=3.4

Table 1.5: Multiple choice: Results for *Sitotroga cerealella* and *Plodia interpunctella*

Variety	<i>S. cerealella</i>		<i>P. interpunctella</i>	
	% adults emerged		% kernels infested	% Survival to adult
Ca	29.7	a	24.0	a 25.9
Hy w	5.2	c	25.1	a 18
Hy y	11.9	c	18.4	b 20
Pop 80	22.3	ab	13.1	c 5.1
Pop 84	20.6	b	9.9	cd 5.5
Sin	10.2	c	9.6	d 0

% Survival to adult: % larvae of *P. interpunctella* that were able to complete development on one kernel.

Results were analyzed with ANOVA, and Fishers protected LSD

Means followed by the same letter in the same column are not significantly different (p=0.05)

S. cerealella :df=5, 18 F=12.01 P<0.0001

P. interpunctella : df=5, 18 F=36.04 P<0.0001

Araecerus fasciculatus

Number of progeny for the coffee beetle, *Araecerus fasciculatus*, differed significantly among varieties, at both 90 and 75%R.H.. Their rate of increase was much higher and variation of resistance was better expressed at the high humidity and temperature condition. Values for MDT did not differ much among genotypes, but differed from 37 to about 60 days at 75% R. H and 26.5 °C (Table 1.6). Cacahuazintle was more susceptible at a higher relative humidity (Fig. 1.5). Total number of progeny correlated well with the same parameter for the larger grain borer (0.89 at 90 % R.H. and 0.65 at 70%R.H.), and with the Dobie index for susceptibility of the maize weevil (0.78 and 0.6 at 90 % and 75 % R.H., respectively). The sex ratio of *A. fasciculatus* was female biased (1.6).

Table 1.6 : Susceptibility data for *Araecerus fasciculatus*.

Variety	90% RH, 30°C		75% RH, 26.5°C	
	Progeny	Development time	Progeny	Development time
Hy y	68.4 ^b	37.0	24.0 ^{ab}	68.0
Ox B	74.1 ^b	37.6	27.8 ^a	55.2
Pop 80	51.3 ^{bc}	37.7	12.3 ^{bc}	63.0
Pop 84	30.9 ^c	37.6	4.5 ^c	61.2
Ca	122.7 ^a	37.6	25.0 ^{ab}	59.7

Number of progeny were analyzed by ANOVA.

Means followed by the same letter in a column are not significantly different in Fisher's protected LSD(p=0.05).

At 90% R.H., 30°C: df=4, 30 ; F=11.47; p<0.0001.

At 75% R.H., 26.5°C : df=4, 15; F=4.49; p=0.014

The lack of correlation of measured parameters between Coleoptera and Lepidopteran species suggests that the resistance factors for these insect families are not the same.

Arnason et al. (1994) found that resistance to *S. zeamais* was correlated with phenolic content of maize varieties. He found high concentrations in Sinaloa 35 and Yucatan 7, both very resistant to *S. zeamais*, but susceptible to the Angoumois grain moth . These are both varieties from tropical areas, as is population 84, derived from Cuban and Caribbean accessions.

Urrello et al. (1989) found that resistance traits against maize weevils are more likely encountered in flint varieties from the lowland tropics than in floury highland accessions. The phenolic compounds that seem to confer this resistance to the grain are located in the embryo, the aleurone layer and pericarp of maize kernels (Sen et al., 1994). This location could be the reason that lepidopteran species are less affected. In fact, the life cycle of storage Lepidoptera differs from that of coleopteran species in that the eggs are laid on the grain, and the neonate larvae has to enter the kernel by its own efforts. In contrast, maize weevils oviposit under the pericarp, so that oviposition and first instar larvae are affected by these resistance factors. First instar larvae of maize weevils are small and fragile, the highest mortality during development occurs at this stage (Longstaff, 1981). Larvae of the other coleopteran pests are much less confined to this location, the eggs are laid in tunnels inside the kernel and will be more affected by overall hardness of the endosperm. The quality of the pericarp, its structure (smoothness, thickness), or biochemical components could have a more important impact on Lepidoptera (Russell and Cogburn, 1976), because it is a barrier for first instar larvae. Russell and Cogburn (1977) found resistance to seed penetration in world collections of rice. Larvae could have difficulties to grip the smooth surface of the pericarp with their mandibles. Cogburn (1974) showed that grains of rough rice with damaged hulls were more susceptible to infestation by the Angoumois grain moth than kernels from which hulls had been removed. We observed that entry holes were frequently located close to the pedicel, or base of the kernel. If a single layer of kernels is placed in a petri dish, neonate larvae will enter the kernel on the side of the kernel that is in touch with the petri dish. This could be explained by their need for some stable support to be able to successfully attack the grain. Observations of the more resistant CML264x CML254 (Hy w) kernels under a microscope revealed a number of unfinished entry holes indicating that at this stage mortality was high. First instar larvae of the Angoumois grain moth are relatively large and mobile, as compared to maize weevil larvae,

and, once passed the barrier of the pericarp can move fast beyond the protective layers that contain the highest concentrations of phenolic compounds, to reach the endosperm. The sensitive first stage is also the reason why *S. cerealella* is outcompeted by *S. zeamais* as soon as the adult of the latter reach a certain population density (Avertey, 1980). There are many authors however that suspect the existence of other mechanisms of resistance in cereals to *S. cerealella*, unrelated to the pericarp (Cogburn et al., 1989). Levels of amylase in the endosperm have a significant influence on life parameters of *S. cerealella* (Peters et al., 1972).

In the long term experiment reported in chapter 3 the moderate resistance of hybrids to *S. cerealella* was confirmed. It showed also that larger numbers of adult grain moth emerging from maize varieties with small kernels. Unlike maize weevils which are known for the clumped distribution of eggs on kernels (Legg et al., 1987) a single moth larvae is usually found per seed, possibly to avoid cannibalism and competition for resources. As already infested kernels are less suitable, they are not found in residual populations (Chapter 6). Varieties with small kernels seem to be more susceptible, if samples of equal weight are compared. Also, for mass rearing wheat is used rather than maize (Morrison, 1985).

The variation of MDT was not very large for *S. cerealella*, nor for *S. zeamais*, but even a difference of a very few days will eventually make a large difference in damage over a couple of months, especially under less favorable climatic conditions. For *P. truncatus* the MDT could not be measured because of the cryptic behavior of the adults that makes it impossible to retrieve them after an oviposition period. Meikle et al (1998) infested different maize genotypes with eggs to measure development time, but found no difference. One is tempted to explain the low number of progeny in resistant genotypes to a slower excavation rate for tunnels required for oviposition by the parent generation.

Plodia interpunctella has been described as a pest of stored whole grain, but (Locatelli and Limonta, 1998) already mentioned that first instar larvae had difficulty attacking the seed

through the pericarp of buckwheat. Larval mortality was high on undamaged maize kernels (Hockensmith et al., 1986), where hard seed coats prevented larva from penetrating the grain (LeCato, 1976). Our investigations confirmed this, with few insects being able to develop on intact kernels (Table 1.5). On damaged grain neither the development time, which was the shortest for all insect measured, nor the survival rate gave any indication of resistance in these maize genotypes if not limited by the quantity of grain. Hockensmith et al. (1986) observed higher resistance to *P. interpunctella* in a *S. zeamais* resistant genotype. It is not clear in his publication whether he used only one or several grain per larvae. In the multiple choice experiment, we observed a preference for larger genotypes (Fig. 1.7). The limited food supply of the germ obliged the larvae to seek another grain. This occurred about 21 days after infestation. The smaller the kernel and its germ the more the larvae moved to another damaged kernel to complete development. This was the only direct effect of genotype on the growth rate of *P. interpunctella* that could be detected. Indirectly, their rate of increase will be affected by susceptibility to primary pests and damage through improper handling at harvest.

Physical measurements

Varieties differed significantly for kernel hardness, kernel weight and equilibrium moisture content, but these parameters were not correlated with each other (Table 1.7). Several varieties obtained from CIMMYT were tested for these parameters with maize from a different growing season. These values differed somewhat from those obtained earlier, but ranks did not change. Variance for force was much higher in open pollinated varieties than in hybrids. Floury genotypes were of course much softer than the flint ones (Table 1.7). Moisture content differed no more than four percent at equilibrium, and differences in size were threefold. Several authors (Dobie, 1974, Arnason et al., 1994) have suggested that for *S. zeamais* kernel hardness is involved in resistance. In our results the correlation of resistance

Table 1.7 : Physical properties of maize varieties

Variety	Moisture content		Weight of 100 kernels		Kernel hardness	
	%	S.E	g	S.E.	kg	S.E.
Azul	11.6	±0.2	33.5	±0.2	11.3	±1.2
Ca	10.0	±0.1	45.9	±0.7	7.7	±1
Hy w	13.9	±0.1	37.0	±0.2	17.1	±0.4
Hy y	14.4	±0.2	35.9	±1.3	17.3	±0.5
Pop 84	12.6	±0.2	21.8	±0.4	21.2	±0.5
Pop 80	11.9	±0.2	22.9	±0.6	18.3	±0.8
Sin	12.1	±0.0	14.6	±0.4	17.1	±0.6
Yuc	12.1	±0.0	16.6	±0.4	15.1	±0.7
PR Pu			26.9	±0.1	9.1	±0.5
PR Cri	12.4	±0.2	27.0	±0.7	16.7	±0.7
Ox 167	10.5		27.1	±0.7	14.5	±0.4
Ox 173	13.3		46.2	±0.5	18.1	±0.4
Ox 174	14.3		35.6	±0.9	12.9	±0.8
Ox 181	13.1		33.0	±0.7	5.2	±0.8
Ox 184	14.5		45.1	±0.8	13.2	±1.8
Ox 190	11.6	±0.4	51.4	±0.5	15.8	±0.6
Ox 201			51.8	±0.6		
Ox 209	11.6	±0.2	35.1	±0.8	11.7	±0.7
Sitio 2			37.2	±0.1		

Moisture content of maize varieties was measured at the end of each series of experiments , with a Dole Model 400B moisture tester (Agri-Tronix Corporation, 2001 N U.S. 31 Franklin Indiana 46131. Siebe)

Kernel hardness was measured with a displacement force test station, Instron (Model 921A, Tricor System Inc.)

Missing values are due to insufficient quantities of maize available. Measurements were analyzed with ANOVA
 % moisture content: df=17, 90 F=13.156, p<0.0001; weight of 100 kernels: df=24, 71 F=494.6 p<0.0001

Kernel hardness: df=18, 345 F= 15.572, p<0.0001

with hardness for *S. zeamais* and *P. truncatus* confirms these observations, but the susceptibility parameters for *S. cerealella* did not correlate with kernel hardness. However, the measure of force to rupture the grain is not only an indication of quality of the endosperm, but also of its thickness at the point of impact. The point where force is applied does not correspond to where maize weevils mainly feed or oviposit. But resistance to *P. truncatus* and *A. fasciculatus*, which have much less of a preference for a particular feeding site was equally well correlated to these hardness measures. More sophisticated physical and biochemical measurements are necessary to clarify and characterize hardness. The method used here, however, is a helpful means to exclude soft genotypes from selection for improvement for resistance. The equilibrium moisture content was not correlated with

resistance but this does not mean that it has no bearing on resistance. In fact, three of the landraces used as susceptible and resistant checks were already used by Arnason et al., (1994) and moisture content (mc) was very low, only 10%, for the resistant accessions, which were described as almost completely resistant. For the susceptible landrace Cacahuazintle, a high mc was measured. *Araecerus fasciculatus* is much more susceptible to dryness than the other pests (Dobie et al., 1991). It had a markedly lower progeny on Cacahuazintle at 75% than at 90% R.H. compared to the other varieties. The low mc of Cacahuazintle at 75% R.H. might have been a limiting factor for its development. Grain moisture content is very important for insect development in storage. Artificially increased grain moisture at the beginnings of development, will increase progeny and decrease MDT of *S. zeamais* (Holloway, 1985), and even resistant grain that is treated this way before infestation will become susceptible (chapters 3 and 4). Differences of equilibrium moisture content of maize genotypes should receive more attention.

2. Maize compared to other cereals

There were significant differences for progeny and Dobie index for *S. zeamais* in an analysis of variance and multiple comparison test (Table 1.8, Fig. 1.7). Nonparametric examination of MDT by the Kruskal-Wallis test revealed small, but significant differences (Table 1.8).

The most susceptible grain proved to be little spelt (*Triticum monococcum*), followed by Cacahuazintle. Undamaged teosinte was highly resistant as weevils were unable to break through the fruitcase and died during the oviposition period. Weevils reproduced well on teosinte deprived, partially or completely, of its fruitcase (Fig. 1.7). Wheat was as susceptible as Cacahuazintle. Approximately the same number of progeny emerged for the granary and maize weevils. However, development was slower for *S. granarius*. More weevil progeny

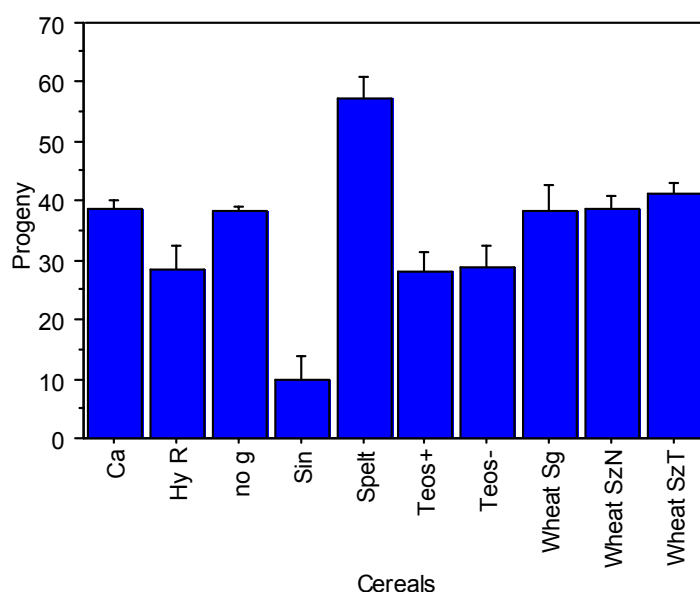


Fig. 1.7: Progeny of *Sitophilus zeamais* on different cereals.

Maize genotypes: Ca, Hy R, Sin, no g : Hy R with germ removed.

Other cereals : Teos: teosinte with fruitcase partially (+) or totally (-) removed, wheat and spelt: little spelt

Sg : *Sitophilus granarius*; Sz N: *S. zeamais* parents reared on maize; Sz T: *S.*

zeamais parents reared on wheat Progeny was analyzed by ANOVA: df= 9, 37 F= 10.3 P<0.0001

Table 1.8 : Susceptibility data of maize and other cereals to *Sitophilus zeamais*.

Grain	Number of progeny		Development time	Index of susceptibility		Insect weight	
Cacahuazintle	38.6	b	33.8	10.9	ab	3.3	a
Hy R	28.6	c	36.3	9.2	c	3.1	ab
Hy R no germ	38.3	b	35.0	10.4	b	2.9	bc
Sin 35	10	d	36.2	6.1	d	3.1	ab
Spelt	57.4	a	33.7	12.0	a	2.6	cd
Teosinte+	28.2	c	35.8	9.1	c	2.4	de
Teosite-	28.8	c	34.1	9.8	bc		
Wheat Sg	38.3	b	38.5	9.4	c		
Wheat Sz N	38.5	b	35.3	10.3	b	1.9	f
Wheat Sz T	41.2	b	35.5	10.5	b	2.2	e

Insect weight: mean weight of emerged adults g*10⁻³

Hy R no germ : Hy R with germ removed; Teosinte with fruitcase partially (+) or totally (-) removed
Sg : *Sitophilus granarius*; Sz N: *S. zeamais* parents reared on maize; Sz T: *S. zeamais* parents reared on wheat

Progeny and Index were analysed by ANOVA, progeny : df= 9, 37 F= 10.3 p<0.0001 index : df=9, 35 F= 9.66 P<0.0001

MDT : with the Kruskal-Wallis test: p=0.0059; Weight was analyzed with ANOVA: df=7, 213 F=46.53 P<0.0001

Means followed by the same letter in column for progeny and Index are not significantly different in Fisher's protected LSD (p=0.05) Means followed by the same letter in column Weight are not significantly different in the Tuckey-Kramer test (P=0.05)

emerged from the maize genotype without germ than from its undamaged counterpart, and MDT was slower on the latter. Sinaloa 35, the resistant maize landrace, was the most resistant grain in this experiment.

For *S. cerealella* the percent survival differed significantly among the cereals, but differences were less important than for the maize weevil, and standard errors were high (Table 1.8, Fig. 1.8). Cacahuazintle was the most susceptible genotype and wheat was within the range of the susceptible maize genotypes. Less than 3 percent of *S. cerealella* survived on undamaged teosinte. When the fruitcase was partially or totally taken off, it ranged close to the most resistant maize hybrid CML364xCML254 (Hy w) for percent survival and index of susceptibility.

Intact hulls or fibrous pericarp of the seed are frequently responsible for resistance to insect attack of many wild relatives of cultivated cereals (Cogburn et al, 1989 ; Locatelli and Limonta, 1998). In the course of domestication, wild maize lost its lignified fruitcase, which protected a relatively large seed from insects, without it, teosinte is a fairly susceptible grain

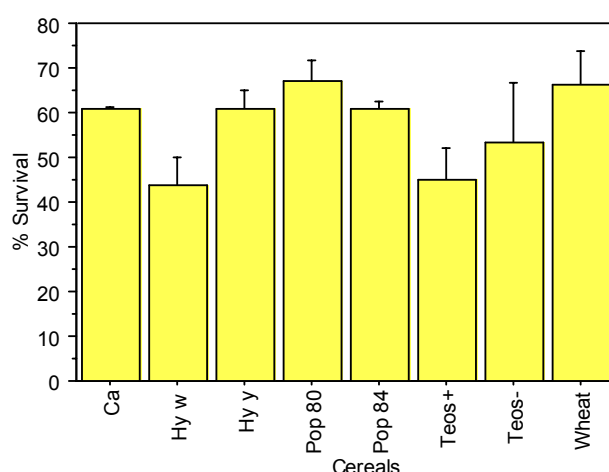


Fig. 1.8: Percent Survival of *S. cerealella* on maize and other cereals

Maize genotypes: Ca, Hy R, Hy w, Pop80, Pop 85

Other cereals: teos+ : teosinte with fruitcase partially, or (teos-) completely removed
Results were analyzed by ANOVA and Fishers protected LSD df=5, 24 F=13.22 p<0.0001
LSD=16.1

Table 1.9 : Susceptibility data for *Sitotroga cerealella* on maize teosinte and wheat

Grain	% Survival	Development time (days)	Index	Weight female g*10 ⁻²
Ca	60.8 ab	31.9	12.9 a	8.78 a
Hyw	43.7 c	38	9.8 d	7.34 b
Hyy	60.7 ab	36.8	11.1 bc	7.36 b
Pop 80	66.9 a	36.6	11.5 bc	7.68 b
Pop 84	60.9 ab	34.8	11.8 b	7.12 b
Teosinte+	45.0 bc	38	9.9 d	4.9 c
Teosinte-	53.4 ab	37.2	10.6 cd	
Wheat	66.2 a	36.7	11.4 bc	4.6 c

%Survival: survival from hatched larvae to adult; index: index of susceptibility $\ln(\% \text{survival}) / \text{MDT} \times 100$; MDT : median development time; Weight: mean weight of emerged adult females g*10⁻²

ANOVA was applied to % survival : df=7, 22 F=2.6 P=0.04 , index of susceptibility: df= 7, 22 F= 11.46 p<0.0001 and weight: df= 9,91 F= 44.31 P<0.0001

Means followed by the same letter in a column % survival and Index are not significantly different for Fishers protected LSD (P= 0.05), and in column weight of females for the Tuckey-Kramer test (P=0.05)
Less than 3% survived on undamaged teosinte which is not listed

to today's storage pests. Its susceptibility lays within the range for domesticated maize, in spite of its smaller size and the large part of hard endosperm. Even if only part of the fruitcase is removed the maize weevil can do well on teosinte. Due to the good protection of the seed there was no evolutionary pressure on teosinte to develop any other resistant traits within endosperm, and not before early races of cultivated maize, deprived of the fruitcase, came under selection pressure of storage pests for resistance traits.

The development of *S. zeamais* on wheat was as good as on susceptible maize and was not influenced by the type of grain on which the parent generation had been reared. No adaptation is needed for the maize weevil to achieve a similar rate of increase on wheat as the granary weevil. The rate of increase for *S. zeamais* was even higher on little spelt (einkorn), a cereal cultivated in the near East, but discontinued in favor of barley and wheat (Harlan, 1992). Maybe its high susceptibility to storage pests contributed to its discontinued use. The population growth rate of maize weevils was higher on maize deprived of its germ, probably due to a higher oviposition rate. Maize weevils are known to oviposit more in the presence of damaged grain (Giga et al., 1995). Weevil larvae will feed to some extent on the radicle and

scutellum portions of the embryo of intact kernels (Kossou et al., 1992, Vowoter et al.,1994), but this feeding has only a limited impact on the germination rate. De and Sarup (1989) examined the quantitative loss of kernel fractions of maize varieties and found that loss of embryo portions vary greatly with genotype, which suggests that preferential feeding on different fractions of the kernel depends on some extent on maize varieties.

MDT of *S. zeamais* on maize deprived of the embryo was not longer than on whole kernels, contrary to observations made by Schoonhoven et al.(1976). This allows the maize weevil to maintain a population on debris of maize, and therefore withstand competition with secondary pests such as *Tribolium castaneum* and *Plodia interpunctella*, which require the germ to complete development.

Some Angoumois grain moths managed to develop on teosinte seeds with an intact fruitcase, but teosinte can still be considered highly resistant to this insect. Because *S. cerealella* infest maize in the field (Weston et al., 1997), occasional infestation of teosinte seed maturing in the same fields in Mesoamerica cannot be excluded. The fruitcase is an efficient barrier against grain moth infestation. As for the maize weevil the growth rate on teosinte lays within the range of susceptible and resistant maize, while wheat was more susceptible than maize.

3. Insect weight

For maize weevils, small significant differences in adult weight were observed between maize genotypes. The two strains of weevil populations did not differ significantly. Some authors have found that weevils emerging from resistant varieties are heavier (Urrello et al. (1989). But we consider that these differences are too small to be attributed to maize genotypes, and may be due to genetic factors of the weevil parent generation or to other in biotic or abiotic conditions. For the weevil strains the ranks of adult weight per maize genotypes were not the same, and earlier observations showed that such small differences were not repeatable.

Differences were much larger when compared with other cereals and teosinte (Table 1.8). Weight of *S. zeamais* was lower, but not significantly when reared on the same genotype without germ. Baker and Mabie (1973) found that a meridic diet containing wheat germ is of superior nutritional quality and increased the weight of larvae of the granary weevil. Weight of emerged weevils was also slightly lower on teosinte than on maize and much lower still on little spelt and wheat. In spite of their small size the strain that was reared on wheat had the same fecundity.

For *S. cerealella* the differences within maize genotypes were very small as well, with the exception of Cacahuazinte where the mean weight of emerged females was higher. (Table 1.4, 1.9) Weight of adults that emerged from wheat and teosinte was significantly lower and can be explained by the smaller size of the grain.

For *P. interpunctella* a lower weight was observed only for adults emerging from Pop 84 (Table 1.4). For males the difference was not significant. These small differences may not be due to the same resistance factor in Pop 84 that acts against the coleopteran pests, as this pest feeds essentially on the germ.

Conclusion

Selection of maize for resistance to the major storage pests *S. zeamais* and *P. truncatus* may result in maize susceptible to other storage pests, and not bring the expected reduction in post harvest losses. To avoid a shift in storage pests, selection programs should include testing resistant varieties for susceptibility to storage pests of minor importance.

Resistance traits to storage pests are unlikely to be found in wild relatives of maize such as teosinte, due to the dramatic structural changes that have affected the seed during the process of domestication.

The relatively large food range that maize weevils and larger grain borers are able to colonize, and increasing resistance to insecticides shows the high potential of these seed predators to adapt to new situations. In the tropics the large number of insect generations over a storage season will increase the risk of overcoming plant resistance. Therefore utilization of resistant varieties should always be accompanied by other measures to protect grain stores efficiently.

Acceptance of resistant varieties by farmers will be conditioned by high yield, but for subsistence farmers in the tropics other factors such as cooking qualities, taste and easy shelling have to be taken into consideration. A successful reduction of post harvest losses of small scale farmers will have to include these aspects and has to be accompanied by additional measures of post-harvest protection.

Implications for parasitic wasps

One of the factors that can contribute to reduce selective pressure on the pests and slow down adaptation to plant resistance is a synergistic interaction of plant resistance and efficiency of natural enemies (Kennedy et al., 1987).

The smaller number of progeny emerging from resistant maize is likely to decrease the number of parasitic wasps such as *Anisopteromalus calandrae* because their number is regulated by host density.

The longer development time of host larvae on resistant varieties is likely to concern mostly early larval instars that are not suitable hosts, but time of exposure of the instars susceptible to parasitoids might be prolonged and favor parasitism in resistant genotypes. Size of the insects was largely unaffected by maize genotype, suggesting that no nutritional inadequacies are present in resistant genotypes.

Because storage parasitoids of *S. zeamais* and *S. cerealella* have to drill their ovipositor into the maize kernel to paralyze and oviposit on their host and later chew their way out of the kernel as emerging adults, kernel size and kernel hardness could influence detectability and handling of host larvae. Large kernels could offer host larvae a more secure environment by staying out of reach of the ovipositor.

Larval parasitoids of *P. interpunctella*, such as *Bracon hebetor*, prefer to attack hosts that are not hidden (Hagstrum and Smittle, 1977). The more time these spend searching for a new feeding site, the more efficient the parasitoids will be. Consequently, grain with a small embryo or resistant to primary pests may increase parasitoid effectiveness.

Acknowledgements

We thank Dr. J. Berthaud (I.R.D.) and Dr. Alfonso Aguirre (INIFAP) for providing the Oaxacan landraces, Silverio Garcia, Sergio Almacende and Nasario Baleras Garcia for technical help, and Jaqueline Moret for help with statistics. The research was conducted at the laboratory of Entomology at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico.

References

- Arnason, J. T., J. Gale, B. C. de Beyssac, A. Sen, S. S. Miller, B. J. R. Philogène, J. D. H. Lambert, R. G. Fuicher, J. A. Serratos, and J. A. Mihm. 1992.** Role of phenolics in resistance of maize grain to the stored grain insects, *Prostephanus truncatus* (Horn) and *Sitophilus zeamais* (Motsch.). J. stored Prod. Res. 28: 119-126
- Arnason, J.T., B. Baum, J. Gale, J.D.H. Lambert, D.J. Bergvinson, B.J.R. Philogène, J.A. Serratos, J. A. Mihm, and D. C. Jewell. 1994a.** Variation in resistance of Mexican landraces of maize to maize weevil *Sitophilus zeamais*, in relation to taxonomic and biochemical parameters. Euphytica 74 : 227-236
- Arnason, J.T., B. Conilh de Beyssac, B J.R. Philogène, D.J. Bergvinson, J.A. Serratos, and J.A. Mihm. 1994b.** Mechanisms of resistance in maize grain to the maize weevil and the larger grain borer. In: Insect Resistant Maize. Recent Advances and Utilisation. CIMMYT 1994, 91-104
- Avertey, J.N. 1980.** Elimination of *Sitotroga cerealella* (Olivier) by *Sitophilus zeamais* (Motschulsky) from mixed laboratory cultures on maize. Res. Pop. Ecol. 22: 101-116
- Baker, J. E. , and J. M. Mabie. 1973.** Growth responses of larvae of the rice weevil, maize weevil, and granary weevil on a meridic diet. Ann. Entomol. Soc. Am. 66: 681-693
- Buckland P.C. 1981.** The early dispersal of insect pests of stored products as indicated by archaeological records. J. stored Prod. Res. 17: 1-12
- Candura, G.S. 1954.** Notes on *Sitotroga cerealella* in Northern Italy, and other moths infesting stored foodstuffs. Rev. appl. Entomol. Am. 42: 35-36
- Classen, D., J.T. Arnason, J.A. Serratos, J.D.H. Lambert, C. Nozolillo, and B.J.R. Philogène. 1990.** Correlations of phenolic acid content of maize to resistance to *Sitophilus zeamais*, in CIMMYT'S collections. J. Chem. Ecol. 16: 301-315
- Cogburn, R.R. 1974.** Domestic rice varieties: Apparent resistance to rice weevils, lesser grain borers and Angoumois grain moths. Environ. Entomol. 3: 681-685
- Cogburn, R.R. 1977.** Resistance to the Angoumois grain moth in some varieties of rough rice from the USDA World Collection. J. Econ. Entomol. 70: 753-754
- Cogburn, R.R., C.N. Bollich and S. Meola. 1983.** Factors that affect the relative resistance of rough rice to Angoumois grain moths and lesser grain borers (*Sitotrogacerealella*, *Rhyzoperta dominica*). Environ. Entomol. 12: 936-942

- Conhil de Beyssac, B. 1990.** Etude de la susceptibilité du grain de maïs (*Zeam mays*) aux attaques du grand capucin *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae) In: La post-récolte en Afrique. Actes du Séminaire International tenu à Abidjan Côte d'Ivoire 29 janvier au 1^e février 1990 (eds) Foua-Bi and Philogène. 146-137
- Cowley, R.J., D.C. Howard, and R.H. Smith, 1980.** The effect of grain stability on damage caused by *Prostephanus truncatus* (Horn) and on three other pests of stored maize. J. stored Prod. Res. 16: 75-78
- De, D., and P. Samp. 1989.** Quantitative losses in the various kernel fractions of some maize varieties in storage due to *Sitophilus oryzae* Linn. J. entomol. Res. 13: 57-59
- Delobel B., and A.M. Grenier 1993.** Effect of non-cereal food on cereal weevils and tamarind pod weevil (Coleoptera: Curculionidae) J. stored Prod. Res. 29 : 7-14
- Dobie, P. 1974.** The laboratory assessment of the inherent susceptibility of maize varieties to post-harvest infestation by *Sitophilus zeamais* Motsch. J. stored Prod. Res. 10: 183-197
- Dobie, P. 1977.** The Contribution of the Tropical Science Production Center to the study of insect resistance in maize. Trop. Stored Prod. Inf. 34: 7-22
- Dobie, P., C.P.Haines, R.J. Hodges, P.F.Prevett and D.P. Rees. 1991.** Insects and Arachnids of tropical stored Products: Their Biology and Identification . Nationale Ressources Institute
- Eden, W.G. 1952a.** Effect of husk cover on rice weevil damage in Alabama. J. Econ. Entomol. 45: 543-544
- Eden, W.G. 1952b.** Effect of kernel characteristics and components of husk cover on rice weevil damage to corn. J. Econ. Entomol. 45: 1084-1085
- Farrell, G. 2000.** Dispersial, phenology and predicted abundance of the larger grain borer in different environments. African Crop Sci. J. 8: 337-343
- Fortier G., Arnason J. T., Lambert J., Nozzolillo C. and Philogène B.J.R. 1982.** Local and improved corn varieties in small farm agriculture in Belize C. A. Phytoprotection 63: 68-78.
- Galinat W.C. 1977.** The origin of corn. In: Corn and Corn Improvement by Sprague (eds.) American Society of Agronomy 1-48
- Giga, D.P. and Y. Katerere. 1986.** Rural grain storage in Zimbabwe. Problems, loss assessment and prevention. Dep. Crop Sci., Univ. Zimbabwe, Harare, Zimbabwe. 96pp.
- Giga, D. P., U. Moyo , and J. Canhao, 1995.** Contribution of the pericarp of maize

- genotypes to resistance to the maize weevil, *Sitophilus zeamais* (M.). Zim. J. of agr. Res. 29 : 87 –93
- Hagstrum, D.W., and B.J. Smittle. 1977.** Host-finding ability of *Bracon hebetor* and its influence upon adult parasite survival and fecundity. Environ. Entomol. 6: 437-439
- Halstead, D.U.H. 1963.** External sex differences in stored products coleoptera. Bull. Entomol. Res. 54: 119-133
- Harlan, J.R., 1992.** Domestication of Seed Crops. In: Crops and Man by J.R.Harlan. American Society of Agronomy, Inc. Crop Science Society of America Inc. Madison, Wisconsin, USA
- Hidayat P., Phillips T. W., and R.H. French-Constant. 1996.** Molecular and morphological characters discriminate *Sitophilus oryzae* and *S. zeamais* (Coleoptera: Curculionidae) and confirm reproductive isolation. Ann. Entomol. Soc. Am. 89: 645-652
- Hockensmith, P.E., I.L. Devine , D.F. Legg and, J.G. Rodriguez. 1986.** Energy consumption and food utilization of the Indian meal moth (Lepidoptera: Pyralidae) on different corn genotypes. J. Kansas Entomol. Soc. 59 : 598 – 603
- Hodges, R.J., and J. Meik. 1984.** Infestation of maize cobs by *Prostephanus truncatus* (Horn) (Coleoptera :Bostrichidae) aspects of biology and control. J. stored Prod. Res. 20: 205-213
- Holloway, G.J., and R. H. Smith. 1985.** Inheritance of the ability of *Sitophilus oryzae* L. (Col., Curculionidae) to feed and breed on yellow split-peas (*Pisum sativum*). Bull. Entomol. Res. 75: 367-375
- Holloway, G.J. 1985.** The effect of increased grain moisture content on some life history characters of *Sitophilus oryzae* (L.) after staining egg plugs with acid fuchsin. J. stored Prod. Res. 24 : 165-169
- Horber, E. 1987.** Methods to detect and evaluate resistance in maize to grain insects in the field and in storage. In: Toward Insect Resistant Maize for the Third World. Proceedings of the international symposium on methodologies for developing host plant resistance to maize insects. CIMMYT, Mexico, 9-14 March 1987.p 140-150
- Howe, R.W. 1971.** A parameter for expressing the suitability of an environment for insect development. J. stored Prod. Res. 7: 63-65
- Joubert, P.C. 1966.** Field infestations of stored-product insects in South Africa. J. stored Prod. Res. 1: 99-100
- Kennedy, G.G., F. Gould, O.M.B. de Ponti, and R.E. Stinner. 1987.** Ecological, agricultural, genetic, and commercial considerations in the deployment of insect-

- resistant germplasm. Environ. Entomol. 16: 327-338
- Kossou, D.K., N.A. Bosque-Perez, and J.H. Mareck. 1992.** Effects of shelling maize cobs on the oviposition and development of *Sitophilus zeamais* (Motschulsky). J. Stored Prod. Res. 28 : 187-192
- Kumar, T.P., S.N. Moorthy, C.Balagopalan, C.A. Jayaprakas, and P.Rajamma. 1996.** Quality changes in market cassava chips infested by insects. J. stored Prod. Res. 32: 183-186
- Le Cato, G.L.. 1976.** Yield , development and weight of *Cadra cautella* (Walker) and *Plodia interpunctella* (Huebner) on twenty-one diets derived from natural products. J. Stored Prod. Res. 12: 43-47
- Legg, D.E., R.J. Barney, P.W. Tipping, and J.G. Rodriguez.1987 .** Factors influencing the distribution of maize weevil (Coleoptera: Curculionidae) eggs on maize. Environ. Entomol. 16: 809-813
- Levinson, A. , and H. Levinson. 1995.** Reflections on structure and function of pheromone glands in storage insect species. Anz. fur Schaedlingsk. Pflanzensch. Umweltsch. 68: 99-118
- Locatelli, D.P., and L.Limonta. 1998.** Development of *Ephestia kuehniella* (Zeller), *Plodia interpunctella* (Huebner) and *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) on kernels and wholemeal flours of *Fagopyrum esculentum* (Moench) and *Triticum aestivum* L. J. Stored Prod. Res. 34 : 269-276
- Longstaff B.C. 1981.** Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): A critical Review. Prot. Ecol. 2: 83-130
- Markham, R.H., N.A. Bosque-Perez, C. Borgemeister, and W.G. Meikle. 1994.** Developing pest management strategies for *Sitophilus zeamais* (Motschulsky) and *Prostephanus truncatus* (Horn) in the tropics. FAO Plant Protection Bulletin 42: 97-116
- Martinez Prieto, P. and H. Velasco Pascuala. 1982.** Observaciones preliminares sobre la incidencia y daño en el maiz por insectos de los granos almacenados en valles centrales de Oaxaca. Folia Entomologica Mexicana 54 : 66-67
- Meikle, W.G., C. Adda, C. Azoma, C. Borgemeister, P. Degbey, B. Djomamou, and R. H. Markham. 1998.** The effects of maize variety on the density of *Prostephanus truncatus* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) in post - harvest stores in Benin Republic. J. stored Prod. Res. 34: 45-58

- Morrison, R.K. 1985.** Effective mass production of eggs of the Angoumois grain moth *Sitotroga cerealella* (Olivier). The Southwestern Entomologist 8: 28-36
- Muhiu, S.K. 1985.** Depth of infestation by *Sitotroga cerealella* (Olivier) into grain layers of wheat, maize and sorghum. Trop. Stored Prod. Inf. 47: 34-37
- Nang'ayo F.L.O., M.G. Hill, E.A. Chandi, N.V. Nzeve, and J. Obiero. 1993.** The natural environment as a reservoir for the larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera : Bostrichidae) in Kenya. African Crop Science Journal 1: 39-47
- Peters, L.L., M.L. Fairchild, and M.S.Zuber. 1972.** Effect of corn endosperm containing different levels of amylose on Angoumois grain moth biology. 1. Life cycle, certain physiological responses, and infestation rates. J. Econ. Entomol. 65: 576-584
- Plumbley, R.A., and D.P. Rees, 1983.** An Infestation by *Aecerus fasciculatus* (Degeer) (Coleoptera: Anthribidae) and *Decadarchis minuscula* (Walsingham) (Lepidoptera: Tineidae) on stored fresh yam yubers in South-East Nigeria. J. stored Prod. Res. 92-95
- Ramirez Martinez, M.F., A. Noguera, R. Zurbia Flores Rico, E. Moreno Martinez, and A. de Alba Avila. 1991.** Ecologia de *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), el gran barrenador de los granos en selva baja caducifolia y selva mediana subperennifolia evergreen woodland. In (Abstract volume) XXVI Congresso Nacional de Entomologia 12-22 de Mayo de 1991, Veracruz, Veracruz, Mexico. Sociedad Mexicana de Entomologia, 490-491
- Russell, M.P., and R.R. Cogburn. 1977.** World collection of Rice Varieties: Resistance to seed penetration by *Sitotroga cerealella*. J. stored Prod. Res. 13: 103-106
- Sen, A., D.J. Bergvinson, S.S. Miller, J.F. Atkinson, R.G., and J. T. Arnason, 1994.** Distribution and microchemical detection of phenolic acids, flavonoids, and phenolic acid amides in maize kernels. J. Agric. and Food Chemistry 42 :1879-1883
- Scholz, D., A. Tchabi, C. Borgemeister, R.H. Markham, H.M. Poehling, and A. Lawson. 1997.** Host-finding behaviour of *Prostephanus truncatus* (H) (Col.,Bostrichidae) - Primary attraction or random attack. J. Appl. Entomol. 121: 261-269
- Schoonhoven, A.V., E. Horber, and R. B. Mills. 1976.** Conditions modifying expression of resistance of maize kernels to the maize weevil. Environ. Entomol. 5: 163-168
- Schulten, G.G.M. 1976.** Insects in stored maize ears. Abstracts on Tropical Agriculture. Review Article: 9
- Taylor, A.T., R.I. Egwutatu, and W.H. Boshoff. 1978.** Significant Infestation by *Araecerus fasciculatus* Degeer (Coleoptera: Anthribidae) following treatment of maize with

- primiphos-methyl for weevil control. J. stored Prod. Res. 14 : 159-161
- Tigar, B. J., P. B. Osborne, G. F. Key, M. F. Flores-S. , and M. Vasquez A. 1994.** Insect pests associated with rural maize stores in Mexico with particular reference to *Prostephanus truncatus* (Coleoptera:Bostrichidae). J. Stored Prod. Res. 30: 267-281
- Urrelo, R., V. F. Wright, R. B. Mills, and C. E. Wasson. 1989.** Screening for resistance against the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera : Curculioidea) in Peruvian maize accessions. Turrialba 39: 9-17
- Vowoter, K. A., N. A. Bosque-Perez, and J. N. Ayertey. 1995.** Effect of maize variety and storage form on the development of the maize weevil *Sitophilus zeamais* Motschulsky. J. Stored Prod. Res. 31 : 29-36
- Waiss, AC. Jr., B.G. Chan, C.A. Elliger, W.W. Wiseman, N.W. McMillan, M.S. Widstrom, M.S. Zuber, and A.J. Keaster. 1979.** Maysine, a flavone glycoside from corn silks with antibiotic activity toward corn earworm. J. Econ. Entomol. 72 : 256 – 258
- Weston, P.A., R.J. Barney , and X.S. Ge. 1997.** Host-plant characteristics and environmental factors influencing flight activity of Angoumois grain moth (Lepidoptera, Gelechiidae) in the Field. Environ. Entomol. 26: 229 – 233

DIRECT BENEFITS FROM PARASITIC WASPS FOR MAIZE SEED

Abstract

Experiments were conducted to determine the capacity of maize seeds to germinate after being attacked by the common storage pests, the maize weevil, *Sitophilus zeamais* (Coleoptera : Curculionidae) and the Angoumois grain moth, *Sitotroga cerealella* (Lepidoptera : Gelechiidae). Maize kernels of different genotypes were tested if they presented a single emergence hole of either the insect itself, or one of its common parasitoids, *Anisopteromalus calandrae* (Hymenoptera : Pteromalidae), for *S. zeamais* and *Pteromalus cerealellae* (Hymenoptera : Pteromalidae) for *S. cerealella*.

Germination rate on wet filter paper varied from 4 to 73 %, when one maize weevil had emerged. There were significant differences among maize genotypes, large landraces had the highest and small seed and hybrids the lowest germination rate. When the immature maize weevil was killed by a parasitic wasp during development the germination rate was not significantly higher, although weight loss of the kernel was reduced. If one Angoumois grain moth had emerged, the germination rate of damaged kernels on wet paper varied from 15 to 71 %, and there were again significant differences among maize genotypes, large ones rating best. When the immature larvae was killed by a parasitoid the germination rate was significantly higher, and weight loss of individual kernels was reduced.

Germination tests for three genotypes damaged by the Angoumois grain moth were carried out in soil with a natural pathogen flora. The germination rate was lower, but up to 22 % of these seeds still germinated, and the rate rose significantly to 17-54 % if the larvae had

been killed by the parasitic wasp. The action of parasitoids has thus resulted in a direct benefit for the stored seed. Possible evolutionary pressure for traits that facilitate host finding and enhance attack rate of parasitoids in stored maize are discussed.

Key words: Maize, germination, storage, *Sitophilus zeamais*, *Sitotroga cerealella*, *Anisopteromalus calandrae*, *Pteromalus cerealellae*, parasitoids

Introduction

Domestication is an evolutionary process influenced by human activities. Besides deliberate human selection for certain characteristics, harvesting and planting seed in a field leads to automatic selection pressures, in which any modification, that would enhance seed recovery, and competition in the new environment, is favored (Harlan, et al., 1973).

While farmers sometimes select seed for planting at harvest, and store them in safer places than grain for trade and consumption, a common form of selection in Mexico is the continual separation of seed for planting (Smale et al, 1998). In any case the seed for planting is not exempt of attack by storage insects until the next planting season. Only those seeds which are undamaged or can tolerate damage will germinate and hence are subjected to automatic selection for post harvest resistance. Plant characteristics may not only affect the pest insects, but also the colonization and effectiveness of their natural enemies. Modifications that enhance parasitoid efficiency will evolve only if a plant with such a trait is more likely to contribute to the next generation than others. For a seed such a benefit would only exist if germination were markedly higher after the host of an attacked grain has been parasitized.

Plant resistance as defined by Painter (1951) is divided up into three categories, antibiosis, non preference and tolerance. Antibiosis and non preference resistance describe the reaction of an insect to a plant's secondary metabolites. Induced resistance is a direct response of a plant to an attacking organism by enhancing its defense (Kogan and Paxton 1983). In the case of seed no defense can be induced by insect attack as in the vegetative stage of the plant, any resistance factors have to be accumulated during maturation of the grain.

Tolerance is defined as the capacity of a resistant plant to withstand or recover from injury by insects. It has been suggested that plant tolerance does not exist in stored grain, because the relatively inert seed cannot regrow nor repair, hence any damage is terminal

(Horber; 1987). This may be based on the fact that any loss through seed predators results in direct loss for trade and consumption by man and livestock, amplified by lower nutritional quality.

The plant produces seeds with the sole purpose of reproduction. From this point of view, tolerance against seed predators exists if the seed is still able to germinate and generate a new plant after suffering damage. Germination being defined as emergence and development from the seed embryo of the essential structures, which for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions (Anonymous 1970). Beans have been shown to germinate after attack and emergence of the bruchids *Acantocelides fasciculatus* (Baier and Webster, 1992), and *Zabrotes subfasciatus* (Kassa, 1993).

In maize, internally developing larvae of the maize weevil (*Sitophilus zeamais*) and the Angoumois grain moth (*Sitotroga cerealella*) feed mostly on the endosperm, at the interface of germ and endosperm (Vowoter et al 1995; Kossou et al., 1992). If the embryo is left relatively intact, the capacity to germinate is not necessarily lost. Seed viability of weevil damaged maize is usually tested with samples that have been exposed to insects for a variable amount of time, but damaged and undamaged grains are not germinated separately (Mora and Petersen, 1976).

Solitary ectoparasitoids that attack and kill larvae of these storage pests can reduce the severity of grain damage (Sanon et al., 1998; Wen and Brower, 1994). This could enhance the capacity of the seed to germinate after insect attack. Biological controls are part of a plant's battery of defenses against herbivores (Price et al., 1980). If the embryo is left intact to germinate, it is important that the endosperm still contains enough energy for seedling establishment in a competitive environment with other seedlings as well as weeds (Harlan, 1992). The emergence holes of parasitoids are much smaller than those of their insect hosts

(Ghani and Sweetman, 1955) and even more likely to be overseen by the farmer at planting time. One hypothesis is that there exists a direct benefit from parasitoid attack which would put an automatic selective pressure on maize to favor traits that enhance parasitoid success.

A major challenge for a seed at germination is to fight off the invasion of soil borne pathogens. A damaged seed is much more vulnerable to pathogens because the protective pericarp is no longer intact. Germination tests on paper are ideal to study the germination potential after damage by different insects. However, standard germination tests are inadequate to completely describe seed quality because constructed under ideal conditions. Field conditions seldom approximate the ideal, more often some form of stress conditions prevails (Delouche, 1973). Therefore, to be representative of field conditions, the ability to germinate in soil with a normal pathogen fauna is the condition under which tolerance to insect attack, or any direct benefit that could result from parasitic wasps, should be evaluated.

The objectives of this work were first, to find out if there are any maize varieties that can tolerate an attack by common storage insects, like the maize weevil (*S. zeamais*) or the Angoumois grain moth (*S. cerealella*), and can still generate a new plant after such an attack. Secondly, we wanted to determine if there is any direct benefit for an infested seed when the herbivore it contains is attacked by a parasitic wasp, in terms of reduced weight loss, and increased germination.

Material and methods

The maize weevil, *Sitophilus zeamais*, and the Angoumois grain moth, *Sitotroga cerealella*, were reared as described in Chapter 1. The parasitic wasps originated from the same localities as their hosts. *Anisopteromalus calandra* was reared on mature maize weevil larvae, and *Pteromalus cerealella* on 23 day old moth larvae in white (CML 244 x CML349) or yellow (CL02420 x CML287) hybrid maize. Maize varieties were provided by CIMMYT or local farmers in Oaxaca (Mexico) (Table 2.1).

Table 2.1 : Maize varieties used

Variety	Abr.	type	Climate, Origin	provided by	Resistance
Cacahuazintle	<i>Ca</i>	landrace	highland	CIMMYT	susceptible
CML 264 x CML 254	Hy w	hybrid	tropical	CIMMYT	susceptible
CML 287 x CML 285	Hy y	hybrid	tropical	CIMMYT	susceptible
CL02400 x CML 287	Hy G	hybrid	tropical	CIMMYT	susceptible
CML 244 x CML 349	Hy R	hybrid	highland	CIMMYT	susceptible
Multiple borer resistant	MBR	population	subtropical	CIMMYT	susceptible
Cuba/Caribbean accesions	Pop 84	population	tropical, Cuba	CIMMYT	resistant
Ejura	Pop 80	population	tropical, Africa	CIMMYT	mod. res.
Sinaloa 35	Sin	landrace	tropical	CIMMYT	resistant
Yucatan 7	Yuc	landrace	tropical	CIMMYT	mod. res.
174 Roagua A.	Ox 174	landrace	Oaxaca	farmer	mod. res.
184 Valdeflores	Ox 184	landrace	Oaxaca	farmer	susceptible

Abr. abbreviations used. Varieties were from Mexico, unless otherwise mentionned; Ox: originating from the state of Oaxaca (Mexico); mod. res. : moderately resistant

A. Kernel weight loss

Sitophilus zeamais

Kernels of a yellow tropical hybrid CL0 2420 x CML 287 (Hy G) were infested for two days with unsexed two- to three- week old adult weevils. After discarding the weevils, kernels were stained with the acid fuchsin stain (Frankenstein 1948), or gentian violet (Goossens 1948), as described by Horber (1987), and examined under a stereo microscope. Kernels with two or more egg plugs were discarded. All kernels with only one egg plug were separated to dry for 24 hours at ambient temperature (22° C) and humidity (40% RH) in the laboratory,

and then for four more days in the incubation chambers; at 26.5° C and 75% RH. An uninfested control sample was found to recover its initial weight from before the stain after this period of time, and the larvae have not hatched yet. According to Sharifi and Mills (1971) the average incubation time before hatching is 6.5 day. The kernels were then individually weighted and placed separately into the cavities of micro-titer plates, covered, and left undisturbed. After 24 days the plates were placed in cages without cover and exposed to a large number of parasitic wasps (*Anisopterus calandreae*) for three days. After this period the plates were again securely covered to avoid the escape of any insect. From day 32 onwards they were checked daily for any emerging insects, which were removed, noted and wasps were sexed. At 55 days after infestation all kernels with one insect emergence were weighted again and weight loss was noted for seed from which a single *S. zeamais* or *A. calandreae*, either male or female, had emerged. Thirty uninfested kernels with no emergence (but with an egg plug) were also weighted.

Sitotroga cerealella

Kernels of the yellow hybrid CL0 2420 x CML287 (Hy G) were infested with an equal number of eggs of *S. cerealella* on wax paper strips, as described in Chapter 1. After five days of oviposition each kernel was examined for entry holes under a stereo microscope and each infested seed was weighted and incubated individually in a micro-titer plate. After 23 days, two thirds of the micro-titer plates were exposed to a large number of female parasitic wasps (*Pteromalus cerealellae*) for two days. The plates were then securely covered and emergence was recorded from day 31 onwards, every 5 days (adult moths do not feed and will not alter the weight of the seed after emergence). At 55 days after infestation all kernels with one emergence hole were individually weighed again, and weight loss was calculated for seeds with emerged *S. cerealella* or *P. cerealellae*, either male or female.

B. Germination

Germination tests were carried out to evaluate the potential of maize seed to germinate after damage and by which factors the germination rate was influenced.

Maize varieties used were those from the experiments for resistance to storage pests (Table 2.1), representing a range of resistance against either of the insect pests used in these experiments (Chapter 1). Varieties were of different origins, including modern hybrids as well as landraces and populations selected for insect resistance. The yellow hybrid, CL0 2420 x CML287 (Hy G), used for the weight loss experiment was also subjected to germination tests.

Sitophilus zeamais

The maize genotypes were infested with adult *S. zeamais* for several days in vials containing 50 kernels, and exposed to *A. calandreae* when most host larvae were in the susceptible fourth larval instar (Smith, 1993). This time varied depending on the degree of resistance of the genotype. Emerging insects were retrieved daily and the samples were frozen after 60 days for four days. Kernels which presented one emergence hole of either *S. zeamais* or a parasitic wasp were selected and used for germination tests. Wasp emergence holes are easily distinguishable because of their small size and circular shape. Any kernels with more than one emergence hole or other feeding damage were discarded.

Sitotroga cerealella

For the Angoumois grain moth, samples of 40 kernels of each genotype were infested with eggs, and exposed to *P. cerealellae* at 22 to 24 days after infestation, when the first emergence “windows” appear. At this time the most developed larvae are fully grown and start excavating their pupal chamber. *P. cerealellae* prefers to parasitize large larvae (Wen et al., 1995). Larger samples of Cacahuazinte, CML264xCML254 and MBR were infested for

germination tests in soil. After emergence of the insects, kernels were frozen, to eliminate a second insect generation, and those presenting single emergence holes of either *S. cerealella* or *P. cerelellae* were separated and stored at ambient conditions until the germination tests were carried out. Infestations and subsequent germination tests for varietal differences were carried out over a period of 18 months.

Germination on paper

Kernels were frozen for four days, to eliminate a second insect generation, and germination tests were carried out within two months after emergence. Until this time they were stored in the laboratory under ambient conditions. Uninfested grain was subject to the same treatments in terms of storage in incubation chambers and freezing. Kernels were neither disinfected nor washed nor did they receive any treatment to avoid microbial or fungal infestation. For the germination test they were placed on wet filter paper in lots of 25, rolled up and incubated at 30°C and 90 % relative humidity. The seed were considered viable when both radicle and folicule had emerged within 5 days. Sixty kernels with one emerged weevil from the weight loss experiment were germinated individually to correlate weight loss with the germination potential.

Wasps emerging (*P. cerealellae*) from the weight loss experiment were separated into male or female, and emergence hole location was recorded (in the germ or in the endosperm). Each of the four groups were germinated separately to evaluate the importance of wasp size and location of emergence site on germination rate.

Germination in soil

Soil germination tests were conducted using untreated alluvial field soil from CIMMYT's experimental station in Pose Rica Veracruz, Mexico (N 20.49, W 97.54).

For this experiment kernels of three genotypes, MBR, Cacahuazintle and CML264x CML 254, were subject to insect infestation (*S. cerealella* and *P. cerealellae*) as above, and tested for germination under ambient conditions (min 11°C, max 26°C) in boxes with soil. The seed were placed at a depth of 4 cm and germination was evaluated three weeks after planting. Each box contained ca 50 insect-damaged seeds, and 7 uninfested kernels as controls.

Effect of time on germination rate of damaged kernels

Kernels of hybrid C CML 264xC CML 254 presenting emergence holes of either *S. cerealella* or its parasitic wasp were stored for one year in the laboratory before the germination test was carried out, to determine the effect of time on damaged seed. They were compared with kernels of the same lot that had been infested at the same time, but stored for less than two months.

The germination rate was calculated on the total number of seed tested for each treatment. For standard errors, t-tests and analyses of variance only complete lots of 25 seeds were considered. The total of germinated kernels for each genotype and insect varied considerably, due to availability and resistance to storage pests on one hand, and low visibility of small exit holes on some varieties on the other. Germination tests were accompanied by testing kernels not exposed to insects, but otherwise maintained at the same conditions (incubation) as infested kernels.

A total of almost 7000 kernels were tested.

Results and Discussion

A. Weight loss

Sitophilus zeamais

Weight loss per grain was 24.1mg when one maize weevil emerged, and it was reduced significantly ($P>0.0001$), by one third (17.1mg) when the larvae was parasitized, and a female wasp emerged; and even more when a male wasp emerged (13.3 mg). (Fig. 2.1 and Table 2.2). All measurements varied considerably (Fig. 2.1). For the maize weevil this can be explained by feeding of the pre-emerged and emerging adult, but the mean weight loss is close to the one observed by Adams (1974) (25.8 mg).

The average larval development time is about 34 days at the experimental conditions, but there can be considerable variation among individuals. Therefore, at the time of exposure to the wasps, the majority of the host larvae were in their fourth stage, but many were smaller, and some might have already pupated. After oviposition by the parasitoid, the larva is paralyzed and its development arrested, so that no further damage occurs to the grain. *A. calandreae* prefers to oviposit on large hosts rather than on pupae and small larvae (Smith, 1993; Ghani and Sweetman, 1955) and the difference of weight loss when a female or male wasp emerges confirms the findings of van dem Assen (1984) that fertilized eggs (resulting in female offspring), are allocated to large hosts, that have already considerably damaged the seed, while unfertilized eggs (male offspring) are laid on smaller host larvae. Some very low values of weight loss for emerged male wasps indicate that the host larvae were very small at the time of parasitization. When no insect emerged, weight loss was measurable only in less than 10 % of the cases, confirming that most larval mortality of maize weevil larvae occurs at an early stage (Birch, 1945; Longstaff, 1981).

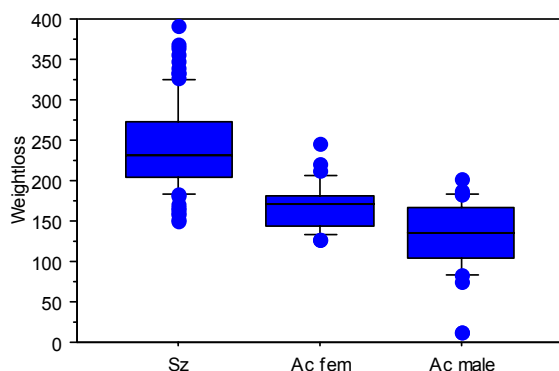


Fig. 2.1

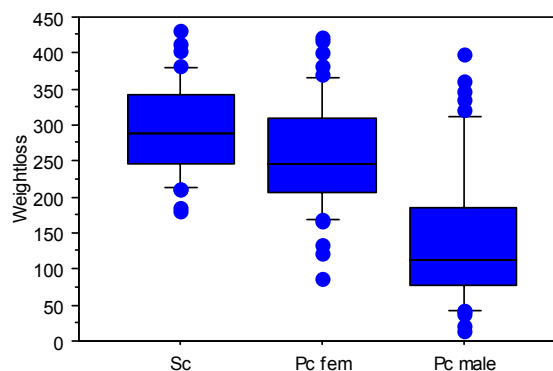


Fig. 2.2

Fig. 2.1: Mean weight loss $g \cdot 10^{-4}$ per maize kernel after emergence of a maize weevil or its parasitoid

Sz: one maize weevil, *Sitophilus zeamais*; Ac fem, Ac male: a female, or male parasitic wasp, *Anisopteromalus calandrae*.

Box plot showing 10th, 25th, 50th(mean), 75th, and 90th percentiles, and outliers for weight loss .
ANOVA showed significant differences: $df=2, 155 F= 78.8 P<0.0001$

Fig. 2.2 : Mean weight loss $g \cdot 10^{-4}$ per maize kernel after emergence of one Angoumois grain moth or its parasitoid

Sc: Angoumois grain moth, *Sitotroga cerealella* ; Pc fem Pc male:its parasitoid: *Pteromalus cerealellae*, either female or male

Box plot showing 10th, 25th, 50th(mean), 75th, and 90th percentiles, and outliers for weight loss.
ANOVA showed significant differences: $df=2, 132 F= 44 P<0.0001$

Table 2.2 : Weight loss ($g \cdot 10^{-4}$) of maize kernels after emergence of a seed predator, or its parasitoid.

Insect emerged	Mean Weight loss	Min. value	Max. value	Count
<i>S. zeamais</i>	241.1 ± 5.6 a	150.0	391.0	88
<i>A. calandrae</i> ♀	170.5 ± 4.9 b	127.0	245.0	33
<i>A. calandrae</i> ♂	133.0 ± 7.3 c	13.0	202.0	37
<i>S. cerealella</i>	293.6 ± 10.2 x	181.0	432.0	40
<i>P. cerealellae</i> ♀	258.3 ± 11.2 y	86.0	423.0	47
<i>P. cerealellae</i> ♂	141.9 ± 13.7 z	13.0	398.0	48

Mean Weight loss: ($g \cdot 10^{-4}$) of individual maize kernels through development of one maize weevil, *Sitophilus zeamais*, or its parasitoid, *Anisopteromalus calandrae*; or one Angoumois grain moth, *Sitotroga cerealella* or its parasitoid, *Pteromalus cerealellae*.

Min. value, Max value : lowest and highest weight loss measured for each insect

ANOVA showed significant differences: $df=2, 155 F= 78.8 P<0.0001$ for the maize weevil and its parasitoid;
and $df=132 F= 44$ LSD 18.1 $p<0.0001$ for the Angoumois grain moth and its parasitoid. Numbers followed by the same letter are not significantly different for Fishers protected LSD.

Sitotroga cerealella

The findings were quite similar for other host parasitoid assemblages, *S. cerealella* and *P. cerealellae* (Fig. 2.2 and Table 2.2). For kernels infested by these insects, weight loss is higher because grown moth larvae are much larger than grown weevil larvae. The weight difference between seeds from which a host, and those from which a female wasp emerged were much smaller than those observed for the maize weevil, but still significant ($P = 0.043$). Adult grain moths do not feed, so when a fully grown host larvae is paralyzed by a parasitic wasp, damage is only reduced in terms of emergence hole size. However, weight loss was reduced by half if a male wasp emerged. Again, female offspring are allocated to larger hosts to obtain optimal reproductive prospects, and *P. cerealellae* prefers to oviposit on large hosts if available (Wen et al., 1995). Therefore male wasps emerged from small hosts.

Development of moth larvae was not well synchronized; some larvae were still small at the time of parasitization. This explains the small mean weight loss for emerged male wasps and the large variation for emerged wasps (Fig. 2.2).

Emerging grain moths differ in body weight. Females, on average, are much heavier than males; which explains the variation of weight loss for kernels.

B. Germination

Sitophilus zeamais

The germination rate within a variety was significantly different ($P=0.05$, t-test) for damaged and undamaged kernels (Table 2.3, Fig. 2.3). Generally more kernels germinated when a parasitoid had emerged instead of a maize weevil, but the difference was not significant between varieties.

There was no significant difference between weight loss of kernels that germinated and those that did not (t-test: $df=57$, $P=0.7$). Thus the ability to germinate was independent of the amount of weight loss caused by the maize weevil, indicating that feeding was more important on kernel fractions that are not relevant for germination. However emergence of more than one maize weevil will very likely reduce germination rate further as observed by Kassa (1993) for the bruchid *Zabrotes subfasciatus*.

Analyses of variance for germination rate of damaged kernels was significantly different between varieties. Larger kernels and landraces were more likely to overcome feeding damage and germinate. An explanation could be the spatial limitation imposed on weevil feeding within a small seed that leads to more damage to the embryo, while in large grains only a small part of the embryo is damaged. Although parasitic wasps lower damage to the grain by arresting the development of their hosts, we observed that they frequently emerge by chewing their way out through the embryo rather than through the endosperm which could undo the benefit the seed has obtained by their action. It is also possible that some small emergence holes in the endosperm area, had been overlooked, and so possibly the tested seeds contained a higher proportion of parasitoids that emerged through the germ. Some varieties may have been more affected than others by the storage conditions in the incubation chamber under high humidity ($>80\%RH$).

No germination tests were made in soil as the overall germination rate on paper under ideal conditions was considered too low to withstand soil pathogens. Moreover, the germination tests using filter paper failed to show direct benefit from parasitism by wasps.

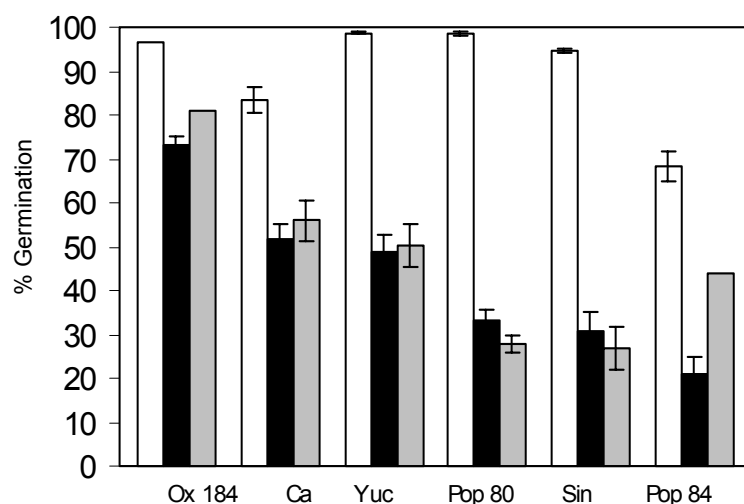


Fig. 2.3

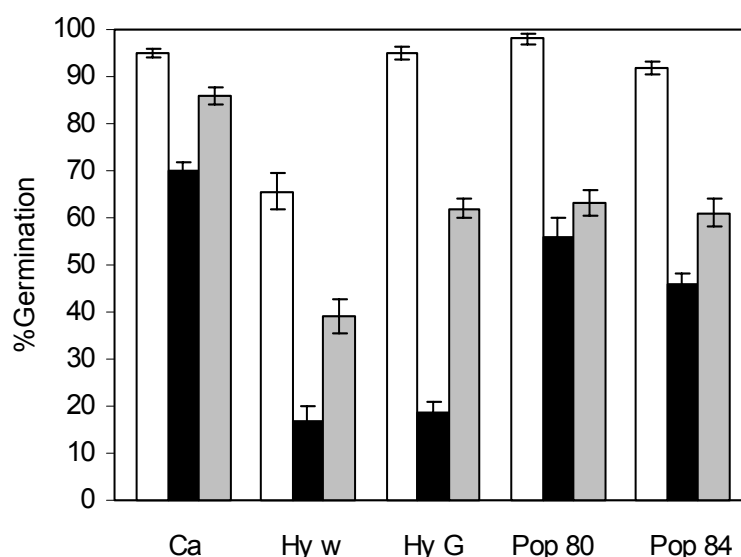


Fig. : 2.4

Fig. 2.3 : Germination of seeds of different maize varieties on paper after damage by *Sitophilus zeamais* or its parasitoid

Black: after emergence of one maize weevil, *Sitophilus zeamais* ; Grey: after emergence of a parasitic wasp, *Anisopteromalus calandrae* ; White: undamaged kernels

Means were analysed with ANOVA and compared with Fisher's protected PLSD. Germination rate for less than 50 kernels were not included in the analyses T-tests were used to compare germination rates of kernels with Sz emergence or Ac emergence within maize genotype, but no significant difference was found.

Fig. 2.4 : Germination of seed of different maize varieties on paper after emergence of one Angoumois grain moth, *Sitotroga cerealella*, or its parasitoid

Black: one *S. cerealella* emerged; Grey: one parasitoid, *Pteromalus cerealellae* emerged; White: undamaged seed. Only genotypes with a total of more than 350 kernels tested are shown.

Means were analysed with ANOVA and compared with Fisher's PLSD. Germination rate for less than 50 kernels were not included in the analyses.

T-tests were used to compare germination rates of kernels with host (Sc) emergence or parasitoid (Pc) emergence within maize genotype. The following varieties showed significant differences: Hy G $P < 0.0001$; Ca $P = 0.005$; Hy w $P = 0.03$

Table 2.3 : Germination rate on paper, of maize kernels after emergence of one *Sitophilus zeamais* or its parasitoid, *Anisopteromalus calandrae*.

Maize Variety	% germination undamaged seed	% germination <i>S.zeamais</i> emerged	%germination <i>A. calandrae</i> emerged	% germination adjusted values		Number tested		
				<i>Sz</i>	<i>Ac</i>	undamaged	<i>Sz</i>	<i>Ac</i>
Ca	83.5 ±5.6 ab	51.8 ±6.5 c	55.9 ±9.3 a	62.0	67.0	194	224	102
Yuc	98.7 ±0.8 a	48.7 ±7.7 c	50.4 ±9.6 a	49.3	51.0	150	150	139
Pop 80	98.5 ±0.7 a	33.1 ±4.7 de	27.8 ±4.1 b	33.6	28.3	150	359	176
Hy	92.1 ±2.5 a	41.1 ±8.0 cd	51.8 ±8.0 a	44.6	56.3	176	56	56
G								
Sin	94.7 ±1.3 a	30.7 ±9.1 e	27.9 ±9.9 b	32.5	27.9	150	205	105
Pop 84	68.1 ±7.6 b	20.8 ±8.2 def	43.8	30.6	64.2	160	120	48
Hy w	49 ±8.8 c	3.6 ±1.3 def	6.0 ±1.2 c	7.2	12.2	100	169	100
Ox174	96 ±1.6 a	87.7 ± 6.0 a		91.4		100	73	
Ox 184	96	73.3 ±3.5 b	81.0	75.9	83.9	29	157	21

undamaged : percentage of kernels that germinated when not infested with maize weevils; *S.zeamais* : percent kernels that germinated with one *S. zeamais* emerged; *A. calandrae* : percent kernels that germinated with one *A. calandrae* emerged; adjusted values : germination rate of insect damaged kernels adjusted to the germination rate of undamaged kernels

Means were analyzed with ANOVA and compared with Fisher's protected LSD. Germination rate for less than 50 kernels were not included in the analyses. Means in the same column followed by the same letter are not significantly different. T-tests were used to compare germination rates of kernels with *S. zeamais* emergence or *A. calandrae* emergence within, but no significant difference was found.

Table 2.4 : Germination rate on paper, of kernels after one emergence of *Sitotroga cerealella* or its parasitoid, *Pteromalus cerealellae*

Variety	% germination undamaged seed.	% germination <i>S. cerealella</i> emerged	%germination <i>P. cerealellae</i> emerged	%germination adjusted values		Numbers tested		
				<i>Sc</i>	<i>Pc</i>	undamaged	<i>Sc</i>	<i>Pc</i>
Ca	95.0 ±1.9 a	70.5 ±3.2 a	82.5 ±3.4 a	74.2	86.8	100	200	150
Pop 80	98.0 ±2.0 a	56.0 ±7.8 ab	63.0 ±5.4 b	57.1	64.3	50	100	200
Pop 84	91.7 ±2.6 a	46.0 ±4.5 bc	61.8 ±5.7 b	50.2	67.4	300	200	280
Sin	76.0 ±13 a	46.0 ±6.0 bc	28.7 ±7.4 d	60.5	37.8	100	50	150
Hy G	95.0 ±2.5 a	18.7 ±4.0 d	62.0 ±4.3 b	19.7	65.3	100	150	250
Hy R	98.0 ±2.0 a	36.0 ±4.0 b-d	51.0 ±9.7 bc	36.7	54.0	50	50	200
Hy y	93.0 ±1.9 a	25.0 ±8.1 cd	44.0 ±12b-d	26.9	47.3	100	100	50
Hy w	65.5 ±7.8 b	15.2 ±5.7 d	32.1 ±7.2 d	23.2	49.0	200	223	218
MBR	96.0 ±4.0 b	24.0 ±3 d	81.0 ±5 a	25.0	84.4	50	250	150

Percentage of kernels that germinated that were either undamaged (undamaged); with one *S. cerealella* emerged (*Sc*); or with one *P. cerealellae* emerged (*Pc*)

Adjusted values. : germination rate of insect damaged kernels based on the germination rate of undamaged kernels. Means were analysed with ANOVA and compared with Fisher's PSLD. Germination rate for less than 50 kernels were not included in the analyses.

Means in the same column followed by the same letter are not significantly different.

T-tests were used to compare germination rates of kernels with *S. cerealella* emergence or parasitoid (*P. cerealellae*) emergence within maize genotype. The following varieties showed significant differences: Hy G $P < 0.0001$; Ca $P = 0.005$; Hy w $P = 0.03$

Sitotroga cerealella

For *S. cerealella* and its parasitoid *P. cerealellae*, germination of damaged kernels was generally higher than for *S. zeamais* (Fig. 2.4), and there were significant differences among genotypes (Table 2.4). T- tests for germination rate within maize genotypes showed significant differences in germination rates between undamaged and damaged kernels and between the genotypes tested. Kernels with parasitoid emergence germinated better than those with emerged grain moths (Hy G: $P < 0.0001$; Ca : $P = 0.005$ Hyw : $P = 0.03$) (Table 2.4, Fig. 2.4). Only in one variety, Sinaloa 35, the reverse was true. The best germination rate was observed for a large land race, Cacahuazintle, followed by some relatively small land races. The tropical hybrids rated lowest.

When seeds of Hy G (CL02400xCLM287) were tested separately, those with male parasitoid emergence or emergence through the endosperm had significantly higher germination rate (Fig. 2.5) than females emerging through germ (Fisher's exact test: $P < 0.0001$). This shows that additional damage due to emergence of parasitoids through the embryo lowers the chance of germination. No other investigations for site of parasitoid emergence were made, except for this experiment where emergence through the germ represents almost half of the cases. Emerging wasps may be forced to choose this emergence

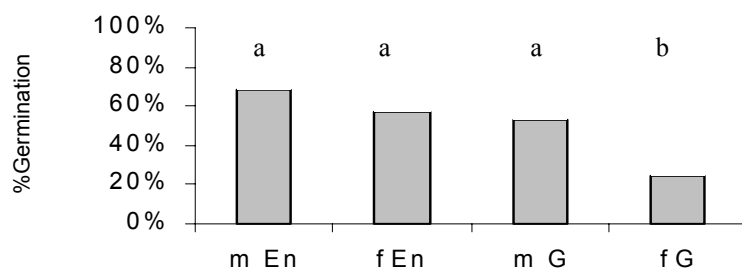


Fig. 2.5 : Germination rate depending on sex and location of emerged parasitoid, *Pteromalus cerealellae*

mEn : male wasps emerged through endosperm; fEn : female wasps emerged through endosperm; mG male wasps emerged through the germ; fG : female wasps emerged through the germ of the seed. Numbers were examined with Fishers exact test. Seeds fG with a female wasp emerged through the germ had a significant lower germination than all others (Fishers exact p-value $P = 0.01$ (with mG), $P = 0.006$ (f En), $P < 0.0001$ (m En).

There were no significant differences among the others.

route, as the endosperm hardness varies with maize genotype (Arnason et al., 1994). This could explain the exceptional results for Sinaloa 35 where seed with moth emergence germinated better than those with wasp emergence. In addition, this grain is a dark red colour and small emergence holes are not easy to locate in the endosperm. This could have led to a higher number of seeds with female emergence through the endosperm in the samples tested, and consequently to a lower germination rate than if all seed with wasp emergence would have been tested.

Germination in soil

Because the germination rate of damaged kernels was high and the action of parasitic wasps improved the germination potential, germination tests using soil were carried out for the three genotypes (Table 2.6 and Fig. 2.6). A large proportion of seeds with wasp emergence managed to generate new plants despite the presence of soil pathogens (Table 2.5). These seedlings appeared at the same time as those from healthy seed and did not look any weaker. One fifth of the seed with moth emergence germinated for genotype Cacahuazintle, and soil tests accentuated the difference in germination rate between kernels with pest damage and parasitoid emergence. These results indicate that a seed that is attacked by a storage insect can tolerate this damage if attacked by a parasitic wasp, and in the case of Cacahuazintle even without the assistance of a parasitoid. But there is a clear benefit for the

Table 2.5: Soil pathogens

Helminthosporium spp.

Fusarium spp.

Pithium spp.

Macrophomia phaseoli (Charcoal Stalk Rot)

CIMMYT experimental station of Poza Rica

(Veracruz, Mexico) Data provided by CIMMYT

seed in terms of fitness, resulting from

parasitoid attack, and evolutionary pressure

may not only favor plant resistance to insects,

but also traits that enhance parasitoid

effectiveness.

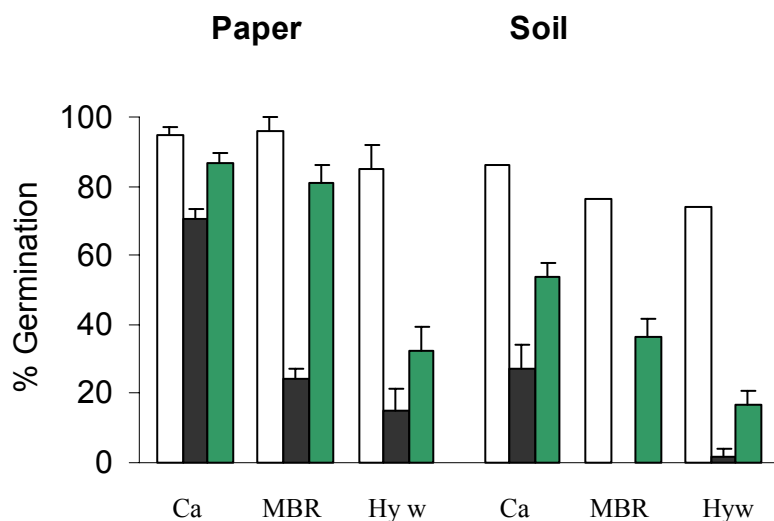


Fig. 2.6 : Germination in soil of maize kernels damaged by *Sitotroga cerealella*, or its parasitoid, *Pteromalus cerealellae*

Three maize genotypes were tested on wet filter paper and in soil.

Black : one *S. cerealella* emerged; Green : one *P. cerealellae* (wasp) emerged; White: undamaged controls

Germination rates of kernels with insects emerged compared by t-tests within maize genotype were all significant. Ca: P=0.035 MBR: P=0.031 Hyw: P=0.026

Table 2.6 : Germination in soil of kernels damaged by *Sitotroga cerealella* or its parasitoid, *Pteromalus cerealellae*

Variety	Damage by	Paper		Soil	
		% germination	Number tested	% germination	Number tested
Hy w	undamaged	85.0±7	100	74.0	50
	<i>S.cerealella</i>	15.2±6	223	2.0 ±2	100
	wasp	32.1±7	218	16.7±4	150
MBR	undamaged	96.0±4	50	76.2	21
	<i>S.cerealella</i>	24.0±3	250	0	150
	wasp	81.0±5	100	36.4±5	165
Ca	undamaged	95.0±2	100	86.0	50
	<i>S.cerealella</i>	70.5±3	200	27.0±7	100
	wasp	86.5±3	150	54.0±4	150

% g : Mean germination rate of three genotypes tested on wet filter paper or in soil. *S.cerealella* : kernels with one *S. cerealella* emerged; wasp : kernels with one wasp (*P. cerealellae*) emerged; undamaged grain was used as control.

Results reported for the wet filter paper tests are only those that were carried out simultaneously with the tests in soil. Numbers of Sc and Pc emerged kernels were compared by t-tests, they were all significant. Ca: P=0.35 MBR: P=0.031 Hyw P=0.026

Effect of time on germination rate of damaged kernels

The ability of a damaged seed to germinate will not last very long (Fig 2.7). Only few damaged seed germinated when they had been stored for a year after insect emergence. Air entering through the emergence hole might lead to accelerated deterioration of the embryo. In storage, damaged grain is attacked by secondary pests which feed on the germ and may prevent germination. Only grain that has been damaged recently will be able to generate a new plant.

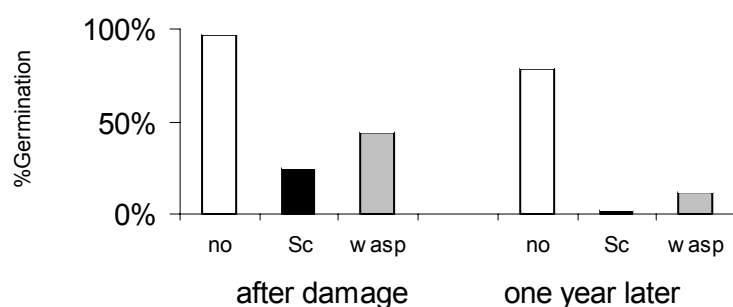


Fig. 2.7 : Effect of time on germination rate of insect damaged grain .

no : undamaged; Sc : one *Sitotroga cerealella* emerged; wasp : one *Pteromalus cerealellae* emerged.

Two way factorial analyses of values, adjusted to the germination rate of undamaged kernels, showed significant interaction of time with type of damage
 $F=10.0$ $P=0.002$ $LSD=12.9$

Conclusion

The objective of this experiment was to find out if domesticated maize seed is subjected to automatic selection pressures that favor modifications which would enhance the effectiveness of parasitic wasps. In the case of the maize weevil and one of its parasitoids, *A. calandrae*, this could not be shown, as no significant advantage resulted from parasitization of weevil larvae despite less grain weight loss in this case. However, for a seed attacked by the Angoumois grain moth there is a clear benefit to be obtained from parasitic wasps, both in terms of reduced grain weight loss and enhanced germination. Selection pressures are likely to favor traits that facilitate host finding and handling.

In both cases, damage by these primary pests concerns mostly the endosperm, and germination potential is still good after insect emergence from the seed. Tolerance to insect damage differs significantly among maize varieties. In regions with high insect pressure, natural selection will not only favour traits for insect resistance, but also resistance to soilborne pathogens that may invade damaged seed.

Acknowledgements:

We thank Dr. J. Berthaud and Dr. Alfonso Aguirre for providing the maize genotypes from Oaxaca, and Nasario Baleras Garcia and for technical help. We are grateful to Jacqueline Moret for statistics assistance, and Jan Hirabayashi for help with identification of the parasitoids. The research was conducted at the laboratory of Entomology at the International Maize and Wheat Improvement Center.

References

- Adams, J. M. 1976.** Weight loss caused by development of *Sitophilus zeamais* Motsch. in maize. J. Stored Prod. Res. 12: 269-272.
- Anonymous. 1970.** Rules for testing seeds. Proc. Assoc. Off. Seed Anal. 60 (2)
- Arnason, J.T., B. Baum, J. Gale, J.D.H. Lambert, D.J. Bergvinson, B.J.R. Philogène, J.A. Serratos, J.A. Mihm, and D.C. Jewell, 1994.** Variation in resistance of Mexican landraces of maize to maize weevil *Sitophilus zeamais*, in relation to taxonomic and biochemical parameters. Euphytica 74 : 227-236
- Baier A.H., and B.D. Webster, 1992.** Control of *Acanthoscelides obtecus* Say (Coleoptera: Bruchidae) in *Phaseolus vulgaris* L. seed stored on small farms-II. Germination and cooking time. J. Stored Prod. Res. 28 : 295-299
- Birch, L.C. 1945.** The mortality of the immature stages of *Calandrae oryzae* and *Rhyzopertha dominica* Fab. in wheat of different moisture content. Aust. J. Exp. Biol. Med. Sci. 23: 141-145
- Delouche, J.C. 1973.** The problem of vigor. I. A look at the germination test. Seedsmen's Digest 24, 8-24
- Dobie, P. 1977.** The contribution of the Tropical Science Production Center to the study of insect resistance in maize. Trop. Stored Prod. Inf. 34: 7-22
- Frankenfeld, J.C. 1948.** Staining methods for detecting weevil infestation in grain. USDA Bureau of Entomology and Plant Quarantine. ET Series No.256.
- Ghani, M.A., and H. L. Sweetman. 1955.** Ecological studies on the granary weevil parasite, *Aplastomorpha calandrae* (Howard). Biologia (Lahore)1 :115 – 139
- Goossens, H.J. 1949.** A method for staining insect egg plugs in wheat. Cereal Chemistry 26, 419-420
- Harlan, J.R., J.M. J. de Wet, and E. Glen Price. 1973.** Comparative evolution of cereals. Evolution 27: 311-325
- Harlan, J.R. 1992.** Domestication of Seed Crops. In: Crops and Man by J.R. Harlan. American Society of Agronomy, Inc. Crop Science Society of America Inc. Madison, Wisconsin, USA
- Horber, E. 1987.** Methods to detect and evaluate resistance in maize to grain insects in the field and in storage. In: Toward Insect Resistant Maize for the Third World.

- Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects. CIMMYT, Mexico, 9-14 March 1987.p 140-150
- Kassa, A.1993.** Damage to weight and germination of haricotbean caused by the Mexican bean weevil, *Zabrotes subfasciatus* Boh. (Coleoptera: Bruchidae) at Bako, Western Ethiopia. Paper presented at the first annual conference of the Crop Protection Society of Ethiopia. Feb. 26-27, 1993. IAR, Addis Ababa.
- Kogan, M., and J. Paxton. 1983.** Natural inducers of plant resistance to insects. In P.A. Hedin (ed.), Plant Resistance to Insects. Am. Chem. Soc. Symp. Series 208, American Chemical Society, Washington, DC. 153-171
- Kossou, D. K., N. A. Bosque-Perez , and J. H. Mareck, 1992.** Effects of shelling maize cobs on the oviposition and development of *Sitophilus zeamais* (Motschulsky). J. Stored Prod. Res. 28 : 187-192.
- Longstaff B.C. 1981.** Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): A critical review. Prot. Ecol. 2: 83-130
- McFarlane, J.A. 1988.** Pest management strategies for *Prostephanus truncatus* (Horn) as a pest of stored maize grain. Present status and prospects. Trop. Pest Management 34: 121-132
- Mora, M.A., and Pedersen, J.R. 1976.** Damage to stored maize infested with *Sitophilus zeamais* Motsch. Agency for International Development United States Department of State. AID/ta-C-1162. Food and Feed Grain Institute, Kansas State University, Manhattan, Kansas 66506
- Painter, R. H. 1951.** Insect Resistance in Crop Plants. University of Kansas Press. Lawrence. 520 pp.
- Price, P.W., C.B. Bouton, P. Gross, B. A. McPherson, J. N. Thompson, and A.E. Weis. 1980.** Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. Ann. Res. Ecol. Syst. 11: 41 – 43
- Sanon, A., A.P. Ouedraogo, Y. Trigault, P.F. Credland, and J. Huignard. 1998.** Biological control of Bruchids in cowpea stores by release of *Dinarmus basalis* (Hymenoptera: Pteromalidae) adults. Environ. Entomol. 27: 717-725
- Sharifi, S., and R.B. Mills. 1971.** Radiographic studies of *Sitophilus zeamais* Mots. in wheat kernels. J. Stored Prod. Res. 7: 195-206
- Smale, M., A. Aguirre, and M. Bellon. 1998.** Farmer management of maize diversity in the central valleys of Oaxaca, Mexico. CIMMYT/INIFAP, 1998 Baseline Socioeconomic Survey.

- Smith, L. 1993.** Host-size preference of the Parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) on *Sitophilus zeamais* (Coleoptera: Curculionidae) larvae with a uniform age distribution. *Entomophaga* 38: 225 – 233
- Van den Assen, J.F.A. Putters and T.C.Prins. 1984.** Host quality effects on sex ratio of the parasitic wasp *Anisopteromalus calandrae* (Chalcidoidea, Pteromalidae). *Netherlands Journal of Zoology* 34 : 33-62
- Vowoter, K.A., N. A. Bosque-Perez, and J. N. Ayertey. 1995.** Effect of maize variety and storage form on the development of the maize weevil *Sitophilus zeamais* Motschulsky. *J. Stored Prod. Res.* 31: 29-36
- Wen, B., and J.H. Brower. 1994.** Suppression of *Sitotroga cerealella* in shelled corn by the parasitoid *Pteromalus cerealellae*. *J. Entomol. Sci.* 29: 254-258
- Wen, B., K. Weaver, and J.H. Brower. 1995.** Size preference and sex ratio for *Pteromalus cerealella* (Hymenoptera: Pteromalidae) parasitizing *Sitotroga cerealella* (Lepidoptera: Gelechiidae) in stored corn. *Environ. Entomol.* 24: 1160- 1166

EFFECT OF MAIZE VARIETIES ON THE PERFORMANCE OF PARASITOIDS OF STORAGE PESTS

Abstract

The effect of maize resistance to storage pests on the performance of storage parasitoids was evaluated for two host- parasitoid assemblages. *Pteromalus cerealellae* (Hymenoptera: Pteromalidae) an oligophagous ectoparasitoid of the Angoumois grain moth, *Sitotroga cerealella* (Lepidoptera: Gelechiidae) showed a 20% higher performance on a more resistant variety, than on a maize genotype that is very susceptible to several important maize storage pests. The higher performance was expressed in successful parasitism per time unit, 9.4 versus 7.6 per female per day. There were no differences in size or development time of parasitoid progeny suggesting that there were no indirect host-plant effects. It was found that the time needed to detect and paralyze a host in the more resistant maize variety, was shorter. Detectability may have been enhanced by higher amounts of volatile compounds emanating from the infested seed, or a better perception of host vibrations or temperature gradients through differences in endosperm quality.

The performance of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) a generalist parasitoid of the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) was similar on all maize genotypes. There was no evidence that kernel hardness prevented the parasitic wasps from finding and successfully parasitizing their internally feeding hosts. However, on average each female of this parasitoid successfully attacked only 3.4 host per day, which seems too low for an efficient control of maize weevils in storage.

Key words : Maize varieties, parasitoids, storage, *Sitophilus zeamais*, *Anisopteromalus calandrae*, *Sitotroga cerealellae*, *Pteromalus cerealellae*

Introduction

Host plant characteristics may affect natural enemies of herbivores dramatically through tritrophic interactions (Price et al., 1980; Turlings and Benrey, 1998). The effects may be positive, enhancing colonization and effectiveness of biocontrol agents such as parasitic wasps and predators. Plants may influence the effectiveness of natural enemies directly, through morphological plant characteristics such as vegetation size, plant surface quality; or visual factors that can reduce their efficiency in searching and controlling herbivores (Obrycki and Tauber, 1984; Botrell et al. 1998). Plant semiochemicals are released by plants under attack from herbivores and parasitoids are able to exploit these plant responses (Turlings et al., 1990). The natural enemies can also be affected indirectly by the host plant through their role as food source for the herbivores. Plants produce secondary compounds to resist insect attack. These compounds, such as anti-feedants or toxins alter host quality, its density and development time and in turn affect the size, longevity, and effectiveness of natural enemies (Benrey and Denno, 1997).

Different species of host plants can be expected to produce the largest range of variable effects on parasitoids or predators, because the above mentioned characteristics show greater variation than within a single species, but varietal differences, especially among cultivated plants, are sometimes large enough to affect the performance of natural enemies (Elzen et al., 1986; Kareiva, 1990). Knowledge of direct and multitrophic effects of plant defense strategies on natural enemies may reveal opportunities to increase the effectiveness of biocontrol agents (Botrell et al., 1998)

The broad genetic range in defense mechanisms in important food crops, such as maize, has allowed the breeding for insect resistant varieties (Bergvinson, 1994). This strategy to increase yield is an environmentally and economically safe method, as it reduces the need for costly pesticides (Smith, 1994). Breeding for resistance is therefore an important component of

integrated pest management and complements other tactics such as biological control. The compatibility of plant resistance with biocontrol agents is crucial for the success of these strategies, and has been the subject of various studies. Many examples show synergistic interaction of plant resistance with parasitoids and predators (Riggin et al., 1994; Kauffman and Flanders, 1985; White and Eigenbrode, 2000). However there are also some examples where plant resistance interacts in an antagonistic way with natural enemies, for example through a high content of toxic plant allelochemicals (Campbell and Duffey, 1970), or dense levels of trichomes (Obryck and Tauber, 1984).

During the vegetative stage of the plant allelochemicals may not only vary in space and time, but plants can also respond to an attack by herbivores with an array of chemical defense reactions (Baldwin and Preston, 1999). In the relatively inert stage of the seed, defense mechanisms are reduced to the metabolites that have been accumulated during maturation of the grain. Natural defense mechanisms such as hulls and thick pericarps have been reduced as a result of domestication because they also contained germination inhibitors that control dormancy, a feature not compatible with agricultural needs of fast and uniform germination (Harlan, 1992). Moreover, the higher quality resource that domesticated cereals provide, and the development of large granaries of early human societies allowed some insects to adapt to storage habitats (Buckland, 1981), and these insects are now responsible for heavy food losses. Several domesticated seed crops could be selected for resistance factors against these storage pests. For example the common bean contains alpha amylase inhibitors that act against the Mexican bean weevil, *Zabrotes subfasciatus* (Ishimoto et al., 1995). In maize, grain resistance against the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) and the larger grain borer, *Prostephanus truncatus* (Coleoptera: Bostrichidae) has been found in some maize landraces (Dobie, 1974; Urello et al 1989; Arnason et al., 1994a) and screening of germplasm at the International Maize and Wheat Improvement Centre (CIMMYT) has led to the discovery

of resistant populations from the Caribbean. The mechanism of this resistance are thought to be the presence of phenolic compounds that contribute to increase grain hardness (Classen et al., 1990), and phenolic acid amines that are toxic to insects (Arnason et al., 1992). These resistance factors result in lower oviposition rate, less progeny and prolonged development time of the maize weevil.

Very few studies have documented the interaction of parasitoids of stored grain with intra- or interspecific variation in seed characteristics. Benrey et al. (1998) found that parasitoids of bruchid larvae performed better on beans of cultivated than of wild species, and attributed it to the higher quality resource on cultivated plants and the thicker seed coat of wild beans that may hinder oviposition by female wasps. Ryoo et al. (1990) found significant differences in performance of parasitoids resulting from intraspecific variation in rice. Cryptic hosts such as stored grain pests may take advantage of variation in size and quality of the seed to escape attack by parasitoids. Price et al. (1980) quote numerous examples of herbivores that stay out of reach of the ovipositor of their natural enemies due to the size of the plant parts they are feeding on. Smith et al. (1995) attributed differences in fecundity of the parasitoid *Anisopteromalus calandrae* to differences of seed size. In stored maize, large kernels may offer more protection to their internally feeding hosts, compared to small kernels. Moreover, maize resistant to storage pest due to kernel hardness, may also directly reduce parasitoid effectiveness, as parasitoids of internally feeding immatures have to insert their ovipositor through the pericarp and underlying layers of endosperm to reach their hosts. For example, a parasitoid of the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae), has difficulty drilling through silicaceous rice hulls (Smith, 1994).

In maize, high post harvest losses due to storage pests, reduce the gains obtained through improved varieties that increase yield in developing countries. A more effective use of natural enemies as a component of integrated pest management may reduce the impact of

insect pests of stored maize. Releases of storage parasitoids have shown promising results (Wen and Brower, 1994; Flinn et al., 1996) and pesticide resistance of some parasitic wasp (Baker and Weaver, 1993; Baker and Throne, 1995) has shown potential for combination with other control methods .

In this study we wanted to examine the effect of genetic variation of maize on the performance of the natural enemies of storage pests, in order to determine if the interaction of grain resistance with parasitic wasps can be synergistic. For this purpose, we studied two parasitoid-host assemblages. *Pteromalus cerealella* (Hymenoptera: Pteromalidae) is an oligophagous ectoparasitic wasp that attacks the immatures of the Angoumois grain moth, *Sitotroga cerealella* (Lepidoptera: Gelechiidae). The female wasp paralyzes the internally feeding host larvae, and subsequently lays a single egg on it. Although *P. cerealellae* is able to attack a number of host species in the laboratory (Brower, 1991) it is generally found in association with the Angoumois grain moth (Espinal et al., 1996). The pteromalid *Anisopteromalus calandrae* is a generalist parasitoid of a wide range of internally feeding storage beetles, among them the maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae). The female wasp attacks only enclosed hosts (Ghani and Sweetmann, 1955), and like *P. cerealellae* it is a solitary ectoparasitoid. The maize varieties used for the study present a wide range of resistance levels to both storage pests and are different in shape, size, hardness and origin.

Material and methods

Insects

Sitotroga cerealella and its parasitoid *Pteromalus cerealellae* were obtained from maize bought at a local market in Pahuatlan (state of Puebla, Mexico). They were reared as described in Chapters 1 and 2. After one year they were replaced with a new colony of the same origin. *Sitophilus zeamais* and its parasitoid *Anisopteromalus calandrae* were collected from infested maize, at CIMMYT's experimental station in Veracruz, unless otherwise specified. They were reared as described in Chapters 1 and 2. The insect colonies were replaced with fresh adults from the same location every six months.

Grain

The maize varieties used were the same as in Chapter 1. They were provided by CIMMYT and represent a range of resistance against *S. cerealella*, and *S. zeamais* (Table 3.1 and Chapter 1).

Table 3.1 : Physical properties and insect resistance level of the maize varieties used

Variety	Abrev.	Kernel weight	Hardness	Resistance to <i>Sitotroga cerealella</i>		Resistance to <i>Sitophilus zeamais</i>	
				%S	Index	Progeny	Index
Cacahuazintle	Ca	45.9 ±0.7	7.7 ±1	71.4	12.9	15.5	11.2
CML 264x254	Hy w	37.0 ±0.2	17.1 ±0.4	39.6	9.8	46.0	10.3
CML 287x285	Hy y	35.9 ±1.3	17.3 ±0.5	59.6	11.1	40.0	9.7
Ejura	Pop 80	22.9 ±0.6	18.4 ±0.8	70.7	12.2	40.0	10.0
Cuba	Pop 84	21.8 ±0.4	21.2 ±0.5	59.3	11.6	12.5	5.5
Sinaloa 35	Sin	14.6 ±0.4	17.1 ±0.6	63.0	11.2	11.3	4.1
Yucatan 7	Yuc	16.6 ±0.4	15.1 ±0.7	59.0	12.0	29.5	9.1

Abrev. : Abbreviation used; Kernel weight: Average kernel weight of 100 kernels; Hardness: average kernel hardness measured as reported in Chapter 1; resistance to *S. cerealella* is expressed as percent survival to adult (%S) and the index of susceptibility; resistance to *S. zeamais* as number of progeny and the index of susceptibility. The lower the index the more resistant is the maize variety.

A - *Pteromalus cerealellae*- *Sitotroga cerealella*

As a first step, the feeding path of the moth larvae was determined in several maize genotypes, as well as the length of the ovipositor of its parasitoid (A.1).

Secondly, female parasitoids were allowed to forage on different maize genotypes with an equal host density to determine any differences in host location and handling efficiency due to maize genotype and mortality, defined as - no host no parasitoid emerged- was evaluated (A.2). A second experiment was conducted in which the parasitoids and their hosts were allowed to interact over several generations on each maize variety (A.3).

In the third part of the study, life time fecundity of female parasitoids was measured on two maize genotypes, with considerable differences in host resistance and parasitoid performance (A.4).

The time female parasitoids needed to locate and paralyze a host in a single infested kernel in a jar filled with uninfested kernels of one of two genotypes, was also determined (A.5).

A .1 - Position of hosts in maize kernels and ovipositor length of parasitoids.

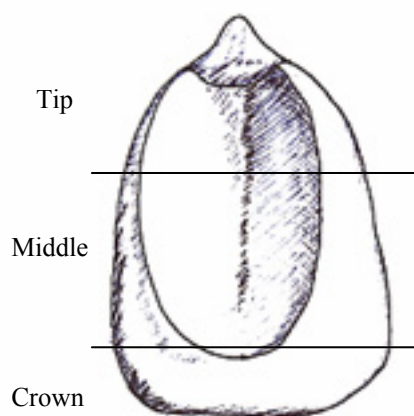


Fig. 3.1 Delimitation of areas in the maize kernel for location of oviposition sites, entry holes, mature host larvae and emergence

Infested shelled kernels of six maize genotypes were examined under a stereo microscope and percent kernels with perforations close to tip (Fig. 3.1 for delimitations) were noted. Four samples of 25 kernels were examined. Locations of emergence holes were noted after examining the samples used in chapter 1 for the evaluation of resistance to *S.cerealella*. The percentage of kernels with emergence holes close to the crown was calculated.

Female *P. cerealellae* were collected from the laboratory colony, 15 of them were dissected, and the length of the ovipositor and frontal head width were measured using a micrometer.

A.2 - Parasitoid performance on different maize genotypes.

In a short term experiment, 500 kernels of each of six maize genotypes were infested with 600 eggs of the Angoumois grain moth, obtained as described in chapter 1. After 15 days the kernels of each genotype were examined under a stereo microscope, and those showing an entry point were used to prepare samples of 40 infested kernels each, which were mixed with 60 uninfested kernels.

When the first “windows” appeared, indicating that the hosts were almost fully grown, they were exposed to the parasitoids. Two female *P. cerealellae*, from the rearing colony, mated, experienced, and 1 to 5 days old were introduced into each vial and allowed to forage for 24 hours. Emerging wasps and grain moths were counted. The experiment was repeated for the two genotypes that had shown the largest difference in parasitoid emergence. Wasps were allowed to forage for 24 hours on half of the samples and then switched to the remaining samples of the other maize genotypes, so that the same wasps had parasitized hosts on either maize variety.

In a subsequent experiment, mortality due to parasitoids was determined by exposing 240 infested kernel of the variety used for rearing to 12 female parasitoids for 24 hours. After emergence of the progeny, all kernels without wasp or grain moth emergence were examined by dissection, and hosts that had been unsuccessfully parasitized were noted.

A.3 - Host-plant effect over several generations.

In a long term experiment, ten jars with 350 g of one of each of the six genotypes were infested twice with 50 eggs of the Angoumois grain moth at an interval of 12 days, to ensure that all immature stages were present at any time. The jars were incubated, and after 40 days when the first adults had emerged, two females and one male *P. cerealellae* were released into half of the jars. This was repeated one week later. The jars were left undisturbed until three month after infestation when they were frozen and all adult moths and wasps were counted, as well as damaged and undamaged grain. Head width of 15 female wasps, emerged on genotypes Hy w and Ca, was measured using a micrometer.

A.4 - Fecundity of *P. cerealellae* on two maize genotypes.

Approximately 650 eggs of *S. cerealella* were used to infest 500 kernels of maize genotypes Cacahuazintle and 850 eggs for an equal number of hybrid Hy w in half liter jars, to obtain a similar host density in each genotype. These egg-kernel proportions were based on the survival rate determined in Chapter 1. This was repeated daily for 12 days. After 14 days, the kernels of each jar were mixed and distributed over 20 vials. Twenty-three days after infestation, one female parasitoid that had been reared on another maize variety and emerged less than 24 hours before was placed in each of the 40 vials, together with one male of the same age. Infestation of Hy w was delayed one day to compensate for the slower development of *S. cerealella* on this genotype. Each pair of wasps was transferred to the next vial every 24 hours, until half of the females had died. Deceased males were replaced once. Surviving females were allowed to forage on infested maize until they died. Emerging wasp progeny and Angoumois grain moths were counted. Head width of 20 female wasps, emerged on day three from different samples was measured using a micrometer.

A. 5 - Host location and paralization.

Glass jars were filled with 800 ml of a white hybrid maize (CML 244 X 349), which accounts for approximately 614 g or 2050 kernels. One single seed of either Cacahuazintle or Hy w containing a grown larvae of *S. cerealella* was marked and placed randomly in the lowest quarter of each jar. Only seeds that had the penetration hole close to the tip (Fig. 3.1) were used. Female *P. cerealellae*, used in this experiment were less than 5 days old, mated and experienced on a yellow hybrid, and starved from six to eight hours prior to the first experiment.

In a series of preliminary experiments we tried to determine the approximate time needed by the parasitoid to locate and paralyze the host. One female was placed on top of the maize in the jar and allowed to forage a varying amount of time, ranging from 1 to 24 hours. The infested seed was then removed and dissected. Any larvae that was found to be paralyzed was considered "found" by the parasitoid. The presence or absence and number of parasitoid eggs was also noted.

Following the outcome of these tests the experimental times were set to two and three hours. Series of six tests (jars) were carried out at a time for each maize genotype. The same females were used twice if possible, once on each genotypes. They were starved for three to six hours prior to a test. A test was not considered valid when the larvae was not in its last instar, as observed by the size of the head capsule, if it had pupated already, was parasitized by mites of the Pyemotes group or was dead. It was not considered valid either if the wasp was dead or not recovered at the end of the experiment, in which case the maize was frozen for 48 hours before re-use.

B. *Anisopteromalus calandrae* – *Sitophilus zeamais*

For the first part of these experiments, the distance of the mature host from the pericarp in different maize genotypes was measured and compared to the range the parasitoids could reach with their ovipositor (B.1). In the second part, infested maize genotypes were exposed to parasitoids in no choice situations and for a limited time interval to detect maize genotypes that eventually reduce or impede successful parasitism (B.2). In the third part maize weevils and parasitoids were allowed to interact for several generations on three different maize varieties (B.3).

B.1 - *Location of host larvae in maize kernels and ovipositor length of parasitoids.*

Shelled grain of seven maize genotypes was infested during a seven day period with a high number of unsexed adult weevils. Approximately 7 to 9 days before the first emergence, when most immatures were in the final instar, kernels were stained with acid fuchsin to reveal egg plugs as described by Petersen (1979, cited by Horber, 1989) and examined under a stereo microscope. Kernels containing egg plugs were dissected. Using a micrometer the following were recorded, larval stage was determined by head capsule width, the position of the larvae within the kernel (Fig. 3.1), the position in relation to the interface of germ and endosperm, and the closest distance to the pericarp.

Two species of pteromalid wasps, *Anisopteromalus calandrae* and *Lariophagus distinguendus*, that commonly parasitize *S. zeamais* were collected from weevil infested maize at the experimental station in Poza Rica of CIMMYT (International Maize and Wheat Improvement of Center). Fifteen randomly chosen females were dissected, and the length of the ovipositor and frontal head width was measured using a micrometer ($\pm 0.025\text{mm}$)

B.2 - Screening of host-plant genotypes.

In a first experiment samples of 15 maize genotypes) were infested for 7 days with six females and three male *S. zeamais*, as described in Chapter 1. The maize varieties included modern hybrids, Mexican landraces, and populations selected for storage resistance (Table 3.1). Four replicates were made at weekly intervals for each genotype. The samples were incubated and when they contained primarily suitable host larvae (fourth instar), they were exposed to two female *A. calandrar*e for 48 hours. To compensate for differences in development time of *S. zeamais* immatures in different maize genotype, the moment of exposure was delayed based on the development times obtained in Chapter 1. Emerging parasitoid progeny and weevils were counted and removed daily. The number of suitable hosts in each vial was estimated by the sum of emerging parasitoid progeny and weevils emerging within 15 days (see Smith and Press, 1992).

In a second experiment the number of maize genotypes was reduced to seven varieties that are grown at CIMMYT's experimental stations for improvement of yield and storage resistance, and show a range of resistance to stored grain pests (Table 3.1). One thousand kernels of each variety were infested with 240 unsexed adult maize weevils for seven days. The infested maize was then subdivided into 10 samples with 100 kernels each. Five samples were exposed to parasitoids as described above, four kept as controls to detect mortality due to parasitism, and one sample was retained to monitor the development of host larvae by dissection. The experiment was repeated with a second maize weevil strain originating from Oaxaca, Mexico

A third experiment was set up to obtain similar host densities in the resistant varieties. One thousand maize kernels of each genotype were placed in water for four minutes and then dried with tissue paper and under a fan. The kernels were infested with 240 unsexed weevils, as described above. Three days after removing the weevils the kernels were spread out on a

plate in the incubation chamber for 24 hours to allow kernels to reach equilibrium moisture again. This treatment will lead temporarily to a higher grain moisture content, which will increase survival and shorten development time (Holloway, 1985) and increase oviposition, if applied before infestation. The grain was then distributed into 60ml vials and incubated. A sample of 100 grains was kept separately to monitor developmental stage and determine host density. When most larvae were in their late instars, the grain of each variety was subdivided into samples of approximately 50 suitable host larvae. If necessary, uninfested kernels were added, so that similar host densities were obtained in all genotypes. The samples were exposed to two female *A. calandreae* and emerging weevils and wasp progeny were recorded as described above.

B.3 - Host-plant effect over several generations.

Three genotypes, Pop 84 (resistant), Ca (susceptible) and Hy R (susceptible) were used for a long term experiment, at CIMMYT's experimental station in Poza Rica, Veracruz. The experiment was carried out inside a building with windows that are permanently open. Extreme temperatures were therefore avoided. Of each variety 350 g was placed in 1 liter glass jars, and infested with 40 unsexed weevils, one to two weeks old. Ten replicates were made for each variety. The jars were closed with wire mesh and tissue paper, and placed in metal dishes filled with a layer of mineral oil to avoid mite infestation. After five, six and seven weeks two female *A. calandreae* and one male were added to half of the jars of each variety. The jars were frozen three months after the begin of the experiment, and all adult weevils were counted, as well as the damaged and undamaged kernels.

Results and Discussion

A - *Pteromalus cerealellae* – *Sitotroga cerealella*

Results

A.1- Position of hosts in maize kernels and ovipositor length of parasitoids

There were no significant differences among maize varieties for the position of the site of entry and site of emergence of *S. cerealella*. The feeding path started most frequently close to the point of attachment of the kernel where 87.3% of entry holes were found (Table 3.2). Most emergence holes were located in the distal third of the seed (crown), where 75.3% of the moths emerged. Analyses of variance showed no differences among varieties, neither for point of entry nor for emergence. Usually only one larvae was found per kernel, but occasionally two were present.

Average length of the ovipositor of *P. cerealella* was 1.5 mm and positively correlated with head width (Table 3.3).

Table 3.2: Location of *Sitotroga cerealella* in the kernel

Variety	Entry through tip	Emergence from crown
Cacahuazinte	87.3 ±3.2	73.1 ±3.7
Hy w	88.3 ±3.0	73.4 ±3.1
Hy y	88.4 ±4.7	79.4 ±2.5
Pop 84	83.5 ±3.2	69.0 ±10.4
Pop 80	87.1 ±2.6	73.6 ±9.4
Sin 35	91.1 ±4.4	88.8 ±0.2

Entry through tip: percent of larvae that entered the kernel close to the point of attachment of the kernel (Tip); Emergence from crown: percent of adults that left the kernel from the crown part. Results were not significantly different among varieties. Entry: df=18 F=0.8 P=0.6; Emergence: df=18 F=1.6 P=0.2

Table 3.3 : Ovipositor length of three common pteromalid parasitoids of stored maize pests

Species	Mean	min-max	corr
<i>Anisopteromalus calandrae</i>	1.2 ±0.03	0.9-1.3	0.7
<i>Lariophagus distinguendu</i>	0.8 ±0.02	0.6-1.0	0.5
<i>Pteromalus cerealellae</i>	1.5 ±0.04	1.3-1.5	0.8

Mean : mean of ovipositor length (anterior valves with serrations) of 15 females; min-max: minimum and maximum values of ovipositor measured; corr: correlation of ovipositor length with frontal head width

A.2 - Parasitoid performance on different maize genotypes.

P. cerealellae performed much better on a resistant genotype (Hy w) than on the most susceptible (Cacahuazintle) in the short term experiment (Table 3.4, Fig. 3.2). Analyses of variance showed that number of parasitoid progeny was significantly influenced by maize genotype ($P>0.0001$). Results for the other genotypes were intermediate. Total available hosts (assumed to be the sum of parasitoid progeny and emerged grain moths) did not differ significantly among genotypes. There were no differences in the average development time of the wasps, 11 days for males and 12 for females. When the experiment was repeated for genotypes Hy w and Ca, with switching parasitoids, significantly more wasp progeny emerged again on genotype Hy w ($P=0.034$), but the difference was smaller than in the previous experiment, and barely significant (Table 3.5). The total host number was slightly, but not significantly higher on Hy w.

Mortality of parasitoids during development was very low, 3.9% of parasitized hosts did not produce an adult parasitoid. Two hosts that were found paralyzed but alive without an egg were not included in this count. It was assumed that the parasitoids, removed after 24 hours had not been able to complete the oviposition process.

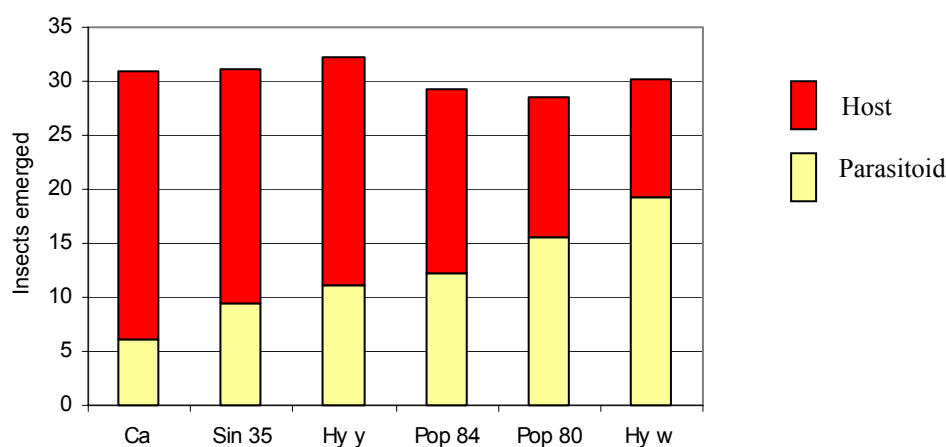


Fig. 3.2: Performance of parasitoid *Pteromalus cerealellae* on different maize genotypes with similar host *Sitotroga cerealella* densities

Yellow: Number of parasitoid progeny . Red : number of hosts that escaped parasitism. Total number of available hosts was not significantly different, but parasitoid progeny differed significantly (df= 29 ; F= 9.6; P<0.0001; LSD 4.9)

Table 3.4: Host-plant varietal effect on performance of *Pteromalus cerealellae* parasitizing its host *Sitotroga cerealella*

Variety	Short term			Long term		
	<i>S.cerealella</i>	<i>P. cerealellae</i>	Hosts	<i>S.cerealella</i>	<i>P. cerealellae</i>	controls
Ca	24.2 ±1.9	5.2 ±1.7 a	29.3 ±3	67.8 ±12 ab	260.8 ±16	355.8 ±13 bc
Hy w	11.0 ±1.1	19.2 ±1.4 d	30.2 ±1.5	39.4 ±4 b	192.6 ±15	277.8 ±17 d
Hy y	21.0 ±1.5	11.2 ±2.2 ab	32.2 ±2.6	57.8 ±3 b	210.6 ±14	300 ±18 cd
Pob 80	10.0 ±2.2	16.3 ±1.5 cd	26.3 ±1.7	54.4 ±4 b	329.4 ±22	389 ±13 ab
Pob 84	16.2 ±1	11.8 ±1.6 abc	28.0 ±2	63.8 ±7 ab	360.4 ±16	354.8 ±12 bc
Sin	21.3 ±1.2	9.0 ±1.3 ab	30.3 ±1.7	92.8 ±12 a	407.6 ±15	441.4 ±22 a
LSD	4.2	4.9	6.2	22.9	48.4	47.6

Short term: Hosts were exposed to two parasitic wasps for 24 hours

Long-term: Hosts and parasitoids were allowed to interact over several generations.

S.cerealella: mean number of grain moth that emerged; *P. cerealellae*: mean wasp progeny; number of suitable hosts (= emerged wasp progeny + emerged grain moths); controls: mean number of *S. cerealella* emerged on control samples that contained no parasitoids.

Numbers followed by the same letter are not significantly different at the 5% level (Tuckey-Kramer test)

ANOVA: short term: *P. cerealellae* : df= 5, 29 F= 9.6 P<0.0001; Host: df= 5, 29 F=0.9 P=0.5 long term: ANOVA : *Pc* differed significantly among varieties. Factorial analyses: *S.cerealella*; Significant interaction of variety and parasitoid: P<0.0001, and for each as main effect P<0.0001. For total emerged insects only variety had a significant main effect P<0.0001. total emerged insects *P. cerealellae* + *S.cerealella* was significantly different from controls only for Pop 84: P=0.015

Table 3.5: Performance of parasitoid, *Pteromalus cerealellae*, when switched between two maize genotypes

	Hy w	Ca
Day 1	a 25.7	b 9.3
Day 2	b 19	a 19.4
Total parasitoids	22.3	14.3
Total hosts	35.2	31

Day 1 and Day 2 show parasitoid progeny of both groups(a and b) of parasitoids, switched to the other maize genotype, as indicated by the letter preceeding the number. Total parasitoids and total hosts indicate mean parasitoid progeny and mean available hosts (= parasitoid progeny + emerged *S. cerealella*) for each maize variety. Means were compared with t-tests: total parasitoids were significantly different: df=10, P=0.034. Total hosts were not significantly different: df=10 P=0.1

A.3 - Host-plant effect over several generations.

In the long-term experiment, parasitoids reduced the number of emerged grain moths 5 to 7 fold as compared to controls (Table 3.4). The total number of emerged insects was not lower for samples with parasitoids than for controls. For Pop 84 it was even higher in samples with parasitoids. Two way factorial analyzes showed significant interaction of parasitism and maize genotype for emerged *S. cerealella* ($P > 0.0001$), where both parasitism and variety had a strong significant effect. There was also a significant interaction of parasitism and variety on the total number of insects (=parasitoids+ *S. cerealella*) ($P = 0.0033$), with variety having a strong influence, but not parasitism ($P = 0.8$). The most resistant genotype, Hy w, had the lowest number of emerged insects in both treatments. From the susceptible genotype, Cacahuazintle, fewer insects emerged than from other genotypes (Table 3.4). Regression of the number of parasitoid progeny against the number of grain moths emerged from controls showed a positive linear relationship (Fig. 3.3). Two genotypes were clearly above (Pop 84) or below (Ca) the line, indicating that the parasitoids had a better (Pop 84) or lower (Ca) performance than could be expected as a function of total host density.

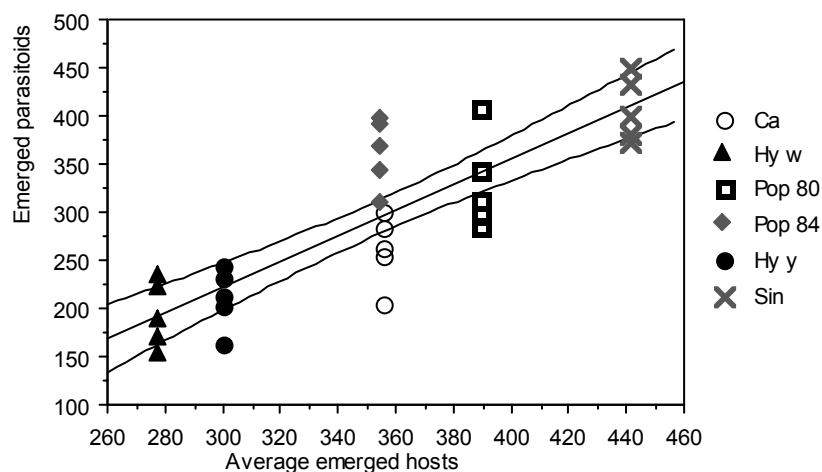


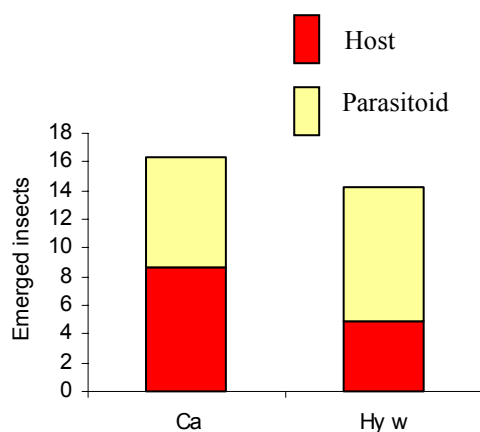
Fig. 3.3: Regression of emerged wasps (*Pteromalus cerealellae*) against mean of emerged hosts (*Sitotroga cerealella*) in controls on six maize varieties in a long term experiment
 $(Y = -177.61 + 1.334 \cdot X \quad r^2 = 0.715)$

A.4 - The fecundity of *P. cerealellae* on two maize genotypes.

Average daily progeny production was 9.4 on Hy w and 7.6 on Ca (Table 3.6, Fig. 3.4, 3.5).

Mean progeny per female during 11 days was about 20% higher in Hy w than in Ca, but varied considerably among individuals, lowest and highest values were lower on Ca than on Hy w (Table 3.6). Maximum progeny per female on a single day was 22 on Hy w and 18 on Ca.

Mean number of available hosts (= emerged wasps + emerged hosts) was slightly but significantly lower in Hy w than in Ca (Fig. 3.4). Half of the female wasps were dead on day 11 on both maize genotypes. Mean longevity was 11.5 (± 0.95) days on Cacahuazintle and 12.3 (± 0.98) days on Hy w (Table 3.6). The Kolmogorov-Smirnov test indicated no differences in distribution of adult longevity. Sex ratio of progeny was female biased (1.9), and not significantly different among maize genotypes. Males emerged earlier (11.7 days ± 0.2) than females (12.4 ± 0.1). There was no significant difference for development time of the parasitoids among the two maize genotypes. Head width of female progeny was not significantly different among maize genotype (Table 3.6) but it was significantly larger than those measured on female parasitoids that emerged in the long term experiment (Kolmogorov-Smirnov test: $P = 0.0008$ for Ca and $P = 0.0002$ for Hy w).



Fig

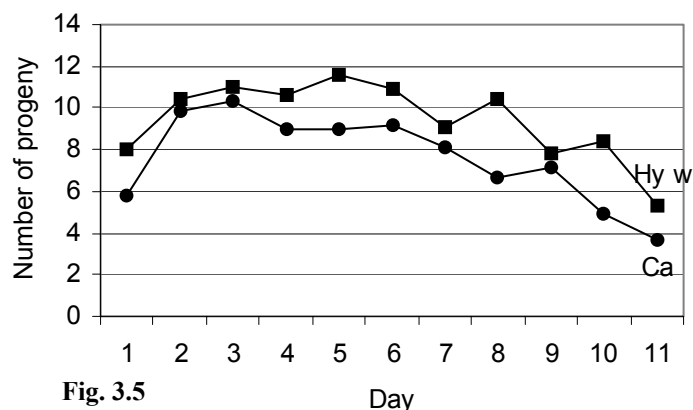


Fig. 3.5

Fig. 3.4 : Mean number of insects that emerged either as progeny of female *Pteromalus cerealellae* or as host, *Sitotroga cerealella* in two maize varieties

Mean of 20 female wasps over 11 days

Yellow: average daily progeny of female *Pteromalus cerealellae* on its host (*Sitotroga cerealella*) in two maize genotypes. Red: number of hosts that escaped parasitism.

Parasitoid progeny differed significantly

(t-test :df=38, t=-2.2, P=0.037). The number of available hosts (= sum of emerged parasitoid progeny and emerged hosts) was also significantly different (t-test: df=38; t=-5.4, P<0.0001).

Fig. 3.5: Mean daily progeny of 20 female *Pteromalus cerealellae* on two maize genotypes, until 50% of the females were dead.

Progeny was significantly lower on genotype Ca (paired t-test: df=10; t=5.5; P=0.0003, mean difference = 1.8)

Table 3.6: Fecundity of *Pteromalus cerealellae* on two maize genotypes on host *Sitotroga cerealella*

Variety	Mean progeny	Progeny/day /female	Survival	Sex ratio	Head width	Hosts
Hy w	103.7 ±7	9.4 ±0.6	12.3 ±1	1.9 ±0.1	850 ±7.1	14.3 ±0.3
Ca	83.5 ±6	7.6 ±0.6	11.5 ±1	1.9 ±0.1	851 ±9	16.3 ±0.8

Mean progeny: mean of 20 females during 11 days. P/day: Survival: Mean survival of female wasps (in days). Head width: mean head width of female progeny : mm*10⁻³.

Hosts: Mean number of available hosts per sample (=sum of emerged parasitoids+ emerged *S. cerealella*)

Mean progeny and progeny per day were compared with t-tests, the difference was significant: df=38 t=-2.2 P=0.037; and df= 20 t=2.2 P=0.045

Survival and head width were compared with the Kolmogorov-Smirnov test: They were not significantly different. Survival: Chi square=0.9 P>0.999 ; head width: Chi square=0.7 P >0.999

Sex ratio was compared with a t-test but was not significantly different

Host density was significantly different: df=38 t=-5.4 P<0.0001

A.5 - Host location and paralization

In most cases, the infested kernel among 2000 kernels was located when exposed to a single female for 5, 8, 12, or 24 hours. Hosts were paralyzed and frequently one or more eggs were found on, or close to the host. Up to five eggs were found on one hosts exposed for 24 hours. Differences among maize genotypes appeared only when the exposure time was reduced from three to two hours. In both series, about 45 valid tests were obtained for each maize genotype.

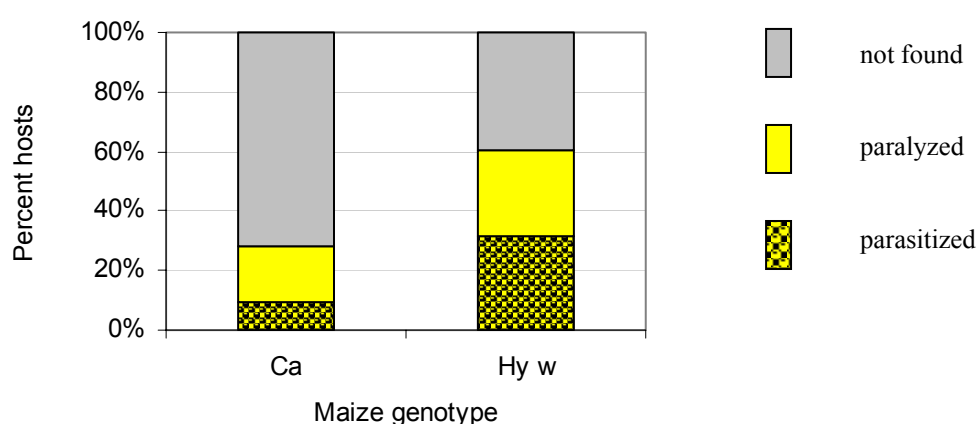


Fig. 3.6 : Hosts found and paralyzed within two hours by *Pteromalus cerealellae* in two maize genotypes. Grey: hosts that were not found; yellow: hosts that were found and paralyzed; black and yellow : hosts that were paralyzed and parasitized after the two hour exposure. Numbers are expressed as percentage of all valid trials (45 for Ca and 44 for Hyw). Significantly more hosts were found (paralyzed) in genotype Hy w than in Cacahuacintle (Fisher's exact test, chi square=11.8 Fisher's exact p-value=0.0009)

Table 3.7: Host finding and paralyzing by *Pteromalus cerealellae* on two maize genotypes

Variety	time	host found	valid tests	eggs
Ca	2	12	47	4
Hy w	hours	29	48	15
Ca	3	14	45	2
Hy w	hours	22	44	8

Time: time of exposure to a parasitoid; host found: number of hosts (*Sitotroga cerealella*) found and paralyzed by the parasitoid in all valid tests; eggs: number of paralysed hosts on which a parasitoid egg had been deposited.

Data were examined with Fisher's exact test. Differences were significant for the 2 hour period : chi square=11.8 Fisher's exact p-value=0.0009; but not for three hours: three hours: chi square=3.3 Fisher's exact p-value= 0.09

More hosts were found paralyzed or even with parasitoid eggs in Hy w, where at least half of the hosts were found, whereas on Ca only about one third of host larvae were detected. The differences were significant only for the two hour period after Fishers exact test ($p=0.0009$). The number of hosts parasitized (host-chambers containing a parasitoid egg) was also much higher for Hy w (Table 3.7, Fig.3.6). When exposure time was further reduced to one hour, only one host was paralyzed in 36 valid tests.

Discussion

The large size of the mature grain moth larvae in relation to the maize kernel (length of grown larvae: 10mm, and 1.9mm body width [Wen and Brower, 1994]) and the length of the parasitoid's ovipositor make it highly unlikely that the host could stay out of reach of the ovipositor, especially in a large maize kernel such as Cacahuacintle. Moreover, the late instar larvae have to approach the surface of the grain when they extend the anterior of their chamber to just beneath the surface of the grain, when they pupate, which allows the adults to emerge by pushing through the translucent seed coat (Dobie et al., 1991). The site of emergence, near the crown, seems to be a consequence of the large size of the pupae, and their feeding preference for the endosperm. Moreover, when maize is stored on the cob, the crown (Fig. 3.1) is the only part of the kernel where grain moths leave the kernel.

The comparison of different maize genotype suggests that some varietal grain characteristics affected the parasitoid efficiency. However, the differences were reduced when the same wasps were used successively on both genotypes (Table 3.5). This suggests that individual differences between wasps, as observed by Smith et al. (1994), or lack of experience contributed to inflated differences in parasitoid efficiency. The higher number of hosts parasitized on the second day may be due to a higher egg load of the wasps after abundant host

feeding on the first day. Results of the long term experiments were seemingly more influenced by the degree of grain resistance and by grain number than by differences in parasitoid performance as a result of host plant variety. The only indication for such an influence was the deviation of genotype Cacahuazintle from the linear relationship between number of parasitoids versus number of hosts. However the final number of insects is not necessarily proportional to the host density at any time during the experiment, and the results have to be interpreted with caution as the host density at the time of the release of the parasitoids could not be determined. Although the parasitoids clearly decreased the number of emerged grain moths, the total number of emerged insects was not affected, or even higher in some varieties than emerged moths in controls, an indication that the parasitoids were released too late to affect the first generation of grain moths.

Mortality due to parasitism was caused by incomplete parasitoid development. It was very low 3.9%, compared to 13.6% observed for the pteromalid *Dinarmus basalis* on its bruchid host in leguminous seed (Damien et al., 2001). Two host larvae were found paralyzed but without parasitoid egg. Van den Assem (1984) observed that another pteromalid, *Anisopteromalus calandrae* sometimes used paralyzed larvae for hemolymph feeding only, and Choi et al. (2001) found that *Anisopteromalus calandrae* used large hosts (*Sitophilus oryzae*) for oviposition and small host larvae for feeding only. However the remains of *S. cerealella* after parasitoid development suggest that this hosts is large enough to support host feeding and parasitoid development. Very likely the host handling was not always completed when the parasitoids were removed.

The evaluation of fecundity on two maize genotypes takes into account the individual variation of progeny production among wasps, and the effect of successive learning processes, as parasitoids are able to learn during a previous oviposition experiment, and increase their searching efficiency (Turlings et al., 1993). Only host-plant varietal differences for parasitoid

effectiveness that are not overcome by successive learning processes can be of value for effective biological control.

The fact that the parasitoid progeny of 20 females was higher on Hy w despite lower host density is a clear indication that the performance of *P. cerealellae* is influenced by the genotype of the host plant. This could occur at the level of host detection, paralysis, acceptance for oviposition, or nutritional suitability (Vinson and Iwantsch, 1980). The similar size and sex ratio of the parasitoid progeny on both maize genotypes and a similar longevity of adult parasitoids indicate the absence of multitrophic effects such as nutritional inadequacy of the host-plant for the host. Acceptance for oviposition is influenced by host age, as pupae or very small larvae are rarely parasitized (Wen and Brower, 1994). The appearance of 'windows' however makes control for host age relatively secure, although there is some variability in host development depending on maize genotype (Chapter 1).

The host stage was controlled for very precisely in the last experiment, as only tests with hosts in the last instar were evaluated. As movement and penetration depth of storage parasitoids can be affected by grain size or shape (Ghani and Sweetman, 1955), the same maize variety was used to hide the infested seed of each variety. Thus, the only factors that could affect the wasps in the last experiment were differences in host finding ability, and paralyzing. If female parasitoids need more time to find and paralyze a host in maize genotype Cacahuazintle than in Hy w, then an exposure time that is shorter than the average time needed for Cacahuazintle, but long enough for Hy w will lead to a different number of paralyzed hosts in each genotype. At a longer exposure time differences will level off. After two hours, more than half of the parasitoids had discovered and paralyzed the single hosts in the kernel of genotype Hy w among 2000 uninfested kernels. This rapid location at low host density confirms that searching by storage parasitoids is not random, as indicated by van den Assen (1984). Strong and reliable signals must guide them through the stored grain mass. The same

author suspected storage parasitoids to be led by cues such as vibrations, chemicals or temperature gradients. Some storage parasitoids, like many other parasitic wasps, use olfactory cues to locate both, storage habitat and infested seed (Cortesero et al., 1993 and 1995; Steidle and Ruther, 2000; Steidle and Fischer, 2000). Female parasitoids will erroneously insert their ovipositor in uninfested kernels if a neighbouring kernel is infested (personal observation) possibly stimulated by volatile compounds. Dispersal of volatiles, from faeces or the kernel itself could differ among genotypes due to chemical composition of the endosperm, or permeability of the pericarp. Vibrations and temperature conductivity could be influenced by kernel hardness or pericarp thickness and enhance the detectability of the larvae within the kernel. Smith et al.(1995) suspected that parasitoids could have difficulties to paralyse very large hosts, and *S. cerealella* was indeed found to be significantly larger emerging from Cacahuazintle (Chapter 1). However, we observed that to paralyze a mature larvae takes about ten minutes, as for *A. calandrea* (Ghani and Sweetman, 1955), so that the major part of the two hour period can be attributed to host-finding. It is therefore more likely that the difference of parasitoid performance between these two maize genotypes is due to a longer time for location of the infested seed, or the location of the host within a kernel.

B - *Anisopteromalus calandrea* – *Sitophilus zeamais*

Results

B.1 - *Host location and ovipositor length.*

Measures for kernel hardness and mean weight of kernels as determined in Table 3.2. Most 4th instar larvae, prepupae and pupae of maize weevils were located close to or at the pericarp

Table 3.8: Location of *Sitophilus zeamais* immatures in maize kernels

Variety	Number	%≤0.2mm	%≥0.4mm
Ca	87	44.8	44.8
Hy w	52	73	11.5
Hy y	60	50	28.3
Pop 84	20	75	20
Pop 80	62	61.3	16.1
Sin	39	94.8	0
Yuc	45	73.3	9.8

Number : number of 4th instar larvae considered; %≤0.2mm: Percent of 4th instar larvae that were located 0.2mm or less from the pericarp; %≥0.4mm : percent of 4th instar larvae that were located 0.4mm or more from the pericarp.

(Table 3.8). Only the landrace Cacahuacintle had a high percentage of larvae more than 0.4mm from the surface. Third instar larvae were not as close to the pericarp (mean= 0.34), but the location of pupae and prepupae was not significantly different from 4th instar larvae (mean= 0.2mm). Measurements on maize genotypes for these stages were pooled. As reported by Gomez et al. (1983), we observed that most eggs were laid in the tip region of the kernel. Newly hatched larvae bored into the floury endosperm which is located at the interface with the embryo (Sass, 1977). On smaller kernels there was more evidence of germ feeding than on large genotypes. Only in one variety, Cacahuazintle, chambers of large immatures were not always in contact with the interface. There was no preference for location of fully grown larvae in any part of the grain (Tip, Middle Crown, distribution was 21%, 45%, 34% respectively). The distribution approximates the volume attributed to each kernel part.

The mean length of an ovipositor of *A. calandreae* was 1.2 mm (Table 3.3). Another parasitoid of the same larval stages of the maize weevil, *Lariophagus distinguendus*, had a much shorter ovipositor (mean= 0.8mm)

B.2 - Screening of host-plant genotypes.

As a consequence of varying resistance, the number of suitable hosts varied significantly among the maize genotypes used. The parasitic wasps were able to reproduce on all varieties within the 48 hours they were allowed to forage. On the varieties with fewer hosts they produced fewer progeny. The number of hosts available (assumed to be the number of emerged maize weevils plus the number of emerged wasps), correlated positively with the number of emerged parasitoid progeny (Correlation coefficient: 0.7) (Fig. 3.7).

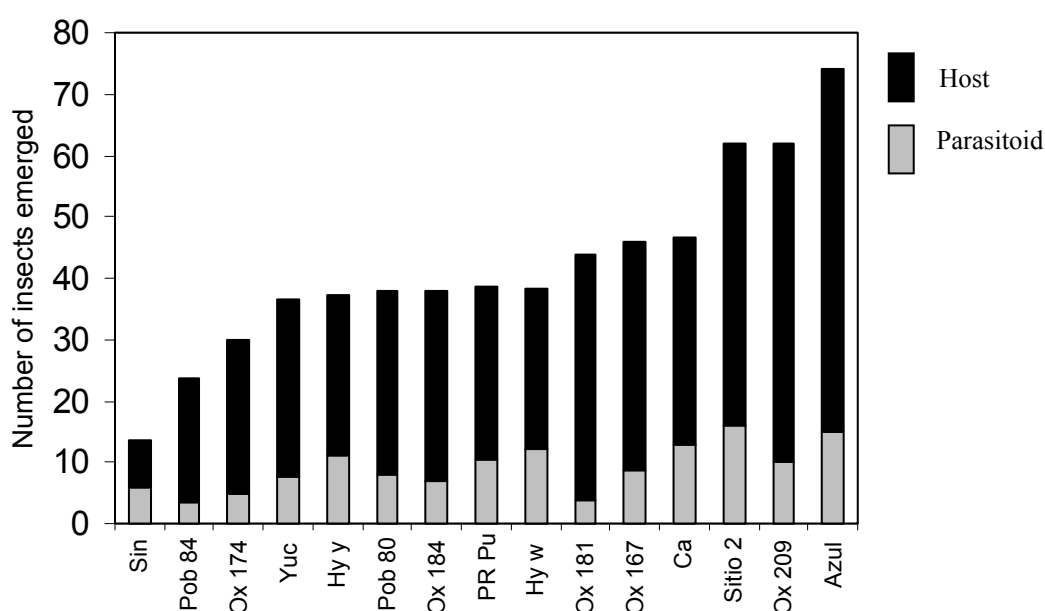


Fig. 3.7: Performance of parasitoid *Anisopteromalus calandrae* on maize genotypes with a varying degree of resistance to *Sitophilus zeamais*.

Maize genotypes with few insects emerged are more resistant.

Number of insects = number of suitable hosts: sum of emerged parasitoids (grey) and emerged maize weevils (black). Number of suitable hosts differed significantly among genotypes, being lower in resistant genotypes (ANOVA: $df=14, 45$ $F=8.0$ $p<0.0001$). Number of parasitoid progeny differed significantly among genotypes: (ANOVA: $df=14, 45$ $F=3.1$ $p=0.002$). The correlation coefficient between mean host number and mean parasitoid progeny was 0.7

Table 3.9: Screening of 15 maize varieties for antagonistic interaction between grain resistance and a parasitoid of *Sitophilus zeamais*, *Anisopteromalus calandrae*.

Maize variety	Hosts	<i>A. calandrae</i>
Ca	46.2 ±6.4	12.8 ±2.2ab
Hy w	38.3 ±9.6	12.3 ±2.8ab
Hy y	28.8 ±5.7	11.3 ±1.3ab
Pop 80	37.5 ±2.9	8.0 ±1.1ab
Pop 84	23.8 ±6.4	3.5 ±0.7a
Sin	13.5 ±7.5	6.0 ±3.5ab
Yuc	36.0 ±2.6	7.5 ±2.1ab
Azul	75.0 ±2.9	15.3 ±1.3b
Ox 167	44.6 ±5.6	8.8 ±2.1ab
Ox 174	29.5 ±9.6	4.8 ±1.7ab
Ox 181	43.8 ±2.2	3.8 ±1.9a
Ox 184	37.8 ±2.4	7.0 ±2.5ab
Ox 209	62.3 ±4.5	10.0 ±4ab
PR Pu	38.0 ±2.3	8.0 ±1.1ab
Sitio2	61.0 ±3.1	16.0 ±2.1b
LSD	28	11.3

Host: number of suitable hosts, estimated by the sum of emerging parasitoid progeny *A. calandrae* and weevils emerging within 15 days.

Number of suitable hosts differed significantly among genotypes : ANOVA: df=14, 45 F=8.0 p<0.0001. *A. calandrae* : Number of parasitoid progeny from 2 females during 48 hours. Number of parasitoid progeny differed significantly among genotypes ANOVA: df= 14, 45 F= 3.1 p=0.002. Numbers followed by the same letter are not significantly different at the 5% level (Tuckey-Kramer test) The correlation coefficient between mean host number and mean parasitoid progeny was 0.7

The second experiment with two different maize weevil strains yielded similar results. The total number of hosts varied among maize genotypes and maize weevil biotypes (Table 3.10). Mean number of progeny correlated with mean host number (correlation coefficient= 0.85 for the strain from Oaxaca and 0.92 for the biotype from Poza Rica). A regression of the combined numbers of emerged *A. calandrae* and maize weevils against the mean number of maize weevils emerged from controls, (all maize genotypes and weevil biotypes combined) indicates a one to one relationship ($Y=0.954 \cdot X + 2.7$; $R^2= 0.954$), suggesting that *A. calandrae* caused no mortality of maize weevil other than by successful parasitism. T-tests that were used to compare combined numbers of emerged *A. calandrae* plus maize weevils with emerged weevils of control samples for each maize genotype and weevil strain, revealed no significant differences either.

There were no differences in development time of parasitic wasps among maize genotypes.

The first males emerged 24 hours before the first females.

In the third experiment, high host densities were obtained for resistant varieties as well. Due to clumped distribution of maize weevil immatures in maize kernels, host number varied considerably (Table 3.11 Fig. 3.8). Only samples containing from 40 to 70 suitable hosts were included in the analyses. The overall mean density was 51.4 (± 0.9). Average progeny per female wasp per day at this host density was 3.6 (± 0.16). Analyses of variance showed that the progeny per female wasp per day was not significantly different among maize genotypes ($P=0.5$) (Table 3.11 Fig. 3.8)

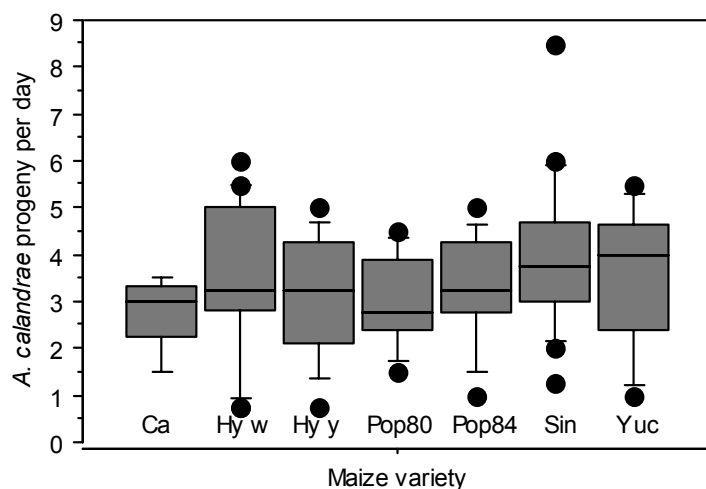


Fig. 3.8 : Daily progeny of *Anisopteomalus calandrae* on 7 maize genotypes with different grain resistance but similar host (*Sitophilus zeamais*) densities.

Lines display the 10, 25, 50, 75 and 90th percentiles and outliers of parasitoid progeny per female per day on each of the maize genotypes. ANOVA showed no differences for parasitoid progeny (df=6, 76 F= 0.9 P=0.5).

Table 3.10: Interaction of seven maize genotypes with two host biotypes, *Sitophilus zeamais* and a parasitic wasp, *Anisopteromalus calandrae*

Biotype		Oaxaca			Poza Rica	
Variety	<i>A. calandrae</i>	<i>A. calandrae</i> + <i>S. zeamais</i>	hosts (controls)	<i>A. calandrae</i>	<i>A. calandrae</i> + <i>S. zeamais</i>	host (controls)
Ca	18 ±1.1	71.2 ±4.3	70.5 ±1.2	12.4 ±1.6	55 ±4.1	49.2 ±4.4
Hy w	15.3 ±7	53.5 ±8.9	59.5 ±1.7			
Hy y	16.5 ±3.4	118.0 ± 4.8	117 ±5.9	10.2 ±2.	45.3 ±3.2	44.0 ±3.5
Pop 84	0.6 ±0.4	0.6 ±0.4	1.0 ±0.4	4.3 ±1	10 ±1	10.5 ±0.6
Pop 80	2.6 ±1.3	3.6 ±1	2.5 ±1.3	9 ±3.2	24 ±5.8	21.8 ±0.0.5
Sin	2.4 ±0.7	9.8 ±0.8	10.3 ±2.0			
Yuc	7.8 ±1.2	13.6 ±0.8	13.6 ±2.0			
LSD	7.5	10.7	7.8	6.0	10.2	9.1

Host biotypes were *Sitophilus zeamais* strains collected in Poza Rica (Veracruz) and in the state of Oaxaca (Mexico)

A. calandrae : mean number of parasitoid progeny; *A. calandrae* + *S. zeamais*: mean number of emerged maize weevils +parasitoid progeny, hosts: number of maize weevils that emerged from controls.

Correlation of host number with parasitoid progeny was 0.9 for maize weevil biotypes. Host numbers of samples exposed to parasitoids (= the sum of emerged parasitoids+emerged maize weevils) were compared to controls with T-tests. They were not significantly different for any of the genotypes, indicating that from all large weevil larvae that died a parasitoid emerged.

Table 3.11 : Performance of *Anisopteromalus calandrae* on 7 maize genotypes with different grain resistance and similar host density (Host : *Sitophilus zeamais*)

Variety	<i>A. calandrae</i>	Host	Number of samples
	per day		
Ca	2.8 ±0.4	49.4 ±4.1 ab	5
Hy w	3.5 ±0.4	51.3 ±2.0 ab	19
Hy y	3.3 ±0.4	46.4 ±1.8 a	11
Pop 80	3.0 ±0.4	55.9 ±3.0 ab	8
Pop 84	3.2 ±0.4	56.8 ±1.3 b	10
Sin	4.0 ±0.4	49.7 ±1.8 ab	17
Yuc	3.5 ±0.4	52.2 ±2.6 ab	13
Critical difference	not significant	10.4	

Ac/day: indicates the number of parasitoid progeny per female wasp per day; Host : number of suitable hosts at the time of exposure to parasitoids; N : number of samples.

ANOVA showed no differences for parasitoid progeny: df=6, 76 ;F= 0.9; P=0.5. ANOVA for hosts showed a weak significance df=6, 76 F=2.2 p=0.51. Numbers followed by the same letter are not significantly different in Tukeys multiple comparison test

Table 3.12: Interaction of three maize genotypes with *Sitophilus zeamais* and a parasitoid, *Anisopteromalus calandrae* over several generations.

Variety	No maize weevil		% damage	
	no wasps	with wasps	no wasps	with wasps
Ca	452.7 ±48a	248.2 ±25a	91.8 ±2a	67.3 ±7.8a
Hy R	686 ±131a	253 ±47a	88 ±2.6a	73.9 ±11.6a
Pop 84	50.5 ±2.5b	62.2 ±4b	3.5 ±0.3b	3.9 ±0.5b

No wasps: no parasitoids were added; with wasps: parasitoids were added to maize infested with maize weevil larvae (untransformed data); % damage: percent damaged grain at the end of the experiment. Significant differences within columns are indicated by different letters (Tukey-Kramer)

B.3 - Interaction of parasitoids with maize genotypes and maize weevils over several generations.

In the long-term experiment, damage and number of maize weevils were significantly different among treatments. Maize weevil numbers were log transformed for analyses (Table 3.12). Their number was significantly different between the resistant (Pop 84) and the susceptible (Ca and CML244x349) genotypes, but not among the susceptible genotypes for either parasitoid treatment (Tukey-Kramer multiple comparison). Parasitic wasps had a significant impact only on the susceptible genotypes Ca and Hy R (Table 3.12).

For damage there were again significant differences between the same resistant genotype and the two susceptible ones, but not among the susceptible genotypes (Table 3.12). Although mean damage was lower for the susceptible genotypes in presence of parasitic wasps this difference was not significant (unpaired t-test, 95% confidence level).

Discussion

The location of large maize weevil larvae close to the pericarp and the relative length of the ovipositor of *A. calandrae* seems to reduce the possibility of spatial refuge, although some kernels were large enough to allow the host to stay out of reach of the ovipositor of *A. calandrae*. Smith et al. (1994) suspected easier host detection in smaller kernels, as the host

larvae would be forced to be closer to the surface, due to the large size of their tunnels, which attain 2mm at pupation (Smith, 1993). Lower attack rates of *A. calandreae* when foraging on immature maize weevils in corn than on bruchid hosts in beans, observed by Smith (1994) were attributed to the lower detectability of maize weevil larvae. Weevil larvae produce no windows before pupation, like bruchids, which settle directly beneath the seed coat, and thus facilitate host handling by the parasitoid *A. calandreae* (Ryoo and Chun, 1993). Therefore, even if the host is in reach of the ovipositor, being at some distance from the pericarp may lower its detectability in a large maize genotype like Cacahuazintle. It may also defend itself easier if only part of the host chamber is close to the surface and accessible to the parasitoid. In fact, Urello and Wright (1989) and van den Assen et al. (1984) observed aggressive or escape behavior if the larvae was touched. The relative closeness of host larvae to the grain surface decreases the hypothetical impact of endosperm hardness on parasitoids. The parasitoid *L. distinguendus* which occupies overlapping niches with *A. calandreae* (Ryoo et al., 1996), has a much smaller ovipositor. In a field experiment where both parasitoids competed, *L. distinguendus* ended up being the dominant species (Chapter 5), and Ryoo et al. (1996) found that *A. calandreae* and *L. distinguendus* were able to co-exist in a competitive situation. Consequently, ovipositor size is either not important beyond a certain limit, or can be compensated by higher host finding efficiency.

The experiments with different maize genotypes, showed that resistant varieties with pronounced kernel hardness, such as Sinaloa 35 and Population 84, did not impede successful attack by *A. calandreae*. Parasitoid progeny was a function of host density even when hosts were few as a consequence of grain resistance (Table 3.9, 3.10).

We did not observe any indirect effects of genotype quality as a food source for the host on parasitoid mortality or development rate. Ghani and Sweetman (1955) found that parasitoid development was slower when the host, *Sitophilus granarius* was reared on maize

than when reared on wheat. Ryoo et al. (1990) found only differences in fecundity of the parasitic wasp, not in development time, when two different varieties of rice were used as host food. Host quality on all maize genotypes was adequate to support the development of larval parasitoids and feeding of adult wasps on all genotypes, indicating that hosts quality was not affected by grain resistance. Hassell et al. (1985) observed a host mortality of 5.8% when the same wasp parasitized *Callosobruchus maculatus* (Coleoptera: Bruchidae) on beans. This could possibly be explained by the smaller host size of the bruchid, which may not allow development of a parasitoid larvae after hemolymph feeding by the adult.

Maize weevil biotype did not influence our results. Baker and Throne (1995) observed that *A. calandreae* was more successful at parasitizing a strain of relatively large rice weevils (*Sitophilus oryzae*) on wheat. The Oaxaca strain is slightly larger (Chapter 1), but there was no indication of a higher parasitism rate.

Host numbers varied considerably among samples, experiments, and maize weevil biotypes. Similar observations were made by Smith (1992) and Throne (1994). Adjustment for host density is difficult, due to the aggregated distribution of maize weevil larvae (Legg et al., 1987; Dobie 1974). Frequently, several larvae are found in the same kernel, while others remain uninfested. Moreover, individual variations among wasp progeny were important, as observed by Smith (1994). While it was found that resistant varieties do not negatively affect the parasitoids, detection of very subtle differences among maize varieties were not possible under these experimental conditions. Number of progeny per female was low, 3.5 per day, as compared to *Pteromalus cerealellae*, which parasitized twice as many grain moths. Higher rates were only achieved at much higher host densities (unpublished results). Therefore it is questionable whether this parasitoid can efficiently control maize weevils infestations, as the host densities used in the experiments already are far beyond the threshold at which grain is severely damaged, and biological control agents are expected to exert control at much lower

densities (Smith, 1994). Fecundity of the weevil parasitoid *Lariophagus distinguendus* varies considerably among strains of different geographical origin (Steidle and Schoeller, 2002), so that comparison of different strains of *A. calandreae* may be a way to find a more efficient biootype.

The long term experiment gave no additional information on maize genotype interaction with parasitic wasps. Host numbers on the resistant variety were too low to allow establishment of a parasitoid population. However, the results show, that grain resistance is an efficient means to prevent extensive damage in stored maize.

Conclusion

The experiments conducted revealed no antagonistic interaction of parasitic wasps with maize resistant to storage pests, for either of the two host-parasitoid assemblages examined. The susceptible maize genotype had a negative effect on performance of *Pteromalus cerealellae*, as compared to a resistant genotype. Successive experiments suggested that these differences could be attributed to a lower detectability of hosts in the susceptible genotypes. Further studies will be necessary to determine if olfactory clues contributed to this difference, or if differences in kernel structure influenced the detectability. This parasitoid had the ability to quickly detect a host at very low host density. This quality, combined with a high number of progeny and a short development time make it a good candidate for biological control of the Angoumois grain moth.

Kernel hardness, frequently mentioned as resistance factor to *Sitophilus zeamais*, had no negative impact on its parasitoid *Anisopteromalus calandrae*. The effect of kernel resistance on the rate of increase of the maize weevil far outranked the effect of the parasitoid, whose reproductive rate was much lower than that of *P. cerealellae*. *A. calandrae* has been described as a good candidate for biological control (Press et al., 1984), a comparison of strains of different geographical origin of this parasitoid may be an appropriate way to find the most efficient biotype. To protect stored grain from damage parasitic wasps must have a high searching efficiency at low host densities.

Acknowledgements:

We thank Mary Fritzsche Hoballah for collecting parasitoids, Jan Hirabayashi for help with the identification of the parasitoids, Dr. J. Berthaud and Dr. Alfonso Aguirre for providing the maize genotypes from Oaxaca, and Nasario Baleras Garcia for technical help. We are grateful to Jacqueline Moret for help with the statistics. The research was conducted at the laboratory of Entomology at the International Maize and Wheat Improvement (CIMMYT) .

References

- Arnason, J.T., B. Baum, J. Gale, J.D.H. Lambert, D. J. Bergvinson, B.J.R. Philogène, J.A. Serratos, J. A. Mihm, D. C. Jewell. 1994.** Variation in resistance of Mexican landraces of maize to maize weevil *Sitophilus zeamais*, in relation to taxonomic and biochemical parameters. *Euphytica* 74 : 227-236
- Arnason, J. T., J. Gale, B. C. d. Beyssac, A. Sen, S. S. Miller, B. J. R. Philogène, J. D. H. Lambert, R. G. Fuicher, J. A. Serratos, and J. A. Mihm. 1992.** Role of phenolics in resistance of maize grain to the stored grain insects, *Prostephanus truncatus* (Horn) and *Sitophilus zeamais* (Motsch.). *J. Stored Prod. Res.* 28 : 119-126
- Baldwin I.T., and C.A. Preston. 1999.** The eco-physiological complexity of plant responses to insect herbivores. *Planta* 208: 137-145
- Baker, J. E., and J. E. Throne. 1995.** Evaluation of a resistant parasitoid for biological control of rice weevils in insecticide-treated wheat. *J. Econ. Entomol.* 88: 1570 - 1579
- Baker, J. E., and D. K. Weaver, 1993.** Resistance in field strains of the parasitoid *Anisopteromalus calandrae* (Hymenoptera : Pteromalidae) and its host, *Sitophilus oryzae* (Coleoptera: Curculionidae), to malathion, chlorpyrifos-methyl, and pirimiphos - methyl. *Biol. Control* 3: 233-242
- Benrey, B., A. Callejas, L. Rios, K. Oyama, and R. F. Denno. 1998.** The effects of domestication of *Brassica* and *Phaseolus* on the interaction between phytophagous insects and parasitoids. *Biol. Control*, 11:130-140
- Benrey, B. , and R. F. Denno 1997.** The slow-growth-high-modality hypothesis: A test using cabbage butterfly. *Ecology* 78 : 987 -999
- Bergvinson, D. J. 1994.** Windows of maize resistance In: *Insect Resistant Maize: Recent Advances and Utilization; Proceedings of an International Symposium held at the International Maize and Wheat Improvement Center (CIMMYT) 27 Nov - 3 dec. 1994 Mexico DF. CIMMYT*
- Botrell, D.G., P. Barbosa, F. Gould 1998.** Manipulating natural enemies by plant variety selection and modification: A realistic strategy? *Annual Review of Entomology* 43 : 347-367
- Brower, J. H. 1991.** Potential host range and performance of a reportedly monophagous parasitoid, *Pteromalus cerealellae* (Hymenoptera: Pteromalidae). *Ent. News* 102 (5) , 231 –235

- Buckland P.C. 1981.** The early dispersal of insect pests of stored products as indicated by archaeological records. J. Stored Prod. Res. 17: 1-12
- Campbell, B.C. and S.S. Duffey.1979.** Tomatine and parasitic wasps: Potential incompatibility of plant antibiosis with biological control. Science 205 : 700-702
- Choi, W.I.; T.J. Yoon, and M.I. Ryoo. 2001.** Host-size-dependent feeding behaviour and progeny sex ration of *Anisopteromalus calandrae* (Hym., Pteromalidae). J. Appl. Ent. 125: 71-77
- Classen, D., J.T. Arnason, J.A. Serratos, J.D.H. Lambert, C. Nozolillo, and B.J.R. Philogène 1990.** Correlations of phenolic acid content of maize to resistance to *Sitophilus zeamais*, in CIMMYT's collections. J. Chem. Ecol. 16 : 301-315
- Cortesero, A. M., J. P. Monge, and J. Huignard 1993.** Response of the parasitoid *Eupelmus vuilleti* to the odours of the phytophagous host and its host plant in an olphactometer. Entomol. Exp. Appl. 69,109-116
- Cortesero, A. M., J. P. Monge , and J. Huignard. 1995.** Influence of two successive learning processes on the response of *Eupelmus vuilleti* (Hymenoptera, Eupelmidae) to volatile stimuli from host and host plant. J. Insect Behaviour 8 : 751 – 762
- Damien, D., E.Imbert,C. Bressac, C.Thibeau and C.Chevrier, 2001.** Egg-laying, pre-imaginal growth dynamics, and mortality in *Eupelmus orientalis* and *Dinarmus basalis*, two solitary ectoparasitoids of *Callosobruchus maculatus*. Entomologia Experimentalis et Applicata 99 : 97-105
- Dobie, P. 1974.** The laboratory assessment of the inherent susceptibility of maize varieties to post-harvest infestation by *Sitophilus zeamais* Motsch. J. Stored Prod. Res. 10: 183-197
- Dobie, P.,C.P.Haines, R.J. Hodges, P.F.Prevett and D.P. Rees, 1991.** Insects and Arachnids of tropical stored Products: Their Biology and Identification . National Ressources Institute
- Elzen, G.W., H.J. Williams and S.B.Vinson, 1986.** Wind tunnel flight responses by hymenopterous parasitoid *Campoletis sonorensis* to cotton cultivars and lines. Entomol. Exp. Appl. 42, 285-289
- Espinal, R., R.H. Markham and V.F. Wright. 1996.** Honduras-Summary of activities on the larger grain borer and storage pest status in meso-America. 109-124 .In Hill, G.& G. Farrell (eds.), Proceedings of the East and Central Africa storage pest management workshop, Naivasha, Kenya 14-18 April 1996. CAB Wallingford, UK.
- Flinn, P.W., D.W. Hagstrum and W.H. McGaughey. 1996.** Suppression of beetles in

- stored wheat by augmentative releases of parasitic wasps. *Environ. Entomol.* 25 : 505-511
- Ghani, M. A. , and H. L. Sweetman. 1955.** Ecological studies on the granary weevil parasite, *Aplastomorpha calandreae* (Howard). *Biologia (Lahore)*1 :115 – 139
- Gomez, L.A., J. U. Rodriguez, C. U. Poneleit, and B. F. Blake 1983.** Relationship between some characteristics of the corn kernel pericarp and resistance to the rice weevil, (Coleoptera:Curculionidae). *J. Econ. Entomol.* 76 : 797-800
- Harlan, J.R. 1992.** Domestication of Seed Crops. In, *Crops and Man* by J.R. Harlan. American Society of Agronomy, Inc. Crop Science Society of America Inc. Madison, Wisconsin, USA
- Hassell, M.P.,C.M. Lessells, and G.C. McGavin. 1985.** Inverse density dependent parasitism in a patchy environment: a laboratory system. *Ecol. Entomol.* 10 : 393-402
- Holloway, G.J. 1985.** The effect of increased grain moisture content on some life history characters of *Sitophilus oryzae* (L.) after staining egg plugs with acid fuchsin. *J. Stored Prod. Res.* 24, (4), 165-169
- Horber, E. 1987.** Methods to detect and evaluate resistance in maize to grain insects in the field and in storage. In: *Toward Insect Resistant Maize for the Third World.* Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects. CIMMYT, Mexico, 9-14 March 1987.p 140-150
- Ishimoto, M. , K. Suzuki, M. Iwanaga, F. Kikuchi and K. Kitamura. 1995.** Variation of seed alpha-amylase inhibitors in the common bean. *Theoretical & Applied Genetics.* 90, (3-4), 425-429
- Kareiva, P. R.Sahakian.1990.** Tritrophic effects of a simple architectural mutation in pea plants. *Nature* 326: 388-390
- Kauffman, W. C. , and R. V. Flanders. 1985.** Effects of variably resistant soybean and Limabean cultivars on *Pediobius foveolatus* (Hymenoptera: Eulophidae), a parasitoid of the Mexican bean beetle, *Epilachna varivestis* (Coleoptera: Coccinellidae). *Environ. Entomol.* 14 : 678 – 682
- Legg, D. E., R. J. Barney, P. W. Tipping, and J. G. Rodriguez. 1987.** Factors influencing the distribution of maize weevil (Coleoptera:Curculionidae) eggs on maize. *Environ. Entomol.* 16: 809-813
- Levinson, A. , and H. Levinson. 1995.** Reflections on structure and function of pheromone glands in storage insect species. *Anz. fur Schaedlingsk. Pflanzensch. Umweltsch.* 68 (5) , 99-118

- Obrycki, J.J. and M. J. Tauber. 1984.** Natural enemy activity on glandular pubescent potato plants in the greenhouse: An unreliable predictor of effects in the field. *Environ. Entomol.* 13, 679-683
- Press, J. W. 1984.** Suppression of residual populations of the rice weevil, *Sitophilus oryzae*, by the parasitic wasp, *Anisopteromahis calandreae*. *J. Georgia Entomol. Soc.* 19: 110-113
- Price, P. W., C. B. Bouton, P. Gross, B. A. McPheron, J. N. Thompson, and A. E. Weis. 1980.** Interactions among three Trophic Levels: Influence of plants on interactions between insect herbivores and natural enemies. *Annual Research Ecological Systems* 11: 41 – 43
- Riggin, T. M., B. R. Wiseman, D. J. Isenhour, and K. E. Espelie. 1992.** Incidence of fall army worms (Lepidoptera : Noctuidae) parasitoids on resistant and susceptible corn genotypes. *Environ. Entomol.* 21: 888-895
- Ryoo, M. I., T. J. Yoon , and S. S. Shin. 1996.** Intra- and Interspecific competition among two parasitoids of the rice weevil (Coleoptera:Curculionidae). *Environ. Entomol.* 25 : 1101 – 1108
- Ryoo, M., C.K. Yoo, and Hong, Y.S. 1990.** Influences of food quality for *Sitophilus oryzae* (Coleoptera : Curculionidae) on life history of *Lariophagus distinguendus* (Hymenoptera: Pteromalidae). *International Working Conference on Stored Product Portecion.* Bordeaux, France Sept 1990 : 211-219
- Ryoo M. I. and Y.S.Chun. 1993.** Oviposition behavior of *Callosobruchus chinensis* (Coleoptera: Bruchidae) and weevil population growth: Effects of larval parasitism and competition. *Environ. Entomol.* 22 : 1009-1015
- Sass J. E., 1977.** Development of the caryopsis. In *Corn and Corn Improvement*. D.F. Sprague (Editor) American Society of Agronomy Inc. Publisher. Madison, Wisconsin, USA, 1977. p 89-99
- Smith, C.M. 1994.** An overview of the mechanisms and bases of insect resistance in maize. In: *Insect Resistance in Maize: Recent Advances and Utilization; Proceedings of an International Symposium held at th International Maize and Wheat Improvement Center (CIMMYT) 27 Nov - 3 dec. 1994 Mexico DF. CIMMYT.*
- Smith, L., and J. W. Press. 1992.** Functional Response of *Anisopteromalus calandreae* (Hymenoptera : Pteromalidae): influence of host numbers versus host density. *J. Entomol. Sci.* 27: 375 – 382
- Smith, L. 1993.** Host-size preference of the parasitoid *Anisopteromalus calandreae*

- (Hymenoptera: Pteromalidae) on *Sitophilus zeamais* (Coleoptera: Curculionidae) larvae with a uniform age distribution. *Entomophaga* 38 : 225 – 233
- Smith, L. 1994.** Temperature influences functional response of *Anisopteromalus calandrae* (Hymenoptera : Pteromalidae). *Ann. Entomol. Soc. Am.* 87 : 849- 855
- Smith, L., D. K. Weaver, and R. T. Arbogast. 1995.** Suitability of the maize weevil and Angoumous grain moth as hosts for the parasitoids *Anisopteromalus calandrae* and *Pteromalus cerealella*. *Ent. Exp. Appl.* 76 : 171-177
- Steidle, J. L. M. , and M. Schoeller. 1997.** Olfactory host location and learning in the granary weevil parasitoid *Lariophagus distinguendus* (Hymenoptera : Pteromalidae). *J. Insect Behaviour* 10 : 331 – 342
- Steidle, J.L.M., and Schoeller, M. 2002.** Fecundity and ability of the parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae) to find larvae of the granary weevil *Sitophilus granarius* (Coleoptera: curculionidae) in bulk grain. *J. Stored Prod. Res.* 38 : 43-53
- Steidle, J.L.M., and J. Ruther. 2000.** Chemicals used for recognition by the granary weevil parasitoid *Lariophagus distinguendus*. *J. Chem. Ecol.* 26 : 2665-2675
- Steidle, J.L.M., and A.Fischer. 2000.** Quantity does matter: How feces are used for host stage selection by granary weevil parasitoid *Lariophagus distinguendus*. *J.Chem. Ecol.* 26 : 2657-2664
- Throne, J. E. 1994.** Life history of immature maize weevils (Coleoptera:Curculionidae) on corn stored at constant temperatures and relative humidities in the laboratory. *Environ. Entom.* 23: 1459-1471.
- Turlings, T.C.J., J. H. Tumlinson , and W. J. Lewis. 1990.** Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250 : 1251 – 1253
- Turlings, T.C.J., F.L. Waeckers, L.E.M. Vet, W.J. Lewis and J. H. Tumlinson, 1993.** Learning of host-finding cues by hymenopterous parasitoids. In D.R. Papaj and A.C. Lewis (eds), *Insect Learning*. Chapman and Hall, NY; 51-78
- Turlings, T.C.J. , and B. Benrey. 1998.** Effects of plant metabolites on behaviour and development of parasitic wasps. *Ecoscience* 5 : 321 –333
- Urrelo, R., V. F. Wright, R. B. Mills , and C. E. Wasson 1989.** Screening for resistance against the maize weevil *Sitophihts zeamais* Motschulsky (Coleoptera : Curculioidea) in Peruvian maize accessions. *Turrialba* 39, 9-17
- Urrelo, R., and V. F. Wright, 1989.** Development and behaviour of immature stages of the maize weevil (Coleoptera: Curculionidae) within kernels of resistant and susceptible

- maize. Ann. Entomol. Soc. Am. 82, 712-716
- Van den Assen, J. F.A. Putters and T.C. .Prins. 1984.** Host quality effects on sex ratio of the parasitic wasp *Anisopteromalus calandrae* (Chalcidoidea, Pteromalidae). Netherlands Journal of Zoology 34 : 33-62
- Vinson, S. B. , and G. F. Iwantsch. 1980.** Host suitability for insect parasitoids. Ann. Rev, of Entomol. 25 : 397 –419
- Wen, B. , and J. H. Brower. 1994.** Suppression of *Sitotroga cerealella* in shelled corn by the parasitoid *Pteromalus cerealellae*. J. Entom. Sci. 29 : 254-258
- White, C., and S.D. Eigenbrode. 2000.** Effects of surface wax variation in *Pisum sativum* on herbivorous and entomophagous insects in the field. Environ. Entomol. 29: 773-780

OLFACTORY ATTRACTION OF PARASITIC WASPS TO STORED MAIZE

Abstract

In a series of experiments carried out with a 6-arm olfactometer the chemically mediated attraction of the parasitoid *Anisopteromalus calandrae* to maize seeds was investigated. Female *A. calandrae* that had an oviposition experience on immature maize weevils (*Sitophilus zeamais*) developing inside maize kernels, showed a significant olfactory attraction to maize seeds infested with maize weevil larvae, but also to odors emitted by undamaged maize. In contrast, wasps that had experience with this host developing in wheat kernels, were only attracted to infested maize, but not to undamaged maize kernels. In a second experiment, *A. calandrae* could choose among the odors emitted by six different maize varieties that have different levels of resistance to the maize weevil. The maize varieties were either all infested or all uninfested. The wasps showed no preference for the odor of any variety, whether these were infested by weevils or not, and irrespective of a previous experience with the host and seed.

Volatile compounds of seeds of the six maize varieties were collected over a period of ten hours. Detectable amounts of volatiles were collected only from infested maize. The four major volatile compounds, tridecane, pentadecane and two other compounds that very likely are (Z)-7-pentadecene and (Z)-6-pentadecene, were the same for all six maize genotypes. They were emitted in similar amounts, indicating that there is apparently no detectable

difference in quantity or quality of volatile blends between resistant and susceptible maize kernels infested by maize weevil larvae.

Key words: Olfactometer, parasitoids, stored maize, volatiles, *Sitophilus zeamais*, *Anisopteromalus calandrae*

Introduction

Parasitic wasps of stored grain pests have to locate first the appropriate habitat of their hosts, secondly the infested seed among all uninfested seeds, and finally the host within the seed. Endophytic hosts often leave few or no signs of infestation, until they emerge as adults, therefore the parasitoid may have to rely on a complex set of subtle and indirect stimuli. Host location and acceptance within the storage habitat may include chemical stimuli directly from grain or the host, or from by-products of the host (Vinson, 1976). Bristles or faeces of the host affects the behavior of a number of parasitoid species (Parra et al., 1996; Steidle and Schoeller, 1997), mandibular gland secretions can be involved in host recognition (Corbet, 1971), and cuticular hydrocarbones are thought to be used for host quality recognition and acceptance (Howard, 2001). Van den Assen et al. (1984) suspected storage parasitoids to be guided by vibrations and by chemicals or temperature gradients. *Lariophagus distinguendus* (Foerster) (Hym., Pteromalidae), a parasitoid of many storage Coleoptera, can be elicited to oviposit in artificial domes treated with extracts from the cocoons of the host (cigarette beetle, *Lasioderma serricone*), without other physical stimuli, such as acoustic signals (Jimenez et al, 1996). Steidle (2000) reported that these parasitoids show drilling activity on artificial grains treated with extracts of faeces from another host, the granary weevil, *Sitophilus granarius*. The importance of cues emanating from the host-plant and the host is well documented (Vet and Dicke, 1992).

Plants are able to react to an attack of herbivores by producing specific volatile compounds (Dicke, et al., 1990; Turlings et al., 1991). In some cases, these plant reactions can be induced by insect oral secretions (Alborn et al., 1997). These induced odours are attractive to parasitic wasps (Turlings et al., 1990), and allow parasitoids not only to recognize the plant in the field (Kessler and Baldwin, 2001), but also to discriminate among herbivore species (De Moraes et al., 1998). Induced odor blends vary in quantity and quality

among different plant species but also among varieties of the same species (Gouinguéné et al., 2001). Such differences can affect the attractiveness of the plants. For instance, Rapusas et al. (1995) found that a predator of planthoppers, *Cyrtorhinus lividipennis*, is more attracted to volatiles of certain rice genotypes. Furthermore, plants may also affect the components of kairomones that are attractive to parasitoids of the herbivore (Nordlund and Sauls, 1981)

Stored seed, such as maize, becomes metabolically active only upon hydration (Watson, 1987). Therefore a phytochemical induction by insects, as in the vegetative stage of the plant is unlikely. Parasitoids will have to rely on cues from the host itself or from its interaction with substances already present in the seed. Volatiles emanating from infested grain are likely to be a product of the interaction of the herbivore with specific parts of the seed, such as embryo or endosperm, that are very different in quality, and /or with the micro-organisms that inevitably follow such an attack. It is likely that the quantity and quality of odors varies with seed and insect species. Seitz and Sauer (1996) collected volatiles of sorghum infested with different storage pests and found large quantitative differences among the insect species. The intact pericarp layers of uninfested seed may prevent volatile substances, attractive to grain pests, from being released (Trematerra et al., 1999).

The use of chemicals by parasitoids to locate their hosts can be genetically fixed, but learning often plays an important role (Turlings et al., 1993). In addition to learning from oviposition experiences as an adult, parasitoids can learn cues of the host-plant at the time of emergence (Hérard et al., 1988; Kester and Barbosa, 1991). For parasitoids of endophytically living hosts, a pre-emergence learning process can determine how females respond to host and host-plant cues (Caubert and Jaisson, 1990; Cortesero et al., 1995; Monge and Cortesero, 1996). Most ectoparasitoids of stored grain pests develop entirely within the cavity excavated in the seed by their host and are intimately in contact with host and seed related odours, until they emerge as adults.

In the developing world, post-harvest losses have limited the success of improved high yielding maize varieties. The use of resistant genotypes is a strategy of integrated pest management to control storage pests without the use of insecticides. The compatibility of this method with naturally occurring biocontrol agents is an important factor for their success. The parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) is a generalist ectoparasitoid of many endophytically developing storage beetles of cereals and beans (Gani and Sweetman, 1955), among them the maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae) one of the most destructive storage pests in the world. *A. calandrae* is frequent in stores of maize in tropical climates, where it considerably reduces infestation of the maize weevil (Chapter 6, Arbogast and Mullen, 1990).

In this study we investigated first how this parasitoid responds to volatiles of infested and uninfested maize seed in an olfactometer and how parasitoid rearing and experience on the same host, but on a different host-seed (wheat), affects this response. Secondly, we investigated whether odors emitted from seeds of resistant maize varieties, infested or not, are as attractive as the odors emitted from more susceptible maize genotypes. Furthermore, we collected and analyzed the volatiles emanating from six different varieties to find out if there are differences in odour quality or quantity between infested and uninfested seed, that parasitoids may use and that could be exploited to enhance the attractiveness of stored maize to biological control agents.

Material and methods

Origin of insects and grain

Both insect colonies, *Sitophilus zeamais* and the parasitic wasp *Anisopteromalus calandrae* were collected at the CIMMYT (International Maize and Wheat Improvement Center) experimental station in Poza Rica (Veracruz, Mexico). These strains had been maintained in the laboratory for 16 months prior to the experiment. The maize weevils were reared in an incubation chamber at 26.5° C and 75 % R.H. on a yellow dent hybrid maize, grown in France for animal feed, or on wheat of the same origin. The parasitoids were reared on 4th instar maize weevil larvae that had infested either maize or wheat kernels (maize reared or wheat reared wasps). They were between one and four days old, mated and experienced on their respective rearing medium. The other maize varieties used in the experiments originated from tropical and subtropical climates, had different degrees of resistance to the pest *S. zeamais* and were provided by CIMMYT (Table 4.1)

6-arm olfactometer

A six-arm olfactometer as described by Turlings and Tamò (in prep.) was used. This apparatus consists of three interconnected levels (shelves), with the odor sources on the bottom, the insect release point in the middle and the actual odor choice chamber on the top. The wasps were released on the second shelf from a glass vial placed on the bottom of the central choice chamber. This vertically placed glass tube extends to the top shelf of the apparatus where it is connected to six arms that carry the odorous air flows among which the parasitoids can choose. The wasps would move to the top of this chamber as they are initially attracted by a light source placed above it. Each of the six arms leads to two successive connected glass vessels, the first one with a vertical trapping bulb, and the second with a

vertical port through which the air flow arrives from the bottom shelf *via* corrugated Teflon tubing. A double wire mesh screen impedes access to the last glass compartment of the arm. For these particular experiments the odor source was not placed in the odor source vessels on the bottom shelf but instead in the last glass compartment of an arm on the top shelf receiving the air flow. The temperature was between 25°C and 30°C. After the tests the olfactometer was cleaned with detergent, rinsed with acetone and pentane and, after the solvents had evaporated, sterilized at 200° C for 30 minutes.

Choice between infested and uninfested maize kernels

For the first olfactometer experiment, 30 maize kernels (Hy F), infested with 4th instar larvae of *S. zeamais* were used as one odor source and in the opposite arm an equal number of uninfested maize kernels constituted the second odour source. The other four arms were left empty. Six female wasps were released at a time into the olfactometer. Their position was noted after twenty minutes and they were removed from the olfactometer and discarded. A choice was considered valid if they had entered an arm and were at least at a distance of 3 cm from the choice chamber. Three groups of wasps reared on maize and three reared on wheat were tested alternating between them. The wasps were only used once. The experiment was replicated 6 times and the odor source position was changed between replicates, but not with respect to each other. For statistical analyses the Friedman test was applied where replicates were treated as blocks, and if significant means were compared with multiple comparison test (Student-Newman-Keuls).

Choice among infested or uninfested kernels of 6 different maize varieties

For the second experiment, female wasps reared either on maize or on wheat, were allowed to choose among the odors emitted by kernels from each of six maize genotypes. For infestation,

large quantities of each maize variety were exposed to unsexed *S. zeamais* for 10 days. To achieve high infestation rate even in very resistant genotypes, such as Sinaloa 35 and Population 84, the grain of all varieties was soaked in water prior to infestation and air dried (Chapter 3). More resistant genotypes were infested earlier, because of the longer development time of maize weevil larvae. The development of the larvae was monitored through dissection of kernels every three or four days. The wheat and maize used for rearing of the wasps were monitored in similar manner and the grain was eventually incubated at lower temperatures to slow development and obtain emergence of all parasitoids at the begin of the experiments. Forty kernels, all of them presenting signs of infestation, were used as odor source for the variety with the largest kernels. The number of kernels for the other varieties was adapted to the respective kernel size and amount of void volume, which was previously measured in vials of 60 ml filled with maize of each genotype. This way an equivalent true volume of each variety was used as odor source, both for infested and uninfested maize.

Groups of six female wasps were released at a time, and their position was noted after they had all chosen an arm, or after 15 minutes. The shorter time as compared to the first experiment was chosen because of a higher ambient temperature, which led to more activity among the wasps. Again 3 groups of 6 females reared on maize, and 3 groups reared on wheat, were tested alternating between them. Eight replicates were made in between which the olfactometer was cleaned. The maize kernels were replaced by fresh samples for each replicate. Between replicates, the positions of the odor sources were changed randomly. For statistical analyses the Friedman test was applied where replicates were treated as blocks, and if significant, multiple comparison was used to compare number of wasps attracted to different odor sources.

Volatile collection

For the volatile collection two maize samples that had been used previously for the olfactometer experiments were placed into the odor source vessels at the bottom shelf of the olfactometer. They were held in place by squares of wire mesh placed above the air inlet and 12 cm below the trapping filters, which contained 25mg of Super- Q absorbent powder for volatile collection (as described by Heath and Manukian, 1992) The port to the top of the olfactometer was closed with a Teflon-coated septum in a screw cap, but not tightly, to let excess air escape. Almost all of the incoming air (1.2l/min) was pulled through each trap. Collections lasted for 10 h and 15 min. The larval activity in the maize kernels generates heat so that the infested maize measured 32° C while the uninfested reached only 30° C prior to the volatile collection. Due to the air flow through the odor vessels the temperature of the infested maize kernels dropped from 32° C to 27° C. To maintain the ambient temperature at 30° C 6 florescent light tubes placed above the odor vessels were lit during the collection time. The volatile collection was replicated 4 times and 1 control collection was made with empty vessels containing only the squares of wire mesh used to hold the grain in place. After each collection the traps were extracted with 150µl methylene chloride, and 10µl of methylene chloride containing the internal standards n-octane and nonyl acetate (10 ng/µl) was added to the sample. Three µl of each sample were injected into a Hewlett Packard model HP 6890 gas chromatograph. The apolar EC-1 capillary column (30m 0.25mm I.D., 0.25µm film thickness, All tech Associates Inc. USA) was held at 50°C for 3 min and then programmed at 8°C per minute to 230°C where it was maintained for 9.5 minutes. Helium was used as a carrier gas (24cm/s). HP GC Chemstation software was used to quantify the components by comparison to the known quantity of internal standards. For volatile analysis see also Gouinguéné et al. (2001). Data was ln transformed and the total amount of volatiles was compared with

ANOVA and the Student -Newman -Keuls post-hoc tests. To compare single compounds among varieties the Kruskal-Wallis test was used.

A sample of each of two infested maize varieties (Ca and Pop 80) was also injected into a GC system with an Agilent 6890 on-line coupled to an Agilent 5973, transfer line 230°C, source 230°C quadrupole 150°C, ionisation potential 70 eV, scan range 0-400 amu, mass selective detector for identification of the detected volatile compound in infested maize.

The infested maize samples used for volatile collection were incubated at 26° C and 75% R.H. for 18 days to allow all third and fourth instar larvae to complete development and emerge. Samples were then frozen and all adult maize weevils were counted. Numbers were compared with ANOVA and a post-hoc test (Tuckey–Kramer).

Results

Choice between infested and uninfested maize kernels

The wasps responded well to the odors of infested and uninfested maize. Female parasitoids that had been reared on maize significantly preferred the odors of maize over the pure air from the empty arms of the olfactometer. They significantly more often chose the arm with the odors from infested maize than from uninfested kernels (Friedman Test: $P=0.0003$, Fig. 4.1, Table 4.1). Wasps reared on wheat had a significant preference for odors from infested maize, but did not distinguish between pure air and the odor of uninfested maize kernels (Friedman Test: $P=0.005$, Fig. 4.2). Few wasps stayed in the central choice chamber without entering any of the arms.

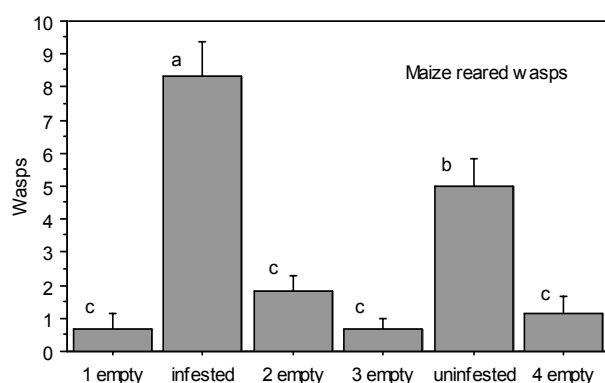


Fig. 4.1

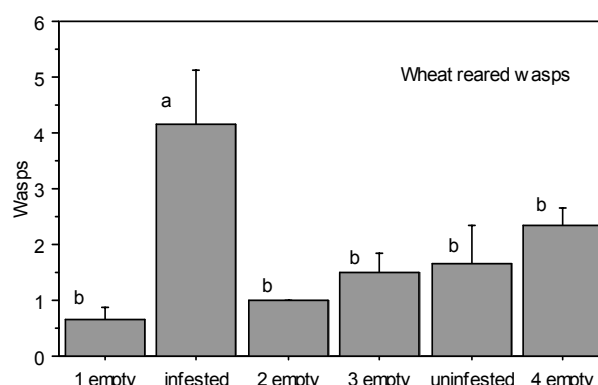


Fig. 4.2

Fig 4. 1: Preference of the female parasitoids *Anisopteromalus calandrae*, reared on maize, for odours in a six arm olphactometer

Results were analyzed with the Friedman test (DF= 5 Chi Square corrected for ties=23 Tied p-value=0.0003) and multiple comparisons were made with the Student-Newman-Keuls post hoc test.

Fig 4. 2 : Preference of the female parasitoids *Anisopteromalus calandrae*, reared on wheat, for odours in a six arm olphactometer

Results were analyzed with the Friedman test (DF= 5 Chi Square corrected for ties=16.7 Tied p-value=0.005) and multiple comparisons were made with the Student-Newman-Keuls post hoc test. Columns with the same letter are not significantly different.

Infested : maize containing maize weevil larvae (*Sitophilus zeamais*); uninfested: maize free of insect infestation; empty: arms containing no odour source.(Mean of six replicates) with the Student-Newman-Keuls post hoc test.

Columns with the same letter are not significantly different.

Table 4. 1 : Attractivity of maize varieties infested, or uninfested by larvae of *Sitophilus zeamais* to the parasitic wasp *Anisopteromalus calandrae* in a six-armed olphactometer.

Variety	Abrev.	Maize reared wasps		Wheat reared wasps		Mean host number	Resistance to <i>S.zeamais</i>
		infested	uninfested	infested	uninfested		
Cacahuazintle	Ca	2.5	3.4	2.3	2.8	188.5 ±9.1 a	susceptible
CML264xCML254	Hy w	2.9	2.6	2.0	2.5	220.3 ±10.6 ab	susceptible
CML287xCML285	Hy y	2.9	1.9	4.8	3.0	242.8 ±7.6 bc	susceptible
Eujura	Pop 80	2.1	2.3	2.4	1.8	220.5 ±8.6 ab	m. susceptible
Population 84	Pop 84	2.4	2.3	1.8	2.4	293 ±16.2 d	resistant
Sinaloa 35	Sin	1.9	2.9	1.8	2.4	281.5 ±7.9 cd	resistant

Numbers show the mean number of female wasps, reared either on maize or on wheat, that chose the variety. Mean host number shows the mean number of maize weevil larvae that emerged from samples used for measurement of volatiles. Numbers followed by the same letter are not significantly different for the Tuckey - Kramer post hoc test. Resistance indicates the grain resistance to the maize weevil. (m. : moderately)

Friedman test was applied where each release of 6 wasps into the olphactometer was treated as a block. There were no significant differences: maize reared wasp: infested $p=0.5$, uninfested $p= 0.4$, wheat reared wasps : infested $p=0.1$, uninfested $p= 0.6$

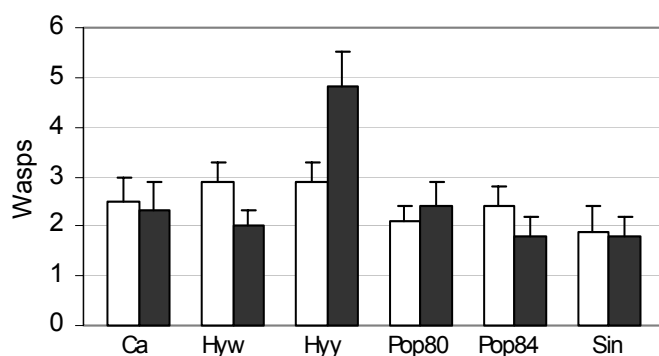


Fig. 4.3a Infested maize varieties

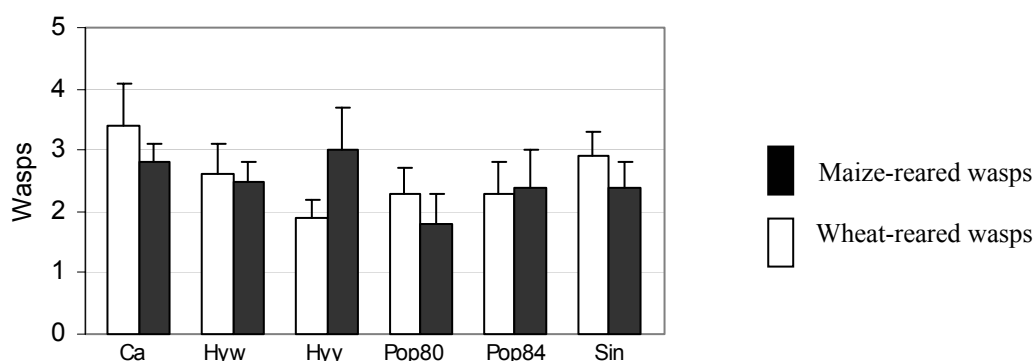


Fig. 4.3b Uninfested maize varieties

Fig. 4.3 Mean number of female wasps, *Anisopteromalus calandrae*, that were attracted to each maize variety, in a 6 arm olfactometer.

a: all varieties were infested with *Sitophilus zeamais*

b: maize was not infested

White: wasps that were reared on *S. zeamais* in maize

Black: wasps that were reared on *S. zeamais* in wheat

Numbers were not significantly different when compared with Friedman's nonparametric test.

Choice among infested or uninfested kernels of 6 different maize varieties

Wasps reared on maize or on wheat showed no preference for any of the odors of the six maize varieties, neither for infested nor for uninfested kernels (Friedman test, $P=0.5$, 0.4 , 0.1 , and 0.6 respectively for maize reared wasp infested and uninfested and wheat reared wasps infested and uninfested; Fig. 4.3 and Table 4.1). The wasps were more active than during the first experiment with choice between infested and uninfested maize.

Volatile collection

Infested grain emitted significantly more volatiles than uninfested grain, for which hardly any were detected (Student-Newman-Keuls post-hoc test after ANOVA $P<0.0001$, Fig. 4.4).

When infested, all six varieties emitted the same four compounds and in a similar ratio (Fig.4.5). The total amount of volatiles collected from infested kernels of the six maize varieties was not significantly different (ANOVA : $df=5$, 18 $F=2.3$ $P=0.6$, Fig. 4.6). None of the four individual compounds compared with the Kruskal-Wallis test differed significantly among varieties ($p>0.06$). The number of maize weevils that emerged from the samples used for the volatile collections varied significantly among varieties (ANOVA, $P<0.0001$, Fig. 4.7). The more resistant varieties contained about 25 to 30 percent more maize weevil larvae than the more susceptible due to the treatments prior to infestation that reduces resistance. However there was no correlation of weevil numbers with total amount of volatiles that were collected on these samples (Spearman rank correlation 0.02), or with the number of female parasitoids that chose the corresponding arm in the olfactometer (0.14).

Based on the mass spectra, two compounds were tentatively identified as tridecane and pentadecane. Two other substances showed very similar fragmentation patterns and may be (Z) -7-pentadecene and (Z) -6-pentadecene which were reported by Ruther and Steidle (2000), as substances released by faeces of the granary weevil, *Sitophilus granarius*, and from extracts of associated mites.

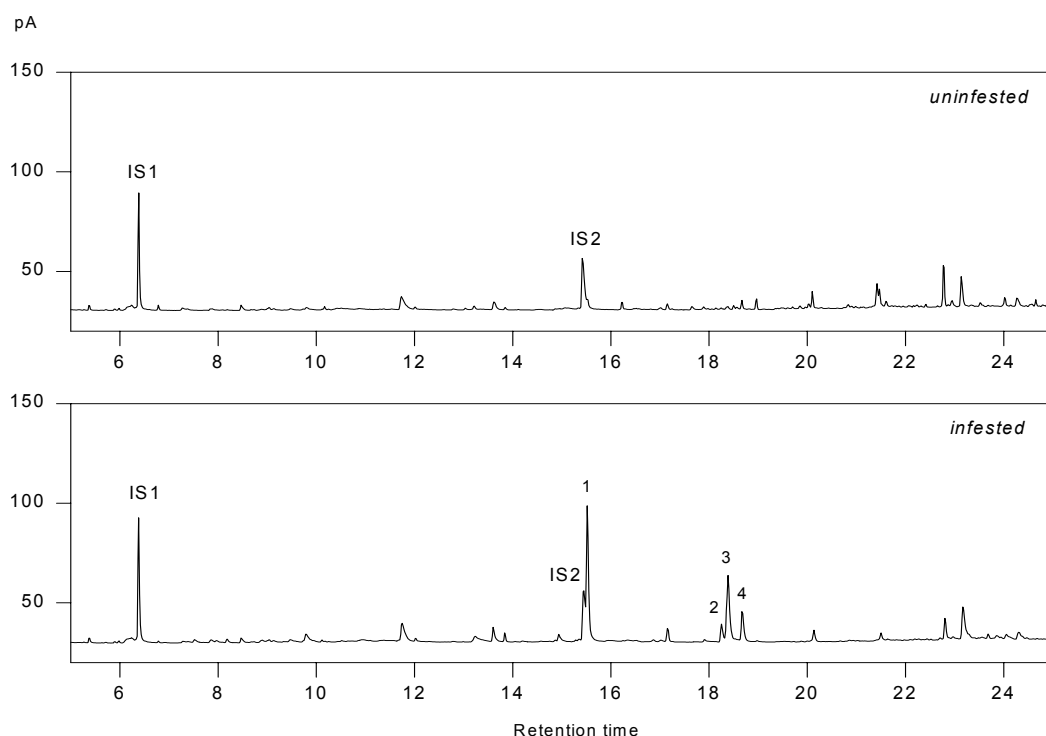


Fig.4.4. Chromatographic profiles of volatiles collected during 10 hours from maize seed (variety Pop 80), either uninfested or infested with mature larvae of *Sitophilus zeamais* .

Numbers indicate the compounds that were all emitted in significantly higher amounts in infested than uninfested maize throughout all varieties. (Student-Newman-Keuls test preceded by ANOVA: $p < 0.0001$) The profiles were the same for all maize genotypes tested (ANOVA $p = 0.06$ for total amounts, Kruskal-Wallis for individual compounds: $p > 0.060$) Mass spectrometry showed high similarities of compound 1 with tridecane and compound 4 with pentadecane. Two other substances showed very similar fragmentation patterns and may be (Z)-7-pentadecene and (Z)-6-pentadecene.

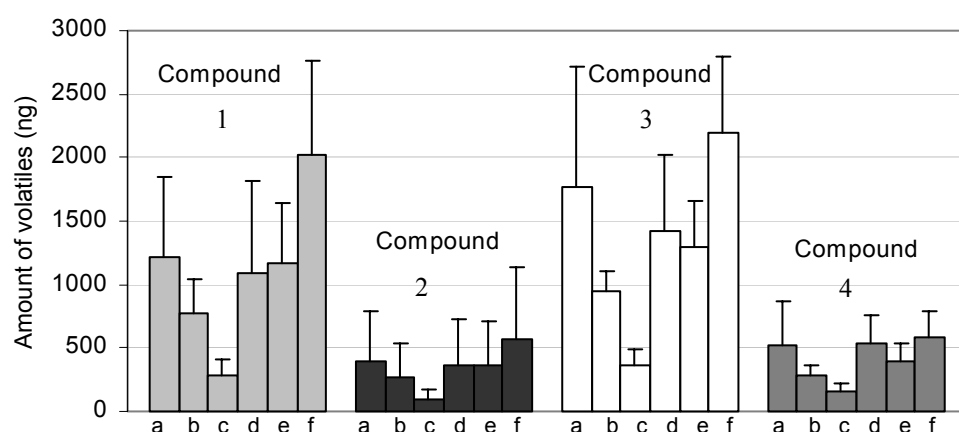


Fig 4.5: Amount of each of four individual compounds trapped on each of six infested maize varieties

Maize varieties: a: Ca, b: Hyw, c: Hyy, d: Pop80, e: Pop84, f: Sin

There were no significant differences for mean amount of each compound among maize varieties ($P = 0.4$, 0.2 , 0.3 and 0.7 respectively for compounds 1, 2, 3 and 4)

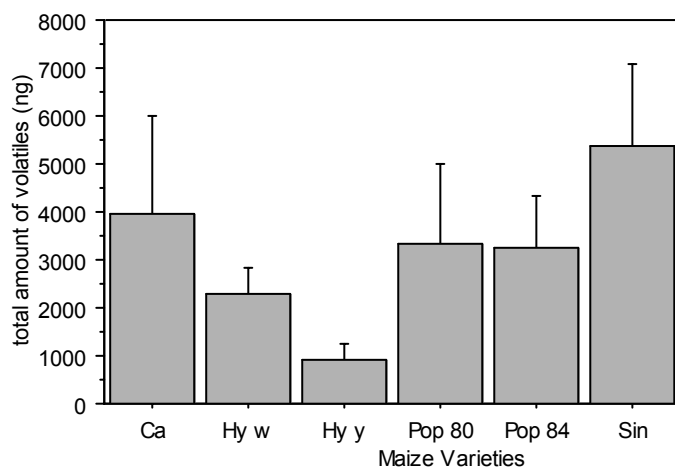


Fig. 4.5

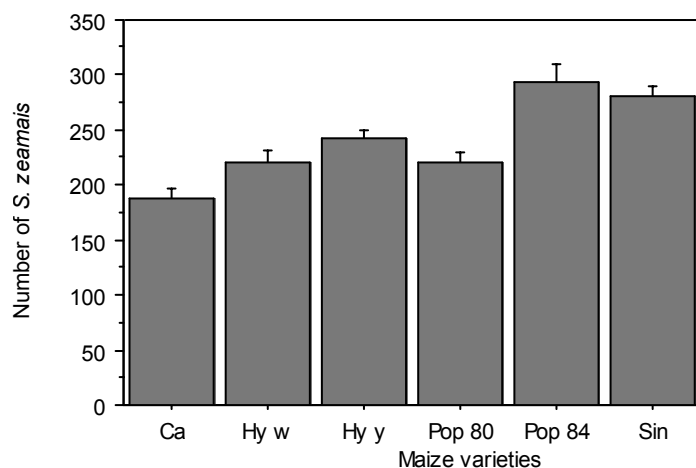


Fig. 4.6

Fig.4.5 : Means of total amount of volatiles (ng) collected during 10 hours from each maize variety, infested with mature larvae of *Sitophilus zeamais*. Amounts were ln transformed prior to analysis. ANOVA showed no significant variation: $df=18$; $F=2.3$; $p=0.6$. Multiple comparison with the Student-Newman-Keuls test showed a significant difference only between the variety with the highest and lowest total amount (Hy y and Sin) The amount of volatiles was not correlated with the number of maize weevil larvae in the grain $r=0.04$

Fig.4.6 Mean number of immatures of *Sitophilus zeamais* that were in the maize samples used for volatile collection. Means differed significantly : ANOVA: $df=18$; $F=14.6$; $p<0.0001$. Bars marked with the same letter were not significantly different with the Tuckey-Kramer post hoc test.

Discussion

Volatiles from host and/or the host-plant complex have long been suspected to be the means by which storage parasitoids and predators locate the host habitat and infested patches (Turlings et al., 1990; Dicke and Vet, 1992; Agelopoulos et al. 1995).

The first experiment in the olfactometer showed clearly that female *A. calandreae* are indeed attracted by olfactory cues, and that through experience they learn to recognize olfactory cues emitted from the seed species within which they found a host.

The volatile compounds that attract maize-reared *A. calandreae* to uninfested maize must be distinct from those of other healthy cereal grains, as wheat-reared wasps were not attracted. Volatiles in stored seed may result from degradation processes such as oxidation of triunsaturated fatty acids that produce “off-odors” in grain containing high levels of it, as has been shown in soybean (Weber, 1990). Different types of starch (of different species or different genotypes) are known to release distinct volatile compounds (Sayaslan et al., 2000). The intact layers of pericarp, however, limit the amount of volatile substances being released from stored cereals (Trematerra et al., 1999). Volatiles emanating from infested seed may originate from the seed itself, from the insects from the interaction of the insect and the seed, or from other organisms that take advantage from the degradation of the grain.

The release of grain odors will increase, through the rupture of the pericarp and increased grain surface by the feeding activity and oviposition by the adult and larval activity of the maize weevil. This is also evident by the fact that storage pests like *Pteromalus truncatus* and *Sitophilus oryzae* are more attracted to damaged maize odors than to intact maize cobs or grain (Scholz et al., 1997; Trematerra et al. 1999).

Chemical information from the host itself may be provided by faeces, exuviae, glandular secretions or pheromones (Vet and Dicke, 1992). Odors from the seed predators, such as aggregation and sex pheromones are present in stored commodities (Seitz and Sauer, 1996;

Levinson and Levinson, 1995) and may act as attractants for their natural enemies (Boeye et al. 1992; Rees et al., 1990; Jiang et al., 1995). *Lariophagus distinguendus* a parasitoid of several storage beetles responds to extracts of cocoons of a host, *Lasioderma serricone* by antennation and oviposition (Jimenez et al., 1995); and *Bracon hebetor*, a parasitoid of several storage lepidoptera was shown to be attracted to host frass in a wind tunnel (Parra et al., 1996).

Female parasitoids that are experienced, defined by Vet et al.(1990) as having had an oviposition experience, may learn to recognize the host plant even when it is not infested. However parasitoids of endophytically living hosts, such as many storage parasitoids, may have a learning experience at an earlier time during their life cycle. Already as larvae they are intimately associated with odors of their host and the host-seed complex as well as preemerged adult, while they remain in seed, in the cavity excavated by its host. Cortesero et al., (1993) found that preemergence learning increased the responsiveness of *Eupelmus vuilleti*, a parasitoid on stored cowpeas to odors of the seed and its host, *Bruchidius atrolineatus* (Cortesero et al., 1995; Monge and Cortesero, 1996). Preemergence learning was also found to be responsible for host preference in *Dinarmus basalis*, a parasitoid of bruchids in stored beans (Caubert and Jaisson, 1990). Even after emergence the confined habitat of a storage facility, is likely to offer the emerging parasitoid a more intense array of host related stimuli than a parasitoid in an open habitat might encounter, although subsequent oviposition experiences will reinforce olfactory learning. For this reason, in our experiments we used parasitoids reared on wheat as a control, as we cannot avoid that wasps, even if collected immediately upon emergence, have experience with odors related to the host-maize complex.

Different volatiles have been shown to emanate from grain infested with different insect species (Seitz et al., 1996). These volatiles may originate from by-products of the host and be attractive to parasitoids (Steidle and Ruther, 2000). Steidle (1997) showed that the

storage parasitoid *Lariophagus distinguendus* (Foerst.) was strongly attracted by stimuli from odor sources such as faeces of the host, *Sitophilus granarius*, host-damaged grain, and slightly by artificially damaged and undamaged grain, but the host itself was not found to be attractive. However Jiang et al. (1995) found that extracts derived from adult *S.zeamais* were attractive to this pteromalid. Volatile infochemicals from faeces of host larvae can differ in quality and quantity (Agelopoulos et al., 1995). It was also found that the relatively large amount of faeces produced by a suitable host larvae elicits a strong response by *Lariophagus distinguendus* whereas a lower amount, which corresponds to unsuitable first or second instar larvae, evoked only little response (Steidle and Fischer, 2000).

Other living organisms, such as fungi and mites that are frequently associated with insect damaged grain are also potential sources of odor production. Maize weevil infestations also increase the development of several species of fungi in stored maize (Barney et al., 1995; Wicklow et al., 1998), which in turn are responsible for odor emissions. Some compounds are mainly produced during early phases of fungal growth and the production of specific compounds varies greatly among different species of fungi (Borjesson et al., 1989). The “minty” smell of mites in stored grain is well known to entomologists (White et al., 1979; Curtis et al., 1981) and several volatiles produced by them have been identified. Ruther and Steidle (2000) showed that females and males of the wasp *Lariophagus distinguendus* were attracted to semiochemicals from host-associated mites containing tridecane.

Volatile compounds and other chemical stimuli may originate from the seed as a result of the interaction of the host with the grain. The degradation of the seed metabolites may be increased by altered physical factors, such as the higher temperature and humidity in the store resulting from respiration and metabolic processes of the host, or through chemical interaction with larval secretions and frass. Steidle (2000) suggested that the seed may have a more active role in the emission of stimuli used by the parasitoid. This idea is indeed very

tempting, because the plant has an evident interest to protect its gene-carrying seed, but the particular physiological state of the seed, and the evolutionary circumstances of domesticated cereals speak against such mechanisms. The maturing seed accumulates energy reserves to increase its chances of germination under competition with other seedlings. The costs of any active defense mechanism would have to be compensated by a direct benefit for the seed. In the case of the maize weevil and its parasitoid no such benefit was found (Chapter 2). The germination rate of damaged maize seed was not significantly higher when the weevil host had been killed by a parasitoid, so that only traits that have a direct effect on the storage pest will be favored by “natural” selection. Such selection pressures affect also domesticated plants and was defined as “automatic selection” by Harlan (1992).

The reliability and volatility of these various odors will determine their specific use at each step of the foraging process, that allows parasitoids to locate the habitat and the host habitat and infested patches. For the recognition of an infested grain and the suitability of the host, additional stimuli, such as non-volatile chemicals and physical factors may be used (Steidle, 2000). *A. calandae* is a polyphagous parasitoid that may attack very different hosts species on seeds of very different plants. With a decrease in diet breadth at the second and third trophic level, variability in the reliability decreases and the need for learned cues may decrease (Vet and Dicke, 1992). In other words, more specialized parasitoids may rely more on genetically fixed responses to cues that are specifically associated with its specific hosts.

There was no significant difference in the attractiveness of the maize varieties, in spite of a higher number of immature hosts in some varieties. This indicates that any genetically fixed or learned chemical cues similar for all maize genotypes, or that they were present in such high quantities that a threshold level was reached beyond which parasitoids will not make a distinction. The fact that they had some tendency to walk out of the chosen tube after some time to enter another may be another indication that they perceived no qualitative or

quantitative odor gradient at the level of the choice chamber. The wasps showed more activity during the second experiment when the ambient temperature was higher. Their position was therefore noted as soon as they had all chosen an arm, usually within 10 minutes. The six armed olfactometer is designed for simultaneous observation of foraging parasitoids and volatile collection of plants (Turlings and Tamò, in prep.). This double function could not be realized for the tritrophic system that was studied, mainly because the collection of volatile emissions required large maize samples and long collection times. However the olfactometer proved to be a useful for behavioral studies of storage parasitoids. Upon release the wasps were observed to walk rather than to fly upwards to the choice chamber, in spiral lines, so that they encountered all odor sources before arriving in the choice chamber. Unlike other species such as *Cotesia marginiventris* (Tamò et al., in prep.) they would not readily enter the trapping bulbs, probably because they are less attracted to light (Cline et al., 1983). The wasps stayed mostly inside the screw caps that connect the arms of the olfactometer to the second glass vessel.

Detectable amounts of volatiles were only collected from the heavily infested grain. The pericarp layers of maize kernels are thought to largely prevent odor emission (Trematerra, 1999). Adult maize weevils lay their eggs under the pericarp but cover it with an egg plug, which might reduce the release of volatiles. However some gaseous exchange must take place to allow respiration by immatures and towards the later stages of development the appearance of small amounts of powder in the vials indicates that the pericarp was ruptured. The quantity and quality of odors produced by developing larvae differs among insect species (Seitz and Sauer, 1996). Some stored grain feeders, such as *Rhyzopertha dominica* and *Tribolium castaneum* increase off-odors in stored sorghum, while infestation by the rice weevil *Sitophilus oryzae* causes hardly any odor (Seitz and Sauer, 1996). This could be due to different feeding habits of the species; weevils of the *Sitophilus* group are mainly endosperm

feeders while *R. dominica* and *T. castaneum* feed on the embryo. These parts of the grain are different in composition; the endosperm contains large amounts of carbohydrates and the germ higher proportions of proteins and lipids (Watson, 1987). Therefore the by-products resulting from feeding and digestion may differ between these species and result in different odor blends.

One of the volatile compounds detected in the infested maize samples was tridecane, a compound found in the headspace of *Acarus siro*, a very common mite in stored grain (Curtis et al., 1981). Mites were observed in all the infested samples, feeding on the germ of maize kernels. The two identified compounds, tridecane and pentadecane, as well as the other two compounds that are likely to be (Z) -7-pentadecene and (Z) -6-pentadecene have also been found in extracts of faeces from the granary weevil, *Sitophilus granarius*, feeding in wheat, and in extracts from associated mites (Ruther and Steidle, 2000). It appears therefore that none of these compounds is specific to the seed or host species.

Such odors of associated organisms, like the ones produced by fungi, could be attractive to storage parasitoids, females as well as males, as indicators of a favorable habitat, or an infested patch, but they may not be reliable enough for the detection of suitable hosts within the bulk of grain. The associated organisms are not always present in infested grain and can therefore not be the only cue on which foraging storage parasitoids rely .

We were unable to detect the volatiles from uninfested maize that attracted experienced (maize-reared) parasitoids. Hence parasitic wasp must be able to perceive volatiles at quantities that cannot be detected by the currently used techniques. This keen ability of the parasitoid to track small quantities of volatile cues should allow it to readily locate potential host habitats (stores) and subsequently the more odorous infested grains within this habitat.

Conclusion

The experiments showed that *Anisopteromalus calandrae* is attracted to infested maize whether it has had previous experience with maize or not. Its attraction to unfested maize after experience shows that they are able to learn and recognize a potential host seed. The parasitoids showed no preference for the odors of any of the six maize varieties, and genotypes with storage resistance showed no deterrent effect on this parasitoid. However the host density used far exceeds the level that these parasitoids would encounter in the field, as a desirable level of damage would have to concern less than 10 percent of the kernels.

Olfactory experiments using low host density, and field observations may be useful to confirm these results. The volatiles collected on infested maize probably originated from associated mites. The techniques used for volatile collection and analyzes procedures may not be appropriate to detect possible differences in odour profiles among maize genotypes, and behavioral experiments may be a more useful means to find such differences as long as the olfactory sensitivity of parasitic wasp has not yet been matched by modern techniques.

Acknowledgements: We thank Dr. Martine Rahier for allowing to use the infrastructure at the Institute of Ecology, University of Neuchatel. We are grateful for technical help, to Dr. Thomas Degen and Dr. Daniel Niederer for helpful comments about the analyzes of the volatiles and to Jacqueline Moret for help with the statistics.

References

- Agelopoulos, N.G., M. Dicke, and M.A. Posthumus. 1995.** Role of volatile infochemicals emitted by feces of larvae in host-searching behavior of parasitoid *Cotesia rubecula* (Hymenoptera: Braconidae) - A behavioral and chemical study. *J. Chem. Ecol.* 21 : 1789-1811.
- Alborn, H. I., T. C. J. Turlings, T. H. Jones, J. G. Stenhagen, J. H. Loughrin , and J. H. Tumlinson. 1997.** An elicitor of plant volatiles from beet armyworm oral secretion. *Science* 276 : 945 – 949
- Arbogast, R. T., and M. A. Mullen. 1990.** Interaction of maize weevil (Coleoptera: Curculionidae) and parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) in a small bulk of stored corn. *J. Econ. Entomol.* 83 : 2462 – 2468
- Boeye, J., M. Wright, and G. A. Laborius (eds.). 1992.** Implementation of and further research in biological control of the larger grain borer. Hamburg, Germany: GTZ
- Borjesson, T., U. Stollman, P. Adamek, and A. Kaspersson. 1989.** Analysis of volatile compounds of molds in stored cereals. *Cereal Chem.* 66: 300-309
- Caubert, Y. and P. Jaisson. 1990.** A post-eclosion learning involved in host recognition by *Dinarmus basalis* Rondani (Hymenoptera: Pteromalidae). *Anim. Behav.* 42 : 977-980
- Cline, L. D., B.R. Flaherty, and J.W. Press. 1983.** Response of selected parasitoids and predators of stored-product pests to whitelight or blacklight traps. *J. Econ. Entomol.* 76: 298-301
- Corbet, S.A. 1971.** Mandibular gland secretion of larvae of the flour moth, *Anagasta kuehniella*, contains an epideic pheromone and elicits oviposition movements in a hymenopterous parasite. *Nature* 232 : 481-484
- Cortesero, A. M., J. P. Monge, and J. Huignard. 1993.** Response of the parasitoid *Eupelmus vuilleti* to the odours of the phytophagous host and its host plant in an olphactometer. *Entomol. Exp. et Appl.* 69 : 109-116
- Cortesero, A. M., J. P. Monge , and J. Huignard. 1995.** Influence of two successive learning processes on the response of *Eupelmus vuilleti* (Hymenoptera, Eupelmidae) to volatile stimuli from host and host plant. *J. of Insect. Behav.* 8 : 751 – 762
- Curtis, R.F., A. Hobson-Frohlock, G.R. Fenwick, and J.M. Beeren. 1981.** Volatile compounds from the mite *Acarus siro* L. in food. *J. Stored Prod. Res.* 17: 197-203
- De Moraes, C. M., W. J. Lewis, P. W. Pare, H. T. Alborn , and J. H. Tumlinson. 1998.**

- Herbivore-infested plants selectively attract parasitoids. *Nature* 393 : 570 – 572
- Dicke, M., M. W. Sabelis, J. Takabayashi, J. Bruin , and M. A. Posthumus, 1990.** Plant strategies of manipulating predator-prey interactions through allelochemicals: Prospects for application in pest control. *J. Chem. Ecol.* 16 : 3091-3118
- Ghani, M. A. , and H. L. Sweetman. 1955.** Ecological studies on the granary weevil parasite, *Aplastomorpha calandreae* (Howard). *Biologia (Lahore)* 1 : 115 – 139
- Gouinguené, S., Degen T., Turlings T.J.C.** Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology* 11: 9-16.
- Hérard , F., M.A. Keller, W.J. Lewis, and J.H. Tumlinson. 1988.** Beneficial arthropod behavior mediated by airborne semiochemicals. IV. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson. *J. Chem. Ecol.* 14 : 1597-1606
- Howard R.W. 2001.** Cuticular hydrocarbons of adult *Pteromalus cerealellae* (Hymenoptera: Pteromalidae) and two larval hosts, Angoumois grain moth (Lepidoptera: Gelechiidae) and Cowpea weevil (Coleoptera: Bruchidae). *Ann. Entomol. Soc. Am.* 94 (1): 152-158
- Ignacimuthu S., F.L. Waeckers, and S. Dorn. 2000.** The role of chemical cues in host finding and acceptance by *Callosobruchus chinensis*. *Entomol. Exp. Et Appl.* 96 (3) 213-219.
- Jiang X.L., D.Y. Cun and W.B. Liu, 1994.** A study on the attraction responses of the adults of *Sitophilus zeamais* to volatile substances. *Journal of the Southwest Agricultural University.* 16 : 511-514
- Jimenez Ambriz, S., M.R. Strand, and W.E. Burkholder. 1996.** Behavioral response of the parasitoid *Lariophagus distinguendus* (Fost) (Hymenoptera: Pteromalidae) to extracts from cocoons of *Lasioderma serricone* Fab. (Coleoptera Anobiidae) and their effect on subsequent oviposition responses. *Biological Control* 6: 51-56
- Kessler, A., Baldwin, J. T. 2001.** Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291: 2141-2144.
- Kester, K.M., P. Barbosa. 1991.** Postemergence learning in the insect parasitoid, *Cotesia congregata* (Say) (Hymenoptera : Braconidae) *J. Insect Behav.* 4 : 727-742
- Levinson, A. , and H. Levinson. 1995.** Reflections on structure and function of pheromone glands in storage insect species. *Anz. fur Schaedlingsk. Pflanzensch. Umweltsch.* 68 : 99-118

- Longstaff B. C., 1981.** Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): A critical Review. Prot. Ecol. 2, 83-130
- Marchand D., and J. McNeil. 2000.** Effects of wind speed and atmospheric pressure on mate searching behavior in the aphid parasitoid *Aphidius nigripes* (Hymenoptera: Aphididae) J. Insect Behav. 13 : 187-199
- Monge J.P. and A.M. Cortesero. 1996.** Tritrophic interactions among larval parasitoids, bruchids and Leguminosae seeds; influence of pre and post-emergence learning on parasitoids' response to host and host-plant cues. Entomologia Experimentalis et Applicata 80 : 293-296
- Nordlund D.A. and C.E. Sauls. 1981.** Kairomones and their use for management of entomophagous insects. XI. Effect of host plants on kairomonal activity of frass from *Heliothis zea* larvae for the parasitoid *Microplitis croceipes*. J. of Chem.Ecol. 7 : 1057-1061
- Parra J.R.P., S.B.Vinson, S.M. Gomes and F.L. Consoli. 1996.** Flight response of *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) in a wind tunnel to volatiles associated with infestations of *Ephestia kuehniella* Zeller (Lepidoptera : Pyralidae). Biological Control, 6, 143-150
- Rapusas, H.R., D.G. Botrell, and M.Coll. 1996.** Intraspecific variation in chemical attraction of rice to insect predators. Biol. Control. 6: 394-400
- Rees, D.P., R. Rodriguez Rivera, and F.J. Herrero Rodriguez. 1990.** Observations of the ecology of *Teretriosoma nigrescens* Lewis (Col: Histerioidae) and its prey *Prostephanus truncatus* (Horn) (Col: Bostrichidae) in the Yucatan peninsula, Mexico. Tropical Science 30 (20): 153-165
- Ruther, J. and J.L.M. Steidle. 2000.** Mites as matchmakers: Semiochemicals from host-associated mites attract both sexes of the parasitoid *Lariophagus distinguendus*. J. Chem. Ecol. 26 (5): 1205-1217
- Sayaslan, A., O. K. Chung, P.A. Seib, and L.M. Seitz. 2000.** Volatile compounds in five starches. Cereal Chem. 77 (2) : 248-253
- Scholz, D., A. Tchabi, C. Borgemeister, R. H. Markham, H. M. Poehling, and A. Lawson, 1997.** Host-Finding Behaviour of *Prostephanus truncatus* (H) (Col.,Bostrichidae) - Primary attraction or random attack. J. of Applied Entomology Zeitsch. fur angew. Entomologie 121 (5) ,261-269
- Seitz L. M. and D. B. Sauer, 1996.** Volatile compounds in grain Sorghum infested with common storage insects. Cereal Chem. 73 : 744-750

- Steidle J.L.M. 2000.** Host recognition cues of the granary weevil parasitoid *Lariophagus distinguendus*. Entomologia Experiment. Et Applicata 95: 185-192
- Steidle, J. L. M. , and M. Schoeller. 1997.** Olfactory host location and learning in the granary weevil parasitoid *Lariophagus distinguendus* (Hymenoptera : Pteromalidae). J. of Insect Behav. 10 : 331 – 342
- Steidle J.L.M., and A. Fischer. 2000.** Quantity does matter: How feces are used for host stage selection by granary weevil parasitoid *Lariophagus distinguendus*. J. Chem Ecol. 26 : 2657-2664
- Steidle, J.L.M., and J. Ruther. 2000.** Chemicals used for recognition by the granary weevil parasitoid *Lariophagus distinguendus*. J. Chem. Ecol. 26 (12) 2665-2675
- Steidle, J.L.M., and M. Schoeller. 2002.** Fecundity and ability of the parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae) to find larvae of the granary weevil *Sitophilus granarius* (Coleoptera: Curculionidae) in bulk grain. J. Stored Prod. Res. 38: 43-53
- Trematerra, P., F. Fontana, M. Mancini, and A. Sciarretta. 1999.** Influence of intact and damaged cereal kernels on the behaviour of rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). J. stored Prod. Res. 35 :265-276
- Trematerra, P. A.Sciarretta, and E.Tamasi. 2000.** Behavioural responses of *Oryzaephilus surinamensis*, *Tribolium castaneum* and *Tribolium confusum* to naturally and artificially damaged durum wheat kernels. Entomol Exp. Et Appl. 94 (2) 195-200
- Turlings T.C.J. and C. Tamò, 2002** 6-arm olfactometer in preparation
- Turlings, T.J C., F. L. Waeckers, L.E.M. Vet, W.J. Lewis and J. H. Tumlinson. 1993.** Learning of host-finding cues by hymenopterous parasitoids. In D.R. Papaj and A.C. Lewis (eds), Insect Learning. Chapman and Hall, NY : 51-78
- Turlings, T. C. J., J. H. Tumlinson, F. J. Eller , and W. J. Lewis. 1991.** Larval-damaged plants: Source of volatile synomones that guide the parasitoid *Cortesia marginiventris* to the micro-habitat of its hosts. Entomol. Exp. et Appl. 58 : 75 – 82
- Turlings, T. C. J., J. H. Tumlinson , and W. J. Lewis. 1990.** Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250 : 1251 - 1253
- Van den Assen, J. F.A. Putters and T.C. .Prins, 1984.** Host quality effects on sex ratio of the parasitic wasp *Anisopteromalus calandrae* (Chalcidoidea, Pteromalidae). Netherlands Journal of Zoology 34 :33-62
- Vet, L.E.M., and A.W. Groenewold, 1990.** Semiochemicals and learning in parasitoids. J. Chem. Ecol. 16: 3119-3135

- Vet, L. E. M. , and M. Dicke. 1992.** Ecology of infochemical use by natural enemies in a tritrophic context. *Ann. Rev. Entom.* 37 : 141 –172
- Vinson, S. B. 1976.** Host selection by parasitoids. *Ann. Rev. Entomol.* 1976, 109- 133
- Watson S.A. 1987.** Structure and composition. In : *Corn: Chemistry and Technology*. S.A. Watson & P.E. Ramstad (eds) Am. Ass. Cereal Chem., St Paul
- Weber, E. 1990.** Lipids of the kernel. In *Corn: Chemistry and Technology* 311-349
S.A. Watson & P.E. Ramstad (eds) Am. Ass. Cereal Chem., St Paul
- White, N.D.G, L.P. Henderson and R.H. Sinha. 1979.** Effects of infestations by three stored-product mites on fat acidity, seed germination and microflora of stored wheat. *J. Econ. Entomol.* 2: 763-766
- Wicklow, D.T., D.K. Weaver, and J.E. Throne. 1998.** Fungal colonists of maize grain conditioned at constant temperatures and humidities. *J. Stored Prod. Res.* 34 : 355-361

EFFECT OF STORAGE METHOD ON MAIZE STORAGE PESTS AND THEIR NATURAL ENEMIES

Abstract

Maize can be stored in three presentations: husk on, husk off and shelled grain. The objective of this study was to quantify the impact of storage method on the efficacy of three biological control agents. Growth rate and damage associated with the maize weevil, *Sitophilus zeamais* (Coleoptera : Curculionidae), the larger grain borer, *Prostephanus truncatus* (Coleoptera : Bostrichidae) and the Angoumois grain moth, *Sitotroga cerealella* (Lepidoptera : Gelechiidae) were assessed in laboratory experiment to observe the possible interaction between storage method and the performance of their natural enemies, *Anisopteromalus calandrae* (Hymenoptera : Pteromalidae), *Teretriosoma nigrescens* (Coleoptera : Histeroidae) and *Pteromalus cerealellae* (Hymenoptera : Pteromalidae). Population growth and damage by maize weevil was reduced on maize stored on the cob, but its parasitoid had a significant impact only when maize was shelled. Larger grain borer populations increased more rapidly on maize stored as cobs, and its predator was equally efficient on both storage methods. The most significant impact of storage on the efficacy of a natural enemy was observed for the host-parasitoid assemblage *S. cerealella* - *P. cerealellae*. The Angoumois grain moth had a higher rate of increase on shelled grain. However its parasitoid was highly efficient on shelled grain and reduced the moth population ten fold, while it was ineffective when maize was

stored as a cob. It was found that storage on the cob offered the larval host a spatial refuge early in its development.

Based on the results recommendations can be made for the appropriate storage method depending on the predominant storage pest, and the potential success rate of naturally occurring or released biocontrol agents under certain conditions can be predicted.

Key words : Maize storage method, *Sitophilus zeamais*, *Prostephanus truncatus*, *Sitotroga cerealella*, *Anisopteromalus calandrae*, *Pteromalus cerealellae*, *Teretriosoma nigrescens*

Introduction

In temperate regions maize is usually stored as shelled grain in secure storage structures such as silos, which impede migration of storage pests and facilitate the applications of insecticides and fumigants. In tropical regions, where post harvest losses are very high, subsistence farmers lack mechanical devices to shell maize immediately after harvest, and storage structures are often not effective in providing protection from insect immigration (Markham et al., 1994a). Maize is therefore frequently stored on the cob, with or without its husk. This storage practice takes more space and requires higher amounts of pesticides (Farell et al., 1996), but the method is believed to reduce post-harvest losses, sometimes to an acceptable level (Dobie, 1977; McFarlane, 1988).

The firmly attached kernels of maize stored on the cob present a completely different architectural structure than shelled grain. Tritrophic interactions consider the plant-herbivore-natural enemy interactions that include the impact of plant structure on access to specific plant parts, or in this case, stored grain (Price et al., 1980). Numerous examples of concealed hosts show that plant structure may influence the searching behavior of parasitoids or even offer the host a structural refuge, leading to a considerable reduction in the rate of predation or parasitism (Porter, 1928; Ball and Dahlsten, 1973).

The maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae) lays most of its eggs close to the point of attachment of the maize kernel, and avoids the crown (see discussion Fig. 5.9) in shelled grain (Kossou et al. 1992). When maize is stored on the cob this preferred oviposition site is not readily accessible to the adult beetles which are obliged to oviposit on less favored parts of the kernel. Kossou et al. (1992 and 1993) and Vowoter et al. (1995) found that weevil populations were considerably smaller if maize was stored on the cob, because of a lower oviposition and development rate.

In contrast, for the larger grain borer *Prostephanus truncatus* (Coleoptera: Bostrichidae) storage on the cob will enhance adult establishment (Cowley et al., 1980). Richter et al., (1997) found shelled maize to suffer less damage from the larger grain borer than when stored on the cob, with or without husk. Moreover, pesticides that are efficient against the larger grain borer have less effect when applied to maize cobs than to shelled grain (Farell et al., 1996). Following the introduction of this pest in Africa in the late seventies, research conducted by the NRI and the FAO led to recommendations that maize be shelled as soon as possible after harvest to reduce damage by this very destructive pest (Farell et al., 1996).

For the Angoumois grain moth no comparative studies have been made. They infest maize cobs already in the field (Schulten, 1976; Weston et al., 1997). If maize is stored as shelled grain, damage will be limited to the upper layer because the movements of adult moths within grain is limited to short distances and they are unable to penetrate deeper than 12 cm into a bulk of grain (Muhihu, 1986). Stored cobs, on the other hand, offer a much larger space for them to move about.

Because of such differences in pest development the appropriate storage practice will depend on the presence of a specific pest. Choosing the right storage method can be considered as one strategy of integrated pest management to reduce post harvest losses without the application of traditional pesticides.

Another pest management strategy is the use of natural enemies of storage pests. Several parasitic wasps and predators are common in tropical stores, and their potential to control storage pests has been investigated in some cases. A very common storage parasitoid, *Anisopteromalus calandrae*, (Hymenoptera; Pteromalidae) is a solitary ectoparasitoid of many immature Coleoptera developing within stored cereals. Several studies have focussed on its life history (Smith, 1992, 1993 and 1994), resistance to pesticides (Baker and Weaver, 1993, and Baker and Throne, 1995), host-suitability (Smith et al., 1994; Choi et al., 2001)

competition with other parasitoids (Wen and Brower 1995; Ryoo et al., 1996), and suppression of storage beetles by augmentative releases (Flinn et al., 1996; Cline et al., 1985; Press, 1984). Another pteromalid, *Pteromalus cerealellae*, an important ecto parasitoid commonly found in association with *Sitotroga cerealella*, has been considered as a potential biocontrol agent and its ability to suppress the Angoumois grain moth in shelled corn was shown by Wen and Brower (1994). Its potential host range has been studied by Smith et al. (1995) and Brower (1991). These studies suggest that pteromalid parasitoids can contribute significantly to the control of storage pests (Cline et al. 1985).

The predator, *Teretriosoma nigrescens* (Coleoptera: Histeridae) feeds on the larvae of the larger grain borer. It is native to Mesoamerica and was released in Africa, to reduce the high post harvest losses in maize, that occurred after the larger grain borer had been accidentally introduced in the late seventies. Although it is a voracious predator in the laboratory (Rees, 1985, 1987), it is unlikely to achieve an acceptable level of control without supplementary measures (Markham et al. 1994b; Holst and Meikle, 2002).

No research has been done so far to investigate the interaction between storage practice and the natural enemies that could control the most important primary pests of stored maize. Since most tropical regions where maize is an important crop for subsistence farmers contain at least two of these primary storage pests (Boxall and Gillett, 1984; Markham et al., 1994a; Tigar et al., 1994), the magnitude of this interaction could be a determining factor for storage recommendations to reduce losses.

The objective of the current study was to evaluate the compatibility of storage methods (shelled grain or on the cob) and the use of natural enemies to control the three most important storage pests in maize.

Material and methods

Maize

All experiments were conducted in incubation chambers with temperature at 26°C and 75% relative humidity, and maize (Table 5.1) used in the experiments was stored under these conditions for at least three weeks prior to use.

Table 5.1: Maize used for experiments and rearing

Maize Geontype	Type	Color	Use
CML273 x CML264	tropical hybrid	white	experiments
CL02420xCML287	tropical hybrid	yellow	experiments & rearing
MBR	population	yellow	experiments
CML244xCML349	tropical hybrid	white	rearing

All maize genotypes are susceptible to storage pests

Insects

Colonies of the maize weevil, *Sitophilus zeamais*, originated from CIMMYT's experimental station in Poza Rica (Veracruz, Mexico). They were reared on the white hybrid maize CML244xCML349. Colonies were replaced every six months. Its parasitoid *Anisopteromalus calandrae* was collected at the same location and reared on 4th instar larvae of *S. zeamais* as described in Chapter 1.

The larger grain borer, *Prostephanus truncatus*, and its predator *Teretriosoma nigrescens* originated from CIMMYT's experimental station in Tlaltizapan (Morelos, Mexico). The colonies were maintained on white hybrid maize, CML244xCML349, as described in Chapter 1. *T. nigrescens* was reared by introducing adult beetles into jars of maize infested with larger grain borer adults five weeks earlier.

The Angoumois grain moth, *Sitotroga cerealella*, and its parasitic wasp, *Pteromalus cerealellae*, were collected on maize purchased at a local market in Pahuatlán (Puebla, Mexico). Hybrid maize, either CML244xCML349 or CL02400xCML287, was infested with eggs of *S. cerealella*, and parasitoids were reared on 22 day old host colonies as described in Chapters 1 and 2. Both insect colonies were replaced with individuals from the same location after one year. All insect colonies were maintained in the laboratory for at least one generation prior to use for the experiments.

The performance of each of the three major storage pests of maize, *S. zeamais*, *P. truncatus* and *S. cerealella* in the presence of a natural enemy was tested for two storage methods: maize stored on the cob, or as shelled grain, over a storage period of three months. The effect of storage method was also examined in the simultaneous presence of *S. zeamais* and *P. truncatus* at equal initial densities and a common natural enemy, the generalist parasitoid *A. calandreae*.

1. Performance of *Sitophilus zeamais* and a parasitoid, *Anisopteromalus calandreae*

A yellow hybrid, CL02420x CML287, was either only dehusked or dehusked and shelled, and one cob or the equivalent amount of grain was placed into half-liter jars. Sixteen jars were prepared for each of the two storage methods, and infested with 18 adult unsexed maize weevils, less than two weeks old. The jars contained a mesh lid and were stored at CIMMYT's experimental station in Poza Rica (Veracruz) within an open structure. The ambient conditions were similar to those of the direct surroundings, but jars were protected from extreme values (temperature: average 27.5 {17.1-35.3}, R.H. ; 75%) and rain. Six weeks after infestation two female and one male *A. calandreae*, less than three days old, were added to half of the jars, which were then left undisturbed until the end of the experiment. Three months after the initial infestation all the jars were frozen, all adult *S. zeamais* were counted, and percentage of damaged grain was determined.

2. Performance of *Prostephanus truncatus* and its predator *Teretriosoma nigrescens*

A white tropical hybrid (CML273 x CML264) was used for the experiment. Each cob, or the equivalent amount of grain was infested with 20 adult, unsexed *P. truncatus*, originating from six to eight week old colonies. Sixteen jars for each storage method were prepared. A husk leaf was added to each of the cobs because the larger grain borer has difficulties to walk on glass and it helped to avoid movement of the cob during handling of the jars. Shelled grain was weighed down with 12 glass balls of 1 cm diameter to facilitate the establishment of *P. truncatus*. After four weeks, three adult *Teretriosoma nigrescens* were added to half of the jars. The jars were again incubated for the remaining time until three months after infestation. At this time cobs were shelled and all samples were sieved. The powder produced was weighted and the adult predators were counted. The remaining material was frozen for easier counting of adult grain borers.

3. Simultaneous presence of two storage pests and a common natural enemy: *Sitophilus zeamais*, *Prostephanus truncatus* and *Anisopteromalus calandrae*

A white tropical hybrid (CML273 x CML264) was prepared as described and eight replicates for each storage method were infested simultaneously with six pairs of each *S. zeamais* and *P. truncatus*. The adults were sexed based on snout characteristics (Halstead, 1963) using methods described by Shires (1976) and Scholz et al (1997).

Two female and one male *Anisopteromalus calandrae* were added five and six weeks after infestation to half of the jars. The jars were left undisturbed until the end of the experiment, when they were frozen and adult grain borers and weevils were separated and counted. Wasps were not counted, because they are too small and very short lived.

4.- Performance of *Sitotroga cerealella* and its parasitoid *Pteromalus cerealellae*

4.1-Long term experiment

The maize used for all experiments was a breeding population with resistance traits to stem borer and fall armyworm (multiple borer resistant: MBR) grown in 1999 at CIMMYT's experimental station in Tlaltizapan (Morelos, Mexico).

Cobs and shelled grain were infested twice with 50 eggs at an interval of 12 days, to obtain suitable hosts at all times. Two female *P. cerealellae* and one male were added twice, after five and six weeks, when the first adult *S. cerealella* had already emerged to assure continuity of the host species. Three months after infestation the experiment was terminated, all jars were frozen, all adult insect were counted, the number of damaged and undamaged kernels was determined, and losses due to insect infestation was evaluated using the standard volume weight method (Adams, 1977).

4.2 -Temporal refuge for Angoumois grain moth larvae

One maize cob or the equivalent amount of shelled grain was placed individually in a half liter jar and infested with 100 eggs of *S. cerealella* on wax paper strips. These strips were removed after two weeks and the number of hatched eggs was counted. Sixteen jars for each storage method were infested this way. Two mated females of *P. cerealellae*, less than four days old were released into half of the jars of each treatment 22 days after infestation. They were allowed to oviposit for 24 hours, and then removed. All emerging insects were counted at 3-day intervals until 55 days after infestation. The survival rate from hatched egg to emerged *S. cerealella* or to emerged wasp was calculated.

Based on the first results, the experiment was repeated, but this time the wasps were released into the jars at day 25 after infestation, when pupation was imminent (for details see discussion).

Results

1. *Sitophilus zeamais* – *Anisopteromalus calandrae*

As expected, maize weevil population increased considerably more (almost threefold) on shelled grain than on the cob (Table 5.2, and Fig. 5.1) and percentage of damaged grain was higher (Fig. 5.2). The released parasitoids reduced the number of emerged weevils

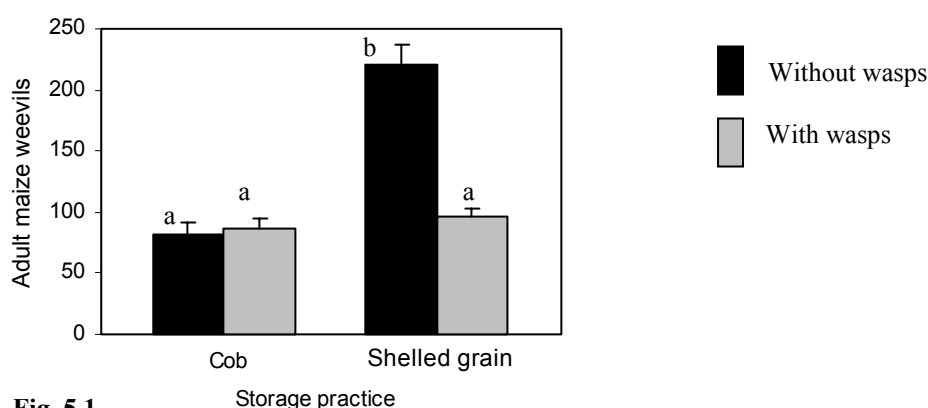


Fig. 5.1

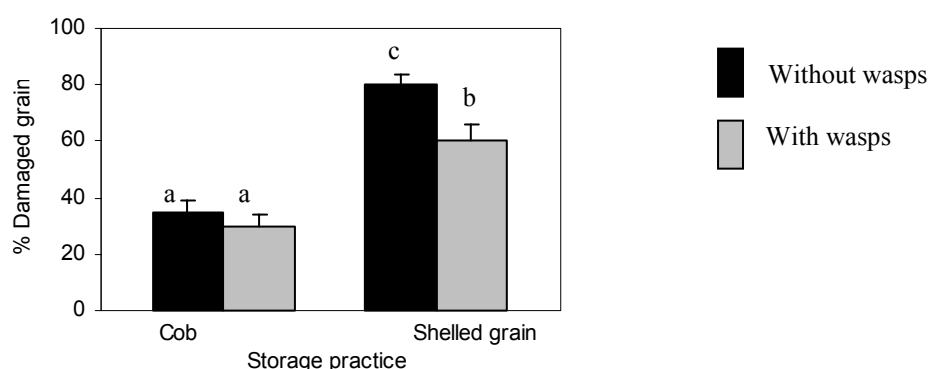


Fig. 5.2

Fig 5.1 : Rate of increase of *Sitophilus zeamais* and impact of a parasitoid, *Anisopteromalus calandrae* depending on storage practice.

Number of maize weevils after three months of storage with (grey) or without (black) parasitoids. ANOVA showed a significantly higher increase of maize weevils in shelled grain ($df=14$; $F=49$; $P<0.0001$). Parasitoids had a significant impact on number of weevils in shelled grain ($df=14$; $F=47$; $P<0.0001$), but not on the cob. Two way factorial analyses showed that the interaction of storage practice and parasitoid significantly affects the increase of maize weevils ($df=28$; $P<0.0001$)

Fig 5.2 : Damage by *Sitophilus zeamais* depending on storage practice and impact of the parasitoid *Anisopteromalus calandrae*

Percent damaged grain after three months of storage with (grey) or without (black) parasitoids. ANOVA showed a significantly higher damage shelled grain ($df=14$; $F=60$; $P<0.0001$). Parasitoids reduced damage significantly in shelled grain ($df=14$; $F=7.8$; $P=0.01$), but not on the cob. Two way factorial analyses showed that the interaction of storage practice and parasitoid had no significant influence on damage. Columns with the same letter are not significantly different with Fisher's protected LSD

significantly, when maize was stored as shelled grain, but had no effect on the populations on the cobs. Damage was reduced by the action of the parasitoids on shelled maize while on the cob the damage was only slightly but not significantly reduced. Although parasitoids were observed for several weeks after release, no living wasps were found at the end of the experiment. A two-way factorial analysis of variance showed a significant interaction of storage method and parasitoids with number of maize weevils, but not with percentage damage (Fig 5.1 and 5.2). Insect numbers were square root transformed prior to statistical analysis to improve normality.

2. *Prostephanus truncatus* – *Teretriosoma nigrescens*

Progeny of the larger grain borer, *P. truncatus* were more numerous on maize stored on the cob than on shelled grain. As an apparent result, flour production by the insects was much higher for maize on the cob (Table 5.1, Fig. 5.3). The predator had a significant impact on the number of the larger grain borer, but not on powder production (Fig. 5.4) Two way factorial analyses showed no significant interaction of storage method with the effect of the predator, *T. nigrescens* (Fig. 5.3 and 5.4)

Table 5. 2: Effect of storage method on number of three storage pests, and the damage they inflict, with or without the presence of a parasitic wasp or predator.

Insects:	Parasitoid or Predator	<i>Sitophilus zeamais</i>		<i>Sitotroga cerealella</i>		<i>Prostephanus truncatus</i>	
		Adult Insects	% Damage	Adult Insects	Damage: Kernel weight	Adult Insects	Powder produced
Cob	None	81.3 ±11 a	34.4 ±4 a	281.6 ±23 b	44.2 ±0.7 b	816.5 ±82 a	39.8 ±4 a
	Yes	85.9 ±9 a	30.1 ±4 a	263.6±15 b	44.9 ±0.4 b	593.3 ±60 b	29.2 ±4 b
Shelled grain	None	220.9 ±17 b	79.8 ±4 c	340.3 ±12 a	42.1 ±0.3 c	508 ±100 b	26.2 ±4 b
	Yes	96.3 ±7 a	60.6 ±6 b	35.4 ±6 c	48.9 ±0.2 a	298.3 ±36 c	22.8 ±2 b

None: without, yes : with parasitoid or predator: *Anisopteromalus calandrae* for *Sitophilus zeamais*; *Pteromalus cerealellae* for *Sitotroga cerealella*; *Teretriosoma nigrescens* for *Prostephanus truncatus*.

Adult Insects: Number of adult insects at the end of the experiment. %Damage: percentage damaged kernels.

Kernelweight: weight of an equal volume of kernels at the end of the experiment. Powder: powder produced

Within each column, values followed by the same letter are not significantly different at $P < 0.05$ (Multiple comparison: Fisher's protected LSD)

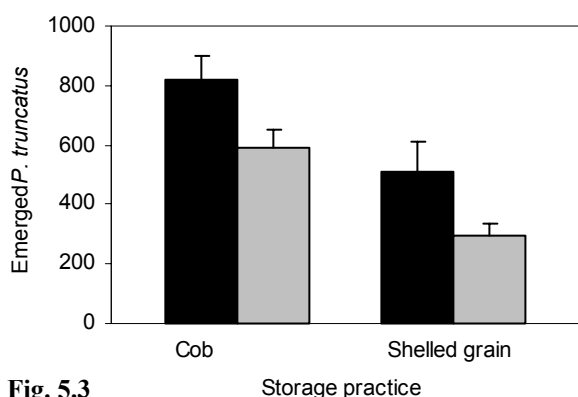


Fig. 5.3

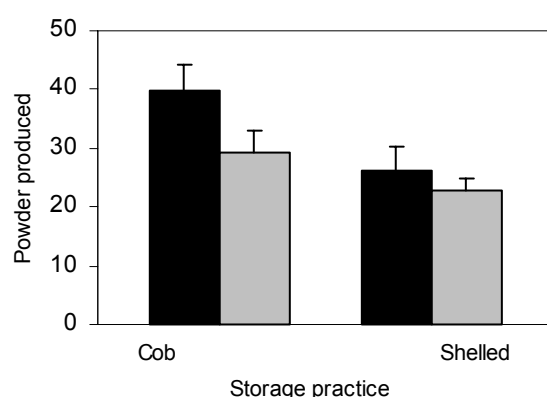


Fig. 5.4

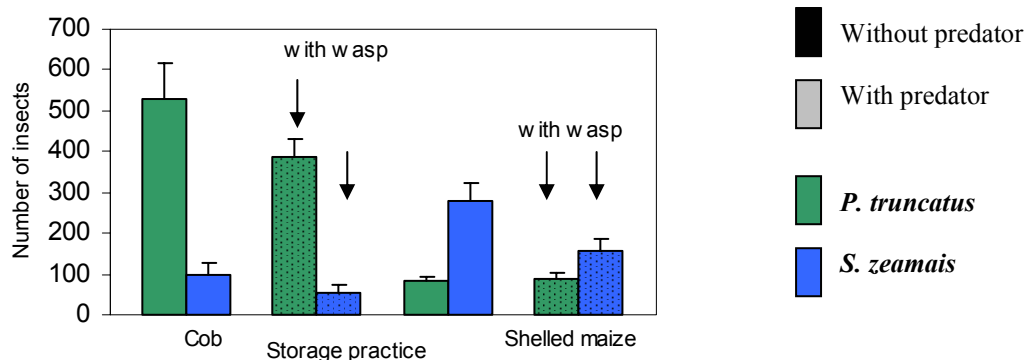


Fig. 5.5

Fig 5.3 : Effect of storage method on the population of the larger grain borer, *Prostephanus truncatus*, in the presence or absence of its predator, *Teretriosoma nigrescens*.

Number (untransformed) of *P. truncatus* after three months in storage with (grey) or without (black) predators. Numbers were square root transformed before analyses. ANOVA showed significant differences of increase of larger grain borers for the storage methods ($df=28$; $F=16.6$; $P=0.0003$) and predator ($df=28$; $F=6.5$; $P=0.016$) Two way factorial analyses showed no significant interaction of storage method with predator. Columns with the same letter are not significantly different for Fisher's protected LSD

Fig 5.4 : Powder production (g) of the larger grain borer, *Prostephanus truncatus*, depending on storage practice and the presence of the predator *Teretriosoma nigrescens*.

Powder produced (mean) by the larger grain borer after three months in storage with (grey) or without (black) predators. ANOVA showed a significant difference in powder production for storage method ($df=28$; $F=3.5$; $P=0.04$) Two way factorial analyses revealed no significant interaction of storage method with predator. Columns with the same letter are not significantly different for Fisher's protected LSD

Fig 5.5: Interaction of the generalist parasitoid, *Anisopteromalus calandrae*, with two storage pests: *Sitophilus zeamais* and *Prostephanus truncatus*, under two different storage methods.

Total number of adults for each species after three months are shown with or without the introduction of parasitoids. Green: *P. truncatus*; Blue: *S. zeamais*

ANOVA: Again significantly more large grain borers emerged on maize stored as cob ($df=14$; $P<0.0001$), and more maize weevils on shelled grain ($df=14$; $P=0.003$). The parasitoid *A. calandrae* reduced both insect species in maize stored on the cob, and maize weevils only in shelled grain, but the differences were not significant.

significant interaction of storage method with the action of the predator for neither of the dependent variables. Both storage method and predator had a significant impact on the number of adult grain borers.

3. *Simultaneous presence of two storage beetles*

When maize was infested with both beetles, *S. zeamais* and *P. truncatus*, simultaneously their number was again different for the two storage methods, and apparently was not affected by the presence of the other pest insect (Fig. 5.5). The larger grain borer was the dominant species when maize was stored on the cob and maize weevils dominated on shelled grain. The parasitic wasp *A. calandreae* was able to reduce the maize weevil significantly in both storage situations. *P. truncatus* on the other hand, was not affected at all by the parasitoid on shelled grain, and although their numbers were also reduced when exposed to the parasitoid on the cob, this reduction was not significant. Growth rate of the larger grain borer was only affected by storage method and the maize weevil was affected by both, but more by storage method than by the parasitic wasps.

4. *Sitotroga cerealella* - *Pteromalus cerealellae*

4.1 *Long term experiment*

Fewer Angoumois grain moths emerged from maize stored on the cob than from shelled maize (Table 5.1 and Fig 5.6). The difference was small, but significant. The impact of parasitoids on *S. cerealella* on shelled grain was very important, reducing the number of adult moths to 10% of the control. Only a modest reduction in emergence was observed for

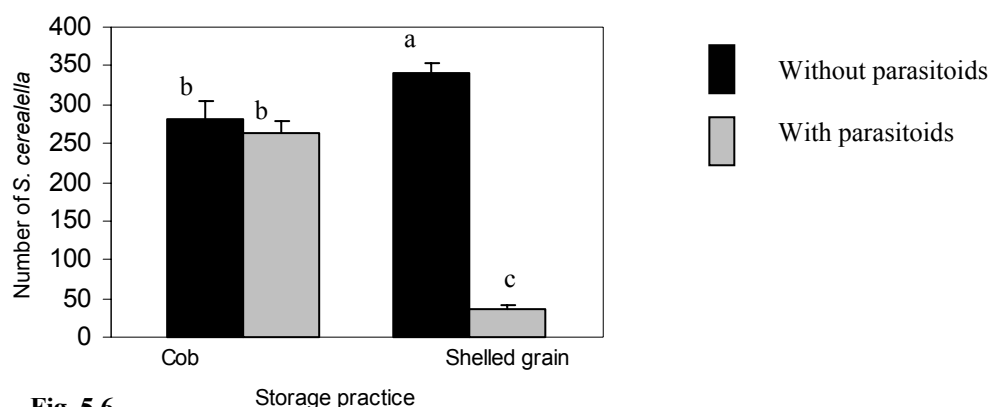


Fig. 5.6

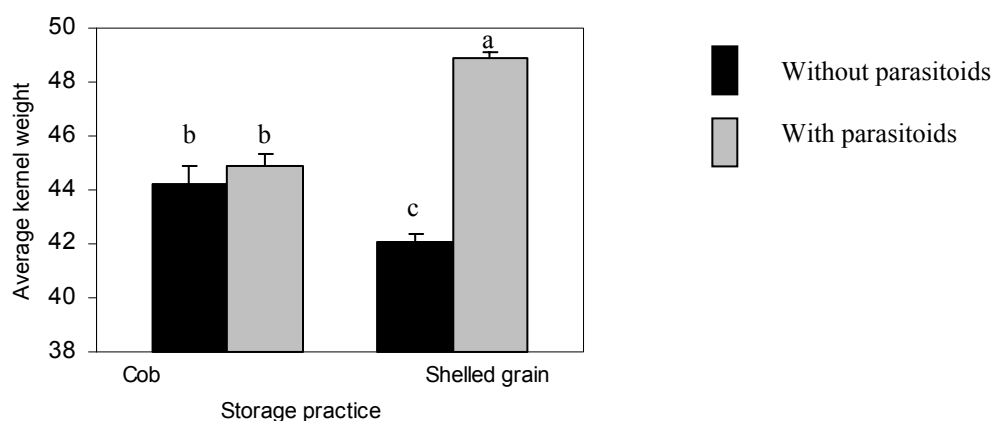


Fig. 5.7

Fig 5.6 : Effect of storage practice and a parasitoid *Pteromalus cerealellae* on number of *Sitotroga cerealella*.

Number of Angoumois grain moths after three months of storage with (grey) or without (black) parasitoids. ANOVA showed slightly higher, but significant increase of grain moths in shelled grain ($df=14$; $F=5.1$ $P=0.04$). Parasitoids had a significant impact on number of grain moths in shelled grain ($df=14$; $F=499.9$, $P<0.0001$), but not on the cob. Two way factorial analyses showed that the interaction of storage practice and parasitoid has a significant influence on increase of grain moths ($df=28$; $P<0.0001$)

Fig 5.7: Effect of storage practice and a parasitoid *Pteromalus cerealellae* on amage by *Sitotroga cerealella*.

Damaged expressed in reduction of average kernel weight after three months of storage with (grey) or without (black) parasitoids. ANOVA showed a slightly but significantly lower kernel weight (more damage) in shelled maize than from the cob ($df= 14$; $F=60$; $P<0.0001$). Parasitoids reduced damage significantly in shelled grain ($df= 14$; $F=330.9$; $P<0.0001$), but not on the cob. Two way factorial analyses showed that the interaction of storage practice and parasitoid had no significant influence on damage ($df=28$; $P<0.0001$)

Columns with the same letters are not significantly different with Fisher's protected LSD

maize stored on the cob. Damage, expressed as weight of an equal volume of grain, was accordingly lowest for shelled grain in the presence of parasitoids, and highest for the same storage method without parasitoids (Fig. 5.8). Total number of insects emerged (wasps and grain moths combined) was clearly reduced in shelled grain (Table. 5.2). Differences are all significant, except within the cob treatment. The two-way factorial analyses showed significant interaction ($P < 0.0001$) between storage method and parasitoid.

Table 5. 3: : Number of parasitic wasps, *Pteromalus cerealellae* that emerged from *Sitotroga cerealella* in infested maize stored either as cob or shelled

	<i>S. cerealella</i>	<i>P. cerealellae</i>	Total
Cob	263.6 \pm 15	18.9 \pm 3.7	282.9
Shelled	35.4 \pm 6	181.4 \pm 22	216.8

Total numbers of emerged insects were significantly lower on maize stored as shelled grain. (df= 14; F=6.7, P=0.02)

4.2 – Short term experiment

The wasp, *P. cerealellae*, was much less efficient in parasitizing its host on maize cobs (Table 5.3, and Fig.5.9). No wasps emerged from maize cobs in the first experiment (released at 22 days after infestation) and only a few (9%) in the second experiment (released 25 days after infestation). The number of adult grain moths that emerged from both storage situations in the treatment without wasps, did not differ significantly, although it was a little lower on maize cobs. The medium development time was not significantly different.

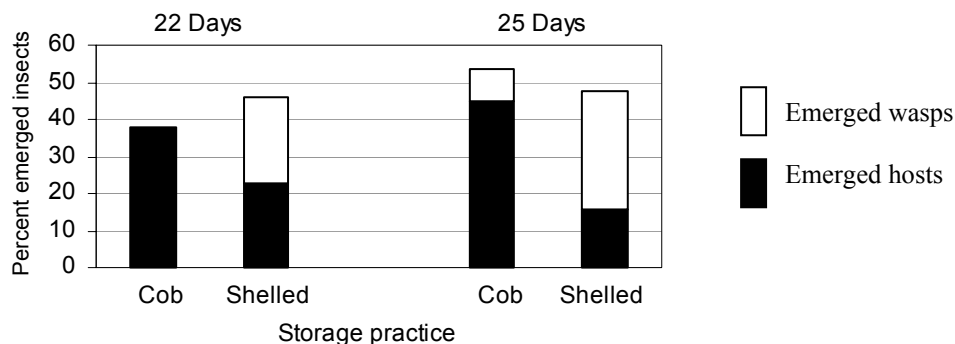


Fig 5.8 :Successful parasitism of *Sitotroga cerealella* larvae of different age in maize by *Pteromalus cerealellae*

Insect numbers are expressed as percentage of successfully hatched larvae that emerged as adults; either as *S. cerealella* (black) or as parasitoid, *P. cerealellae* (white)

When host larvae were 22 days old significantly more were parasitized on shelled grain than on the cob where no wasp emerged ($df=1,12$; $F=12.4$; $P<0.0001$)

From 25 day old hosts on the cob 8 % emerged as parasitoids, but significantly more emerged on shelled grain ($df=1,14$; $F=50.7$; $P<0.0001$)

Total emerged insects were not significantly different on either storage practice

Table 5. 4: Emerged insects from maize, stored as cob or shelled, infested with *Sitotroga cerealella*, and with the parasitic wasp *Pteromalus cerealellae* foraging for one day at 22 or 25 days after infestation

Storage method:	Parasitoid	Parasitoids added 22 days after infestation % emerged insects:			Parasitoids added 25 days after infestation % emerged insects:		
		<i>P.cerealellae</i>	<i>S.cerealella</i>	Total	<i>P.cerealellae</i>	<i>S.cerealella</i>	Total
Cob	None		47.2 ±5 a	47.2 ±5		59.2 ±6 ab	59.2 ±6
	Yes	0	38.6 ±3 a	38.6 ±3	8.8 ±1 a	44.6 ±5 b	53.4 ±5
Shelled	None		46.3 ±2 a	46.3 ±2		60.5 ±6 a	60.5 ±6
	Yes	23.1 ±2	22.9 ±3 b	44.6 ±3	32.2 ±3 b	15.5 ±2 c	47.7 ±5
LSD			9.3	not signif.		14.9	not signif.

Numbers of emerged insects are given as percentage of hatched eggs

% emerged insects : percentage emerged as *P. cerealellae* or as *S. cerealella*; Total : percentage of insects(hosts + parasitoids) emerged

Values followed by the same letter within each column are not significantly different at $P<0.05$ (Multiple comparison: Fisher's protected LSD)

Discussion

As observed by Kossou et al. (1993), the rate of increase of maize weevils was much lower on maize stored as cobs (Fig. 5.1). These authors related a slower larval development, and lower oviposition rate, apparently as a result of preferential selection for oviposition sites. The distribution pattern of egg plugs was significantly different in the two storage forms (Kossou

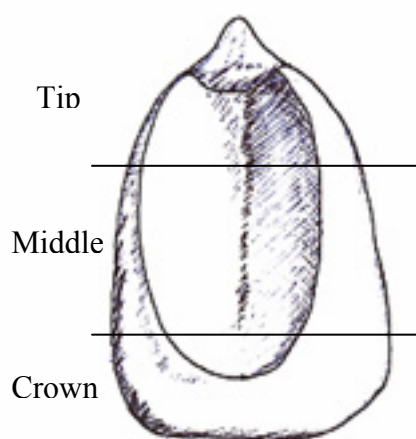


Fig. 5.9 Delimitation of areas in the maize kernel for location of oviposition sites

et al., 1992). In shelled maize, where weevils have equal access to all parts of the kernel they will avoid to oviposit on the crown, while on cobs they have no choice but to oviposit on this site (Fig. 5.9)

An additional factor certainly is that, during the initial phase of infestation, the adult insects are not able to feed to the same extend on cobs as on shelled grain where all parts of the grain are

accessible (Vowoter et al., 1993). This may have a negative impact on the oviposition rate, and adult survival during the initial phase of infestation.

The parasitic wasps had no effect on host number when maize was stored on the cob. This can be interpreted as a direct consequence of storage method, as the host larvae may have moved too far into the kernel and not remain in reach of the ovipositor of the parasitoid. But it could also be explained by the low host density. In fact, the number of available hosts may not have permitted the establishment of the parasitoid population. The observation that no live wasps were seen at the end of the experiment indicates that the population was either extinct or consisted of only a few individuals.

In the experiment with simultaneous infestation by *S. zeamais* and *P. truncatus*, the number of maize weevils was slightly reduced by the parasitoids on maize stored on the cob (Fig. 5.5). The tunnelling by *P. truncatus* adults could have facilitated the parasitoids access to the weevil larvae. The reduction of *P. truncatus* by the wasps was not significant on the cob, and inexistant in shelled maize. Helbig (1998) observed an important reduction of *P. truncatus* in double species cultures on shelled maize, but his field sampling data indicated a close association of *A. calandreae* with the maize weevil, but not with the larger grain borer. Other investigations also indicated a negative correlation between the parasitoids and *P. truncatus* and storage on the cob, with or without husk (Rios Ibarra et al., 1992). Although the ability of *A. calandreae* to attack *P. truncatus* immatures is well documented (Boeye et al., 1988), this pteromalid was not found to be an adequate biological control agent for the larger grain borer in Mesoamerica (Markham et al., 1994c; Espinal et al., 1996). Ghani and Sweetman (1955) found that *A. calandreae* only parasitizes host larvae that are enclosed. Since the larger grain borer larvae frequently evolve outside the maize kernel, in the powder produced by the adults, they may thus escape parasitism. The large amounts of dust and powder created by the intense boring activity of the adult beetles may hinder the searching activity of the parasitoids, principally in maize stored shelled or with the husk on.

Larger grain borer reproduce much better on the cob, grain stability and the possibility to burrow long tunnels may approximate their original boring activity in wood and favour development. The final number of adults may have been underestimated as many adults were encountered within the stalk and not all could be removed for counting.

The predator, *T. nigrescens*, was apparently unaffected by storage method (Fig. 5.3). Its small size as adult and slender shape as larvae allow it to follow its prey through the tunnels and reduce *P. truncatus* immatures in either storage situation, not hindered by powder. High variation in reduction of *P. truncatus* among samples was possibly due to an unequal number

of female predators, as *T. nigrescens* cannot be sexed by external characteristic (Rees, 1987). Dendy et al. (1989) found that *T. nigrescens* were able to locate their host in shelled grain, using host pheromones or through short range chemical stimuli left on the grain by the host (Rees et al., 1990). Avertay et al. (1999) showed that *T. nigrescens* reduced *P. truncatus* but not *S. zeamais* in maize cobs infested with both species, but Holst and Meikle (2002) found that maize weevils were also affected by this predator.

Damage reduction was not proportional to the reduction in numbers of weevils and grain borers for the different forms of storage (Fig. 5.2 and 5.4). Both beetles produce feeding damage as adults and contribute to weight loss for several months in case of the maize weevil (Longstaff, 1991). The larger grain borer is highly destructive as an adult. Demianyk and Sinha (1988) found that one adult completely destroys five maize kernels, which is far more than during larval development. Thus only a part of the damage was produced by the progeny, as adult beetles used for infestation contributed to grain damage.

The Angoumois grain moth was much less affected by storage method, a lower rate of increase was only detected in a long term experiment, but the difference is small compared to that observed for the maize weevil and the larger grain borer (Fig. 5.6). The apparent lack of effect is due to the mobility and small size of first instar larvae which can attain the part of the kernel where they prefer to infest, even in maize stored on the cob.

Emerging adults however are obliged to leave the kernel through the crown (Fig. 5.10). Its parasitoid, *P. cerealellae*, was strongly affected by storage method (Fig. 5.6) with no parasitism being reported for wasps placed on the host at 22 days after infestation on the cob (Fig. 5.8). As shown in Chapter 3, newly hatched Angoumois grain moth larvae penetrate the pericarp close to the tip of the maize kernel and tunnel through the endosperm towards the crown. The mature larvae has to excavate its pupal chamber beyond the pericarp, creating a so-called window to facilitate moth emergence. When maize is stored on the cob only the

crown portion of the kernel is exposed (Fig. 5.10). The ovipositor of *P. cerealellae* measures ca. 1.5 mm (Chapter 3), its range of action is therefore very limited. Moreover the part of the larvae that will be closest is its head with strong mandibles, ready for defence. Very small larvae (less than 0.43 mm) are not attacked by *P. cerealellae* (Wen et al., 1995), which prefers medium to large larvae and prepupae. Storage on the cob offers the developing larvae a safe refuge from attack by this parasitoid until shortly before pupation. The second short term experiment showed that wasps are able to parasitize a number of hosts only at a later stage of their development (25 days after infestation) (Fig. 5.8). When maize is stored as



Fig. 5.10 : Location of growing larvae of *Sitotroga cerealella* in maize on the cob. From left to right: an early, medium and late instar (Pupae)

shelled grain, all parts of the grain are potentially available for the parasitoid to search, and it may not only parasitize the hosts earlier, but also be less confronted with the hosts

attempts to defend itself. The window of action for the

parasitic wasp is therefore considerably longer in time, and the success rate higher on shelled grain. The long-term experiment showed that this pteromalid wasp is a very efficient parasitoid, and the magnitude of the difference in efficiency for both storage methods is such that, although not synergistic, it far outranks the relatively small difference in population increase of its host.

For *S. cerealella*, damage was not reduced as much as the number of progeny would suggest. This is due to the fact that each emerged parasitoid accounts also for some damage done by the attacked host larvae prior to parasitisation. However the total number of insects emerged on shelled grain with parasitoid was significantly reduced, indicating an effective

control of this grain pest (Table 5.2). Angoumois grain moths can have a high rate of increase in maize stores (Markham et al., 1996) and even a small number of adult grain moths produces a large number of eggs (Shazali and Smith, 1985), and assure a new generation and more hosts for the parasitoid. But the total number of insects clearly decreases with this treatment, and storage as shelled grain is strongly recommended in regions where *S. cerealella* and/or *P. truncatus* are the predominant pests.

These experiments have only included two storage methods. A third, storage as cob with the husks on, has been recognized by many authors as a means to reduce damage to storage pests significantly if the quality of the husk cover is good (Eden, 1952; Schulten, 1976; Dobie 1977; Meikle et al. 1998). We observed that the Angoumois grain moth developed only half of 8 infested cobs with husks on (unpublished results). However, this method is difficult to evaluate in a laboratory setting.

Conclusion

The effect of storage practice on the number of maize weevils and subsequent damage is so strong, that it is highly unlikely that this effect can be reversed by the parasitoid, *A..calandrae*, and in regions where the maize weevil is the dominant storage pest, maize stored on the cob will reduce post harvest losses. Further studies will be needed to confirm the negative effect of storage on the cob on the parasitoid (chapter 6).

The predator of the larger grain borer was not affected by storage method. In dry regions with possible larger grain borer outbreaks maize should be stored shelled as already recommended in Africa (Farell et al., 1996).

The storage practice that reduced Angoumois grain moth infestation has an antagonistic effect on its parasitoid *P. cerealellae*. However number of grain moths is only slightly reduced if maize is stored on the cob. On the other hand the efficiency of its parasitoid, *P. cerealleae*, on shelled maize is so important that this outcome is reversed, and maize suffers less damage when stored as shelled grain. *S. cerealella* is more important as a pest in dry climates (Dobie et al., 1991) maize stored as shelled grain will result in much lower damage due to their parasitoids.

These laboratory studies have shown the effect of storage method on storage pest populations and their natural enemies. Field studies under natural conditions will be needed to confirm these results, as they may be modified by other biotic factors, such as maize genotype and microorganisms associated with insect infestation, as well as abiotic factors, like type of storage facility, and climatic variations during the storage period.

Acknowledgements: We thank Nasario Baleras Garcia for technical help with the experiments. We are grateful to Jacqueline Moret for help with the statistics. The research was conducted at the laboratory of Entomology at the International Maize and Wheat Improvement Center (CIMMYT).

References

- Adams, J. M. 1977.** The evaluation of losses in maize stored on a selection of small farms in Zambia, with particular reference to methodology. *Trop. Stored Prod. Inf.* 33, 19-24
- Avertay, J.N., W.G. Meikle, C.Borgemeister, M. Camara, R.H. Markham, 1999.** Studies on predation of *Prostephanus truncatus* (Horn) (Col., Bostrichidae) and *Sitophilus zeamais* Mots. (Col., Curculionidae) at different densities on maize by *Teretriosoma nigrescens* Lewis (Col., Histeroidae). *J. Appl. Entomol.* 123 : 265-271
- Baker, J. E. , and J. E. Throne. 1995.** Evaluation of a resistant parasitoid for biological control of rice weevils in insecticide-treated wheat. *J. Econ. Entomol.* 88: 1570 - 1579
- Baker, J. E., and D. K. Weaver. 1993.** Resistance in field strains of the parasitoid *Anisopteromalus calandrae* (Hymenoptera : Pteromalidae) and its host, *Sitophilus oryzae* (Coleoptera: Curculionidae), to malathion, chloropyrifos-methyl, and pirimiphos - methyl. *Biol. Control* 3: 233-242
- Ball, J.C., Dahlsten, D.L. 1973.** Hymenopterous parasites of *Ips paraconfusus* (Coleoptera : Scolytidae) larvae and their contribution to mortality. I. Influence of host tree and tree diameter on parasitization. *Can. Entomol.* 105: 1453 – 1464
- Boeye, J., S. Burde, H. Keil, G.A. Laborius and F.A. Schulz. 1988.** The possibilities for biological integrated control of the larger grain borer (*Prostephanus truncatus* [Horn]) in Africa, pp 110-139. In G.G.M. Schulten & A.J. Toet (eds.) Workshop on the containment and control of the larger grain borer, Arusha, Tanzania, 16-21 May 1988.
- Boxall, R.A., and R.Gillet. 1984.** Farm level storage losses in Eastern Nepal. *Trop. Stored Prod. Information* 42 : 20-25
- Brower, J. H. 1991.** Potential Host Range and Performance of a reportedly monophagous Parasitoid, *Pteromalus cerealellue* (Hymenoptera: Pteromalidae). *Ent. News* 102 : 231 –235
- Choi, W.I., T.J. Yoon, and M.I. Ryoo. 2001.** Host-size-dependent feeding behaviour and progeny sex ration of *Anisopteromalus calandrae* (Hym., Pteromalidae). *J. Appl. Entomol.* 125: 71-77
- Cline, L. D., J. W. Press, and B. K. Flatherty, 1985.** Suppression of the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae), inside and outside of burlap, woven polypropylene, and cotton bags by the parasitic wasp, *Anisopteromalus calandrae* (Hymenoptera:Pteromalidae). *J. Econ. Entomol.* 78: 835 – 838

- Cowley, R. J., D. C. Howard, and R. H. Smith, 1980.** The effect of grain stability on damage caused by *Prostephanus truncatus* (Horn) and on three other pests of stored maize. J. Stored Prod. Res. 16: 75-78
- Demianyk, C. J. , and Sinha. 1988.** Bioenergetics of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) feeding on corn. Ann. Entomol. Soc. Am. 81: 449-459
- Dendy, J., P. Dobie, J. A. Saidi , and C. Sherman. 1989.** The design of traps for monitoring the presence of *Prostephanus truncatus* Horn (Col.: Bostrichidae) in maize fields. J. Stored Prod. Res. 25 : 187-191
- Dobie, P. 1977.** The contribution of the Tropical Science Production Center to the study of insect resistance in maize. Trop. Stored Prod. Inf. 34 : 7-22
- Dobie, P., C.P. Haines, R.J. Hodges, P.F. Preveet and D.P. Rees. 1991.** Insects and Arachnids of tropical stored Products: Their Biology and Identification . National Ressources Institute.
- Eden, W. G. 1952.** Effect of husk cover an rice weevil damage in Alabama. J. Econ. Entomol. 45 : 543-544
- Espinal, R., R.H. Markham, and V.F. Wright. 1996.** Honduras-Summary of activities on the larger grain borer and storage pest status in meso-America pp. 109-124 .In Hill, G.& G. Farrell (eds.), Proceedings of the East and Central Africa storage pest management workshop, Naivasha, Kenya 14-18 April 1996. CAB Wallingford, UK.
- Farrell, G., M.G. Hill, F.L.O. Nang'ayo, and A. Stabrawa. 1996.** A review of investigations to improve pest management of stored maize in smallholder farms in Kenya. Integrated Pest Management Rev. 1 : 251-263
- Flinn, P.W., D.W. Hagstrum and W.H. McGaughey. 1996.** Suppression of beetles in stored wheat by augmentative releases of parasitic wasps. Environ. Entomol. 25 : 505-511
- Ghani, M. A. , and H. L. Sweetman, 1955.** Ecological studies on the granary weevil parasite, *Aplastomorpha calandrae* (Howard). Biologia (Lahore)1 : 115 – 139
- Helbig, J. 1998.** Ability of naturally occurring parasitoids to suppress the introduced pest *Prostephanus truncatus* (Horn) (Coleoptera, Bostrichidae) in traditional maize stores in Togo. J. Stored Prod. Res. Vol 34 (4), 287-295
- Holst, N. amd W.G. Meikle. 2002.** The population dynamics of the biocontrol agent *Teretriosoma nigrescens*, its target *Prostephanus truncatus* and non-target *Sitophilus zeamais*, and the prospects for successful control in rural maize stores in humid West

Africa. Submitted

- Kossou, D. K., N. A. Bosque-Perez , and J. H. Mareck. 1992.** Effects of shelling maize cobs on the oviposition and development of *Sitophilus zeamais* (Motschulsky). J. Stored Prod. Res. 28: 187-192
- Kossou, D. K., J. H. Mareck, and N. A. Bosque-Perez. 1993.** Comparison of improved and local maize varieties in the Republic of Benin with emphasis on susceptibility to *Sitophilus zeamais* Motschulsky. J. Stored Prod. Res. 29: 333- 343
- Longstaff B. C. 1981.** Biology of the Grain Pest Species of the Genus *Sitophilus* (Coleoptera: Curculionoidea): A critical Review. Prot. Ecol. 2, 83-130
- Markham, R.H., N.A. Bosque-Perez, C. Borgemeister, and W.G. Meikle. 1994a.** Developing pest management strategies for *Sitophilus zeamais* (Motschulsky) and *Prostephanus truncatus* (Horn) in the tropics. FAO Plant Protection Bulletin 42: 97-116
- Markham, R. H., F. Djossou, J.M. Hirabayashi, P. Novillo, V.F. Wright, R.M.Rios, F.J. Trujillo, W. G. Meikle and C.Borgemeister. 1994b.** Biological control in the context of an integrated management for the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera, Bostrichidae) and associated storage pests. In E.Highley, E.J. Wright, H.J. Banks, & B.R. Champ (eds).Proceedings of the 6th international Working Conference on Stored product Protection, Canberra Australia Vol 2, 1106-1111
- McFarlane, J. A. 1988.** Pest management strategies for *Prostephanus truncatus* (Horn) as a pest of stored maize grain. Present status and prospects. Trop. Pest Man. 34: 121-132
- Meikle, W.G., C. Adda, C. Azoma, C. Borgemeister, P. Degbey, B. Djomamou , and R. H. Markham, 1998.** The effects of maize variety on the density of *Prostephanus truncatus* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) in post-harvest stores in Benin Republic. J. Stored Prod. Res. 34 : 45-58
- Muhiu, S.K. 1985 .** Depth of infestation by *Sitotroga cerealella* (Oliver) into grain layers of wheat, maize and sorghum. Trop. Stored Prod. Information, 47, 34-37
- Porter, B.A. 1928.** The apple maggot. U.S. Dep. Agric. Tech. Bull. 66: 1-48
- Press, J. W. 1984.** Suppression of residual populations of the rice weevil, *Sitophilus oryzae*, by the parasitic wasp, *Anisopteromahis calandrae*. J. Ga. ent. Soc. 19: 110- 113
- Price, P. W., C. B. Bouton, P. Gross, B. A. McPheron, J. N. Thompson, and A. E. Weis. 1980.** Interactions among three trophic Levels: Influence of plants on interactions

- between insect herbivores and natural enemies. Annual Research Ecological Systems 11 : 41 – 43
- Rees, D. P. 1985.** Life History of *Teretriosoma nigrescens* Lewis (Coleoptera; Histeridae) and its ability to suppress populations of *Prostephanus truncatus* Horn (Coleoptera; Bostrichidae). J. Stored. Prod. Res. 21: 115-118
- Rees, D. P. 1987.** Laboratory studies on predation by *Teretriosoma nigrescens* Lewis (Coleoptera, Histeridae) on *Prostephanus truncatus* Horn (Coleoptera, Bostrichidae). J. Stored Prod. Res. 23: 191-195
- Rees, D.P., R. Rodriuez Rivera, and F.J. Herrero Rodriguez. 1990.** Observations of the ecology of *Teretriosoma nigrescens* Lewis (Col: Histeroidae) and its prey *Prostephanus truncatus* (Horn) (Col: Boastrichidae) in the Yucatan peninsula, Mexico. Tropical Science 30 : 153-165
- Richter, J., A. Biliwa, and S. Henninghelbig. 1997.** Losses and pest infestation in different maize storage systems with particular emphasis on *Prostephanus truncatus* (Horn)(Col, Bostrichidae) in Togo. Anzeiger Fuer Schaedlingskunde, Pflanzenschutz, Umweltschutz 70: 112-116
- Rios Ibarra, R. M., R. H. Markjam, P. Novillo Rameix , and V. F. Wright. 1992.** Ecology and biological control of the larger grain borer in Mexico and Honduras. In: Implementaion of and further Research on Biological Control of the Larger Grain Borer. J. Boeye, M. Wright and G.A. Laborius (eds) Proceedings of and FAO/GTZ Coordination Meeting. pp.123-136
- Ryoo, M. I., T. J. Yoon , and S. S. Shin. 1996.** Intra- and interspecific competition among the parasitoids of the rice weevil (Coleopotera: Curculionidae). Environ. Entomol. 25 : 1101 – 1108
- Scholz, D., C. Borgemeister, W. G. Meikle, R. H. Markham , and H. M. Poehling. 1997.** Infestation of Maize by *Prostephanus truncatus* initiated by male- produced pheromone. Entomol. Exper. Applic. 83: 53-61
- Schulten, G. G. M. 1976.** Insects in stored maize ears. Abstracts on Tropical Agriculture. Review Article: 9
- Shazali, M. E. H. and R.H. Smith. 1985.** Life history studies of internally feeding pests of stored sorghum : *Sitotroga cerealella* (Ol) and *Sitophilus oryzae* (L.). J. Stored Prod. Res.. 21 :171-178
- Smith, L., 1992.** Effect of temperature on life history characteristics of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). Environ. Entomol. 21: 877 –887

- Smith, L. 1993.** Effect of humidity on life characteristics of *Anisopteromalus calandrae* (Hymenoptera : Pteromalidae) parasitizing maize weevil (Coleoptera: Curculionidae) larvae in shelled corn. Environ. Entomol. 22 (3) 618 – 624
- Smith, L. 1994.** Temperature influences functional response of *Anisopteromalus calandrae* (Hymenoptera : Pteromalidae). Ann. Ent. Soc. Am. 87 : 849- 855
- Smith, L., D. K. Weaver, and R. T. Arbogast 1995.** Suitability of the maize weevil and Angoumous grain moth as hosts for the parasitoids *Anisopteromalus calandrae* and *Pteromalus cerealellae*. Entomol. Exp. et App. 76 :171-177
- Tigar, B. J., G. B. Key, M. B. Flores , and M. Vasquez. 1994.** Field and post-maturation infestation of maize by stored product pests in Mexico. J. Stored Prod. Res. 30 (1) ,1-8
- Tigar, B. J., G. E. Osborne, G. F. Key, M. E. Flores-S. , and M. VasquezA. 1994.** Distribution and abundance of *Prostephanus truncatus* (Coleoptera: Bostrichidae) and its predator *Teretriosoma nigrescens* (Coleoptera: Histeridae) in Mexico. Bulletin of Entomological Research 84 : 555-565
- Vowoter, K. A., N. A. Bosque-Perez, and J. N. Ayertey 1995.** Effect of maize variety and storage form on the development of the maize weevil *Sitophilus zeamais* Motschulsky. J. Stored Prod. Res. 31: 29-36
- Wen, B. ,and J. H. Brower 1995.** Competition between *Anisopteromalus calandrae* and *Choetospila etegans* (Hymenoptera: Pteromalidae) at different parasitoid densities on immature rice weevils (Coleoptera: Curculionidae) in wheat. Biol. Control, 151 – 157
- Wen, B., K. Weaver, and J. H. Brower, 1995.** Size preference and sex ratio for *Pteromalus cerealella* (Hymenoptera: Pteromalidae) parasitizing *Sitotroga cerealella* (Lepidoptera: Gelechiidae) in stored corn. Environ.Entomol. 24: 1160- 1166
- Wen, B., L. Smith, and J. H. Brower. 1994.** Competition between *Anisopteromalus ca/ant/rae* and *Choetospila elegans* at different parasitoid densities on immature maize weevil in corn. Environ. Entomol. 23: 367 –373
- Wen, B., K. Weaver, and J. H. Brower 1995.** Size preference and sex ratio for *Pteromalus cerealella* (Hymenoptera: Pteromalidae) parasitizing *Sitotroga cerealella* (Lepidoptera: Gelechiidae) in stored corn. Environ. Entomol. 24: 1160- 1166
- Weston, P. A., R. J. Barney , and X. S. Ge.1997.** Host-plant characteristics and environmental factors influencing flight activity of Angoumois grain moth (Lepidoptera, Gelechiidae) in the field. Environ. Entomol. 26: 229 – 233

INTERACTION OF MAIZE WEEVILS AND PARASITOIDS WITH DIFFERENT STORAGE METHODS IN THE FIELD

Abstract

Three maize varieties from the International Maize and Wheat Improvement Center (CIMMYT) were used to determine the interaction of storage form with biological control agents found in farmers stores in Veracruz, Mexico. The three storage forms included the husk on, as cob, or as shelled grain and were exposed to natural insect infestation for four months. The insect population, within and between the kernels, and percent damage were monitored at two week intervals. The maize weevil *Sitophilus zeamais* (Coleoptera: Curculinodae) was the most abundant primary pest. Other primary storage pests such as the Angoumois grain moth *Sitotroga cerealella* (Lepidoptera: Gelechiidae) were found only occasionally and in small numbers. Two parasitic wasps, *Lariophagus distinguendus* and *Anisopteromalus calandrae* (both Hymenoptera: Pteromalidae) that attack *S. zeamais* larvae were very common. Secondary storage pests were present, but not frequent.

There were no differences in number of insects and percentage damage among the maize varieties. But significantly more maize weevils emerged from shelled samples than from those stored as cob. Even less maize weevils were found on cobs stored with husk on, but differences between cobs were large. All shelled grain was damaged after only 11 weeks, and for cobs after 15 weeks. Fifty percent damage was observed for grain stored with the

husk after 19 weeks of storage. Parasitic wasps had a significant impact on shelled maize only. They emerged from the first weevil generation that developed and parasitized up to 67 % of exposed maize weevil larvae. The parasitoid populations followed the increase of the maize weevil, but dropped sharply after 11 weeks in storage. *L. distinguendus* was slightly more frequent than *A. calandrae*.

Key words: Maize, farm storage, natural enemies, *Sitophilus zeamais*, *Anisopteromalus calandrae*, *Lariophagus distinguendus*

Introduction

In many developing countries, subsistence farmers lack adequate storage structures and shelling devices (Markham et al, 1994). After harvest, maize is stored either on the cob both with or without the husk on, as this storage practice is believed to reduce post-harvest losses (Dobie, 1977; McFarlane, 1988). Moreover, shelling is labor intensive and time consuming. In the course of domestication, maize seed lost its protective fruitcase, but has developed several leaves which are collectively called the husk. The husk protects the maturing maize cob from attack by birds, diseases and insects pests. Modern breeding programs have considerably improved maize varieties, increasing yield and resistance to pathogens, but frequently neglecting the quality of the husk cover. While the beneficial role of a well developed husk before and after harvest has been known for a long time (Eden, 1952a,b; Wiseman et al., 1970; Schulten, 1976), storage of maize on the cob, even without husk, will reduce damage by the maize weevil, *Sitophilus zeamais* (Kossou et al., 1992; 1993). Meikle et al. (1998) observed that husk cover had little effect on *S. zeamais* densities in a six months field exposure in Africa, suggesting that this protection is limited in time. Other work reported that poor husk cover is a crucial factor for increasing the early colonization by maize weevils (Giles and Ashman, 1971; Dick, 1988), and other primary grain feeders that are more likely to infest maize in the field if the cob is exposed (Eden, 1952a,b) but the drying of the husk cover may reduce its efficiency in protecting from insects (Schulten, 1976). The importance of the husk cover is clear, but protection is only temporary, and selecting for varieties with good husk cover may best be considered as one part of an overall stored product protection strategy (Meikle et al., 1998).

Natural enemies of stored grain pests are another component of integrated pest management strategies to reduce post harvest losses. Predators of the order of the Hemiptera are frequent in tropical stores. Boeye (1988) however found that *Calliodis sp.* (Anthocoridae)

was present too late in the storage season to serve as an efficient natural enemy. The release of *Xylocoris flavipes* (Anthocoridae) on residual populations affected only secondary feeders, but not *Sitophilus* spp., *Rhyzopertha dominica* (Coleoptera: Bostrichidae), or *Plodia interpunctella* (Lepidoptera: Pyralidae) (Brower and Press, 1992).

Several species of parasitic wasps sometimes occur in large numbers in tropical stores and have received close attention. *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) is a cosmopolitan parasitoid of the maize weevil *Sitophilus zeamais*, and frequently found in tropical stores (Dobie et al., 1991). In various laboratory studies it was found to be a potential candidate for biological control of the maize weevil (Smith, 1993 and 1994; Baker and Weaver, 1993 and Baker and Throne, 1995) and experiments with augmentative releases resulted in significant reduction of storage beetles (Flinn et al., 1996; Cline et al., 1985; Press, 1984). *Lariophagus distinguendus* is another cosmopolitan ecto-parasitoid of *S. zeamais*. Hong and Ryoo (1991) found that between 28 and 30°C was the optimal temperature to control the rice weevil, *Sitophilus oryzae*. *L. distinguendus* was found to co-exist with *A. calandrae* in conditions of interspecific competition for a common host, *S. oryzae* (Ryoo et al., 1996). Field studies of insect populations dynamics usually focus more on storage pests populations than on their natural enemies (Storey et al., 1983) and sampling of insects is made with traps or collects from stored maize (Tigar et al., 1994; Meikle et al., 1998b). Both are methods that will lead to an underestimate of insects that, like wasps, fly readily. Arbogast and Mullen (1990) incubated samples collected from maize stores in Georgia (USA) and found that the rate of parasitism by *A. calandrae* responded to changes in host (*S. zeamais*) density, but lagged behind, so that heavy damage occurred. They proposed early releases of the parasitoid to suppress initial build-up.

The interaction of parasitic wasps and predators with storage method, as shelled grain or on the cob have been investigated in the laboratory (chapter 4). While another pteromalid,

Pteromalus cerealellae, was negatively affected by storage on the cob that provided a spatial refuge for its principal host, *Sitotroga cerealella*, the outcome for *A. calandreae* could not be definitely cleared because of a very low host density. It is however very likely, that storage on the cob will also provide more refuge for maize weevil larvae than shelled grain. The effect of husk cover on parasitoids has not been studied yet.

The first objective of this study was to evaluate the importance of parasitoids in a tropical storage environment, their initial build-up and the rate of parasitism of their hosts over a four months storage period and secondly, to quantify the effect of storage practice, as shelled grain, on the cob, with or without husk, on the composition of the insect population and subsequent damage, and on the rate of parasitism.

Material and methods

The field experiment was set up with three different maize varieties from the subtropical breeding program at CIMMYT (International Maize and Wheat Improvement Centre, Mexico) : two three way hybrids, : TWHST (CML320xCML321)xCML311(white) and TWHST (CML323xCML327)xCML335 (yellow), and one population, P 590 cycle 5 (yellow), which was developed for resistance to stem borer and fall army worm. About 120 ears of each variety were harvested at CIMMYT's experimental station in Tlaltizapan, Morelos. Forty ears were left with the husk on, another 40 were dehusked and 40 were shelled to generate three storage methods: husk on, cob, shelled. Two cobs or their equivalent in grain were placed in a nylon mesh bag to constitute one sample. Samples of the same treatments (maize variety x storage method) were placed together in a large nylon mesh bag. Enough samples were prepared to be able to collect at least two at each sampling date per storage method and variety, and three samples for the part that was stored with husk on.

The storage facility used was an open shed (2 x 2m) at CIMMYT's experimental station in Poza Rica, Veracruz. The structure was fitted with wire mesh to impede the entry of rodents and other larger animals, but to allow free movement of insects. The shelter is free standing from any other building and protected by the shade of trees. It had been used for storage of maize before, and storage insects had been sampled over two years prior to the experiment. The facility was cleaned out, and, at the start of the experiment, appeared to be free of any residual insect population.

Because rodents did some heavy damage at the beginning of the experiment, the bags were put inside wire mesh cages five weeks into the experiment. The cages were placed on three shelves in the shed and their positions were switched at regular intervals.

The samples were placed in the shed at the end of June, and sampling started three weeks later. From there on samples were collected every two weeks. The last sampling day was 19 weeks after the start of the experiment. No insects were released at any time. The collected samples were brought to the laboratory in closed cotton bags so that no insects could escape. Upon arrival the material was dehusked and shelled if necessary, and sieved. The interstitial insect population was collected and identified. All samples were then placed in half liter jars covered with wire mesh, and incubated at 26°C and 75 % R.H.. The samples were sieved and insects collected again after 15 days. This is the time needed for the parasitic wasps, *L. distinguendus* and *A. calandreae*, that have attacked their hosts in the field to complete development, and at the same time it allows to separate maize weevil immatures into two size classes. Insects were collected again one month after the collection date. Parasitic wasps that emerged after 15 days were not from field infestation but from reinfestation through emerged adults, and therefore counted as small maize weevil immatures. Subsequently, samples were frozen and percentage of damaged kernels was evaluated.

Insect numbers were log transformed to improve normality and after ANOVA post-hoc comparisons were made using Tuckey-Kramer multiple comparison test. Maize weevils that were found on the maize samples on the day of collection (interstitial population) were not used in those tests, only intra-kernel population was considered. To follow the increase of wasp populations a ratio was calculated using emerged parasitoids per large maize weevil larvae, and percentage parasitism using the number of large immature maize weevil plus the number of emerged wasps as total available host number.

Results

Primary pests

The predominant insects that were collected from maize samples and emerged after incubation were *Sitophilus zeamais* and the two generalist parasitoids (*Lariophagus distinguendus* and *Anisopteromalus calandrae*). Other primary pests, such as *Prostephanus truncatus* and *Sitotroga cerealella* were found only occasionally and never became established (Table 6.1).

Maize weevil population increased rapidly and lead to a 100% damage level in shelled grain after only 11 weeks of storage (Fig. 6.1). There were no significant differences among the maize varieties for number of insects or damage, so the numbers were pooled. The differences of percent damaged grain and number of weevils emerged were significant among storage methods (Table 6.2, Fig. 6.1, 6.2 and 6.3). Shelled grain had a significantly higher percentage of damaged grain than maize stored on the cob, 100 % grain was damaged after

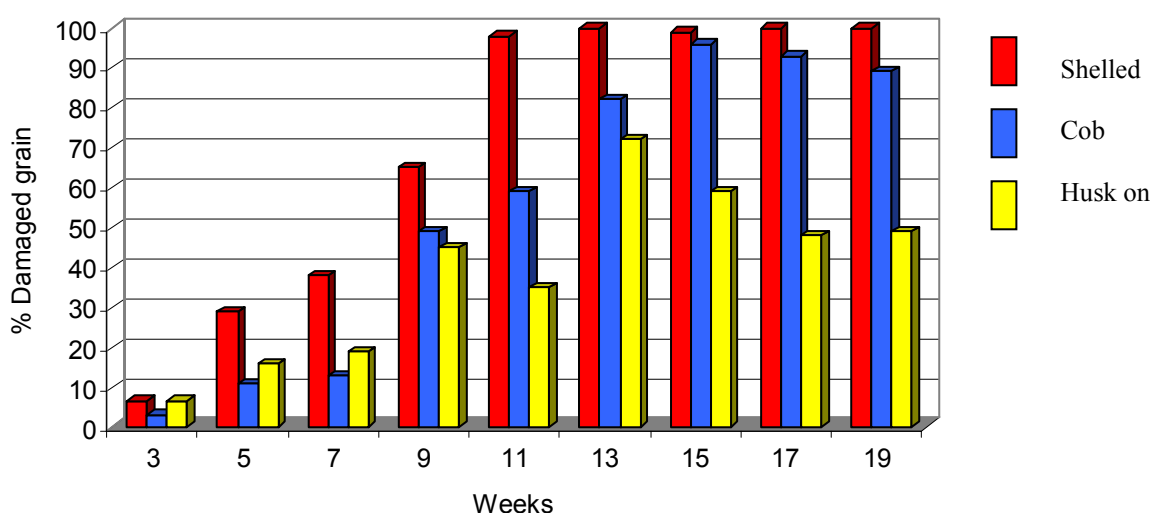


Fig 6.1 : Evolution of damage levels for each storage method

Damage measured as percentage of damaged kernels. Red: shelled grain; blue : on the cob; yellow : with husk on. Means are given of samples of all varieties pooled. ANOVA showed significant differences for storage method of overall damage level (df= 175; F= 13.9; P<0.0001) Significantly less damaged kernels were found for storage with husk from the 11th week onwards, cobs suffered significantly less damage than shelled grain only at this date (Tuckey-Kramer)

Table 5.1 : List of insects found in the stored maize samples in Poza Rica (Veracruz, Mexico)

Family	Genus	Species	Frequency	Feeding
Pest species				
<i>Coleoptera:</i>				
Anthribidae	<i>Araecerus</i>	<i>fasciculatus</i>	*	prim
Bostrichidae	<i>Prostephanus</i>	<i>truncatus</i>	*	prim
Bostrichidae	<i>Rhyzopertha</i>	<i>dominica</i>	**	sec
Bostrichidae	indeterminate		*	sec
Cucujidae	<i>Cryptolestes</i>	<i>ferrugineus</i>	**	sec
Curculionidae	<i>Sitophilus</i>	<i>zeamais</i>	***	prim
Dermestidae	indeterminate		*	scav
Nitidulidae	<i>Carpophilus</i>	spp.	**	fung
Sylvanidae	<i>Cathartus</i>	<i>quadricolis</i>	*	fung
Sylvanidae	indeterminate		*	
Tenebrionidae	<i>Tribolium</i>	<i>castaneum</i>	**	sec/pred
Tenebrionidae	<i>Gnatocerus</i>	<i>cornutus</i>	*	sec/pred
<i>Lepidoptera:</i>				
Gelechiidae	<i>Sitotroga</i>	<i>cerealella</i>	*	prim
Pyalidae	<i>Ephesia</i>	spp.	*	sec
Pyalidae	<i>Plodia</i>	<i>interpunctella</i>	*	sec
<i>Psocoptera:</i>				
Liposcelidae	Liposcelida	indeterminate	**	scav
Predators and Parasitoids				
Hemiptera				
Anthricidae	indeterminate		*	pred
Dermaptera	indeterminate		*	pred
Hymenoptera				
Pteromalidae	<i>Anisopteromalus</i>	<i>calandrae</i>	***	par
Pteromalidae	<i>Lariophagus</i>	<i>distinguendus</i>	***	par
Pteromalidae	<i>Choetospila</i>	<i>elegans</i>	**	par
Braconidae	<i>Bracon</i>	<i>hebetor</i>	*	par
Formicoidea	indeterminate		**	pred

Frequency : *** : species that were very abundant, ** : species that were moderately abundant, with more than 100 individuals found over the storage season, * : species that were found only occasionally

Feeding : feeding activity of the pests on stored maize : prim : primary grain feeders; sec : secondary grain feeders; fung : fungus feeders; scav : scavengers; pred : predators; par : parasitoids

only 11 weeks. Maize stored on the cob reached the same level 4 weeks later. On maize stored with the husk on the rate of increase of the maize weevil was slower and rate of damage reached only 50% after 19 weeks of storage (Fig 6.1 and 6.3). However the standard error for damage and number of weevils was very high for maize stored with husk on throughout the storage period as some individual cobs were encountered almost undamaged at the last sampling date, but others were heavily damaged after only a few weeks (Fig. 6.2).

Parasitoids and predators

Two pteromalid wasps, *Lariophagus distinguendus* and *Anisopteromalus calandrae* emerged in large numbers from maize stored as shelled grain (Table 6.3, Fig. 6.4). Of the weevil immatures that emerged within two weeks and were considered to be suitable hosts exposed to natural parasitism in the field up to 67% were parasitized on shelled maize (Fig. 6.5), but far less on maize stored as cob and the husk on, reaching only 10.7% and 11.2% respectively (Table 6.2). The number of parasitic wasps increased over the first 9 weeks and then stagnated, but the number of maize weevils continued to increase (Table 6.2, Fig. 6.5). The

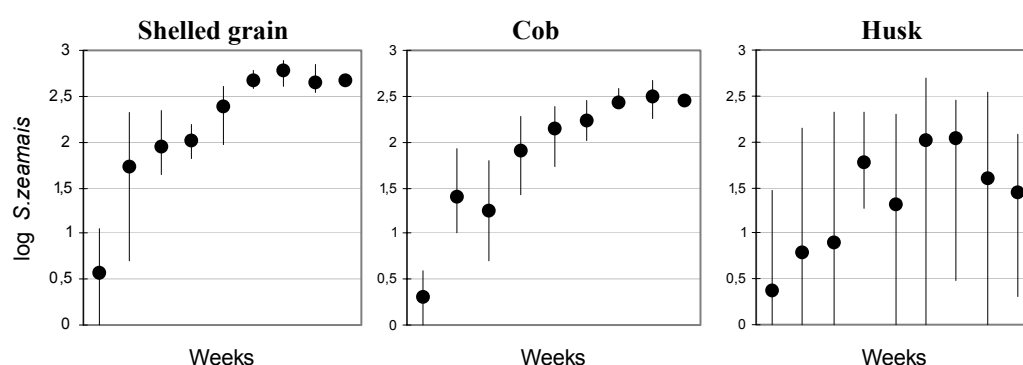


Fig. 6.2 Variation of numbers of maize weevil larvae per sample for each storage method
Maximum and minimum numbers of maize weevils that emerged during the incubation time are given, as well as the mean number per sample for each storage method and sampling date. Notice the large differences for storage with husk on, throughout the storage time.

Table 6.2: Maize weevils and their parasitoids found on, or emerged from maize samples

Storage:	Shelled grain			Cob			Cob with husk		
	Emerged after incubation		on sample	Emerged after incubation		on sample	Emerged after incubation		on sample
Week	wasps	<i>S.zeamais</i>	<i>S.zeamais</i>	wasps	<i>S.zeamais</i>	<i>S.zeamais</i>	wasps	<i>S.zeamais</i>	<i>S.zeamais</i>
3	0	3.8	3.5	0	2	1.3	0	5.7	10.8
5	11.3	90	9.6	0.4	35	7	0.6	35.3	4.6
7	21	111	62.3	1,6	29	15.6	1.9	48.6	11.4
9	53.7	107	46.3	2.1	95	42.6	0.4	76	44.6
11	60.7	269	112.3	4.1	156	52.3	3.3	77.1	71.4
13	47.7	483	136	4.9	180	69.8	0.3	197	154.4
15	84	603	255.5	9.2	275	177.2	1.2	177	96.9
17	15.4	462	138	5.6	324	128.9	0	152.4	106.8
19	108	463	329	1	290	103	0	96	65.4

Number of wasps emerged during the first two weeks on incubation, both species (*Anisopteromalus calandrae* and *Lariophagus distinguendus*) confounded; *S. zeamais* : number of maize weevils emerged over 30 days of incubation; and *S. zeamais* that were recovered from the maize samples at the day of collection.

All numbers are given as mean per sample (one sample = two maize cobs or the equivalent amount of grain) ANOVA was applied to log transformed numbers and showed significant differences among storage methods for insects emerged : wasps : df=2, 175 F=161.4 P<0.0001; emerged: df=175; F= 9.0; P=0.0002; total insects emerged (*S. zeamais* +wasps); df=175; F=32.8; P<0.0001

Table 6.3 : Numbers of parasitic wasps emerged during the first 14 days of incubation

Week	Parasitoid/host ratio	% paraistized	Species ratio	Sex ratio
5	1.1	41.4	0.9	1.0
7	1.9	47.7	0.5	0.8
9	2.7	67.3	1.5	1.0
11	1.3	51.4	1.6	0.9
13	0.4	26	2.1	1.6
15	0.6	28.7	3.9	2.2
17	0.1	9.7	3.9	1.3
19	0.6	39.4	1.8	1.2

Parasitoid/host ratio: wasps per maize weevils emerged during the first 14 days of incubation. % parasitized: percentage large hosts that were parasitized on shelled grain. Species ratio: ratio of *L. distinguendus* per *A. calandrae* calculated for shelled grain only, but ratios on the two other storage methods were similar
Sex ratio: female per male for shelled grain, both species combined. Parasitism on cob and husk treatments reached 10.7% and 11.2% maximum and details are not reported.

ratio of weevils parasitized, calculated as number of wasps per number of large hosts

increased steadily until week 9 and then dropped sharply (Fig. 6.6).

Of the two wasp species *A. calandrae* was less frequent. At the begin of the storage season there was a certain equilibrium of both species, but during the second half *L.*

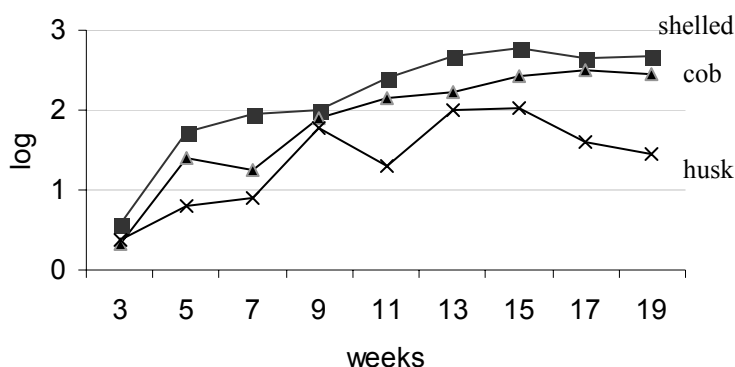


Fig. 6.3

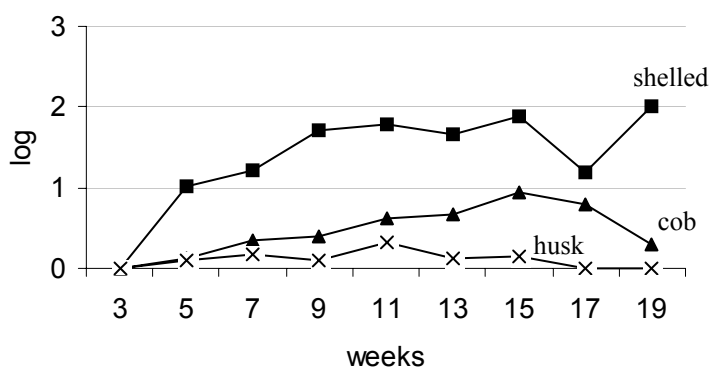


Fig. 6.4

Fig 6.3 : Total number of *S. zeamais* adults emerged from incubated samples on each storage method

Log transformed numbers (n+1) of adult *S. zeamais* that emerged within 30 days from sampled maize were log transformed.

ANOVA was applied and showed significant differences among storage methods for the intrakernel population of immature *S. zeamais* : df=175; F= 9.0; P=0.0002. The difference was significant from the 11th week throughout the 17th week.

Fig 6.4 : Total number of parasitic wasps emerged from incubated samples on each storage method

Log transformed numbers (n+1) of two species of wasps that parasitized maize weevils.

ANOVA was applied and showed significant differences among storage methods for number of parasitoids df=175; F=161.4; P<0.0001.

distinguendus strongly dominated (Table 6.3). The sex ratio was equilibrated at the begin of the storage period, but later on became strongly female biased (Table 6.3).

Other parasitoids were not frequent. Another pteromalid, *Choetospila elegans* never reached high numbers. On average 1.8 wasps per sample were encountered on shelled grain, 4.2 on maize stored as cob and 8.4 on maize stored with husk on (all sampling dates combined). It was clearly more frequent towards the end of the storage period. One single braconid wasp

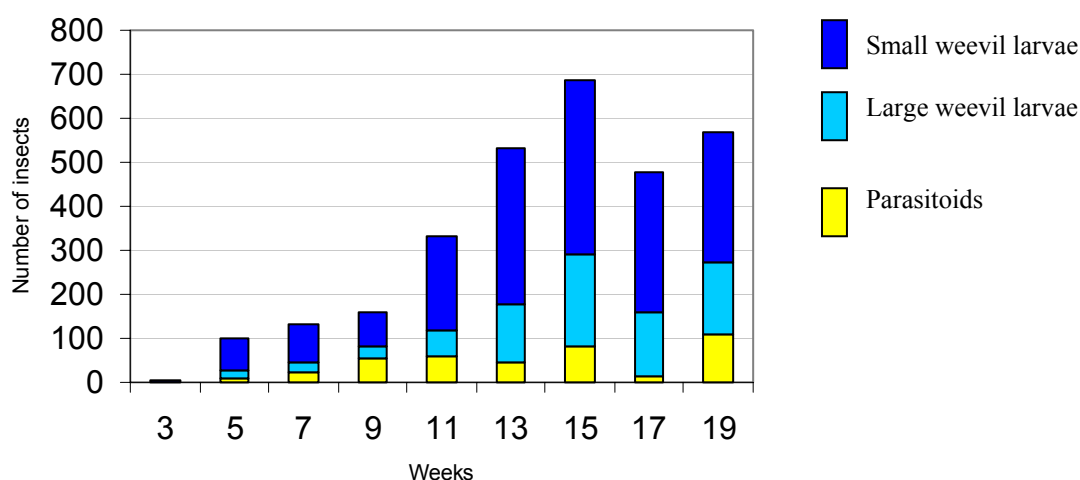


Fig 6.5 : Intra-kernel insect population on shelled grain (mean numbers, untransformed)

dark blue : small weevil larvae that emerged between 15 and 31 days after samples were collected, and were probably too small to be exposed to parasitoids in the field
light-blue : large weevil immatures that emerged as maize weevils and were exposed to parasitoids in the field
yellow : large weevil immatures that emerged as parasitic wasps (two species combined: *Lariophagus distinguendus* and *Anisopteromalus calandrae*) during the first 14 days of incubation and originated from natural infestation in the field.

was found, *Bracon hebetor*, a parasitoid of pyramalid moth larvae, such as *Plodia interpunctella*.

Predators were rarely encountered. Hemiptera that had been very numerous on the residual population before the storage facility was cleaned out, had all disappeared and we found only two in the course of the whole period. Ants were quite frequent at times, and were observed carrying away large maize weevil larvae.

Secondary pests

Secondary pests were not numerous as compared to the number of maize weevils (Table 6.1). They presented only 2% of total insect found on shelled maize and cobs, and 4% on cob with husk on. *Rhyzopertha dominica* immigrated at the start of the experiment but were unable to establish on the undamaged maize. Few were found alive on the samples, but many dead were

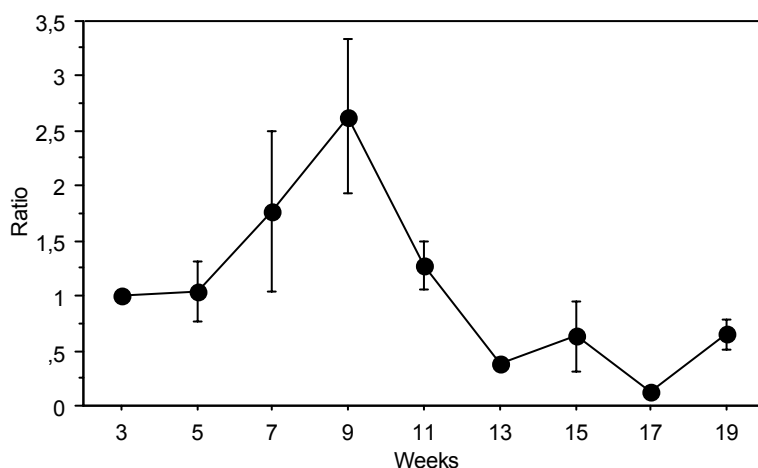


Fig 6.6 : Ratio of emerged wasps per emerged hosts

Number of parasitic wasps (two species combined) emerged over the first 14 days per number of maize weevils emerged over the same period.

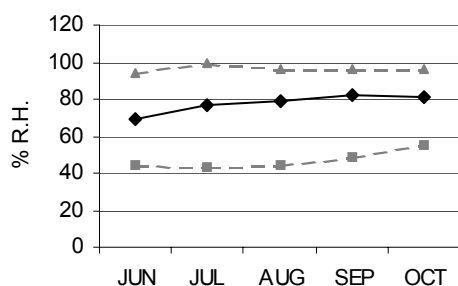


Fig. 6.7

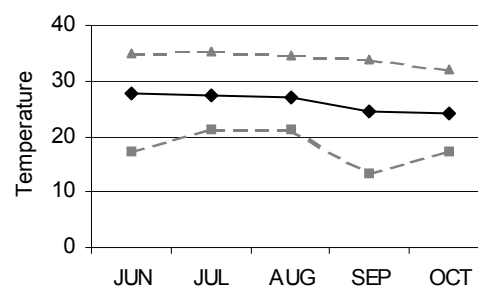


Fig. 6.8

Fig. 6.7 : Relative humidity during the storage period (El Ejido)

med: medium; min :minimum; max: maximum relative humidity foreach month of the storage period

Fig . 6.8 : Temperatures during the storage period (El Ejido)

med: medium; min :minimum; max: maximum temperatures in ° C for each month of the storage period (data provided by CIMMYT)

observed around the boxes. Numbers of *Carpophilus* spp., which feed on mouldy grain or on fungi, increased at in the middle of the storage time but then decreased again. *Tribolium castaneum* was more frequent on maize stored with husk on, and increased towards the end of the storage season, an overall average of 3.9 insects were found per sample. On shelled grain and maize cobs there were less, only 1 and 0.1 respectively per sample, all sampling dates combined. *Cryopolestes ferrugineus* numbers also increased towards the end of the storage season.

Discussion

In the two years before the experiment, *Sitophilus zeamais* was always found to be the dominant pest species in this storage facility, but the Angoumois grain moth, *Sitotroga cerealella* and the Indian meal moth, *Plodia interpunctella* also occurred in large numbers, as well as their natural enemies, *Pteromalus cerealellae* (Hymenoptera: Pteromalidae) and *Bracon hebetor* (Hymenoptera: Braconidae). During this experiment, however, the insect population was much less diverse (Table 6.1). *S. zeamais* was the only primary grain pest of importance. The climatic conditions, high relative humidity and temperatures (Fig. 6.7 and 6.8) favoured a fast increase of *S.zeamais*. Under such condition *S. cerealella* is not able to withstand competition with the maize weevil (Avertey, 1980; Dobie et al., 1991), and it appeared only sporadically.

Although there may have been some residual insect population left in crevices of the shed after cleaning, the initial infestation with maize weevils was so severe, that it can only be explained by immigration from nearby storage facilities. The first sampling date, after three weeks yielded no large *S. zeamais* immatures, an indication that infestation prior to harvest or during the preparation of the experiment was scarce. At five weeks after the beginning of the experiment the intra-kernel population of immature maize weevils averaged already 90 per sample (two cobs) in shelled grain, which is equivalent to about 450 immature maize weevils per kilo. The number of emerging maize weevils then rapidly surpassed the number of adult weevils found on the collected samples of the following weeks (interstitial population), indicating that emerging maize weevils started to emigrate after 7 to 9 weeks.

The lower growth rate of maize weevil populations on artificially infested maize stored as cob and with the husk on had already been observed by Kossou et al. (1993). As all maize was stored in the same facility this may have accentuated the differences between storage forms because adult weevils could move within the facility. It may be that in a no-

choice situation maize stored as cob will suffer heavier damage than what we observed. The insect populations in cobs stored with husk on were highly aggregated. While some cobs were found with heavy damage already at an early stage of the storage period others were almost intact after 19 weeks, hence the large standard error (Fig. 6.2). The differences were also large within the samples, among the two maize cobs they contain. Heavily infested cobs may act as reservoirs for further infestation of still intact cobs, so that the beneficial effect of husk cover is lost over time. Meikle et al. (1998) found that the husk cover had few effects over a period of 6 months of storage under field conditions, and with natural infestation by two storage beetles, the larger grain borer, *Prostephanus truncatus*, (Coleoptea: Bostrichidae) and the maize weevil. The husk may be of very different quality. The selection of maize cobs with good husk covers before storage delays damage through *S. zeamais* (Borgemeister et al., 1994). While it can provide a good protection against field infestation prior to harvest its quality will be modified through handling and drying, and it will eventually become permeable to insects (Schulten, 1976).

Parasitic wasps were found very early in the store and paraitized already the first maize weevil generation. The increase of parasitoids in shelled grain responded well to the increase in large immature maize weevil over several weeks. The rate of parasitism was based on the number of weevils that emerged after 14 days of incubation, assuming that this corresponds to the actual number of hosts that were exposed to parasitoids in the field. This time limit was used by Smith and Press (1992), and is based on the fact that only large larvae are suitable hosts for *L. distinguendus* and *A. calandreae* (Bellows, 1985; Ghani and Sweetman, 1952; Smith 1993; Choi et al., 2001), and on estimates of the relative length of those developmental stages of *S. zeamais* in shelled maize by several authors (Longstaff, 1981; Urello et al., 1989; Vovoter et al, 1995). However here may be some variation due to individual development time and climatic conditions in the field. The relation of small to

large immatures in Fig 6.5 shows an initial increase with much more small immatures during the first weeks, and a more equal distribution in the second half of the experiment, which corresponds to a stagnation and decrease in population growth. The rate of parasitism was very important in shelled grain, and certainly delayed damage, but obviously the impact was not strong enough to suppress the build-up of the weevil population. Several authors found *A. calandreae* to be able to suppress maize weevil population (Press, 1984; Cline et al., 1985), but immigration and the excellent conditions for reproduction of *S. zeamais* may have countered the action of the parasitoids. Their efficiency may be too low to arrest maize weevil population in the tropics. After 9 weeks, the rate of parasitism dropped markedly (Fig 6.6). One explanation for this sudden decrease, in spite of a very high host density, may be the disturbance through high numbers of adult weevils at this time. Host location, paralyzing and oviposition through the shell of the kernel, and hemolymph feeding by the adult parasitoids all take considerable time and effort, and interference by adult weevils may have diminished the number of successful parasitism, and increased mortality of the parasitoids. Functional response data of *A. calandreae* (Smith, 1992; 1994) and *L. distinguendus* (Hong and Ryoo, 1991) was investigated using grain with different host densities, but without the adult beetles that produced them. In such an undisturbed situation the handling time of suitable maize weevil larvae by *A. calandreae* was found to be well over three hours at 25° C. Another explanation may be the presence of young weevil larvae in the same kernel that may kill older weevil immatures including parasitized hosts and parasitoids (Sharifi and Mills, 1971). The populations density of parasitoids reached after 11 weeks may have been the threshold level at which one or both species start to emigrate. Holst and Meikle (2002) found that a predator, *Teretriosoma nigrescens*, of the larger grain borer, *Prostephanus truncatus*, also displayed a negative intra-specific density-dependence in stored maize. Competition among wasps, interspecific or intraspecific may also have an impact on their population dynamics although

resource was not limited, and parasitoid mortality through superparasitism therefore unlikely. Ryoo et al. (1996) reported that *A. calandrae* was the dominant species if both competed for larvae of the rice weevil (*Sitophilus oryzae*) in wheat. Studies of *L. distinguendus* and *A. calandrae* indicate that they occupy overlapping niches. The former is reportedly not frequent in tropical stores (Dobie et al., 1991) but was commonly reared from larger grain borer larvae in Mexico (Espinal et al., 1996). In this field experiment *L. distinguendus* seemed to be better adapted to the very high insect density, wasps and weevils, during the second half of the storage period.

Another factor may be the occurrence of predatory mites of the genus *Pyemotes* spp. which attacks the parasitoids not only in their larval stages but also as adults, whereas adult maize weevils exposed to replete females of *Pyemotes* spp. had no higher mortality rates over 14 days than weevils that were not exposed to mites (unpublished results). The shift of the sex ratio to female biased ratio could be explained by the very small size of male wasps. They can be easily overlooked among the hundreds of insects emerging from one sample, or remain hidden within heavily damaged grain.

The third parasitoid encountered, *Choetospila elegans*, had no significant impact although it was present from the beginning. Wen and Smith (1995) found that this parasitoid had no effect on immature maize weevils when competing with *A. calandrae* in shelled maize, through its small size it may have a niche on cobs stored with husk on, where the other parasitoids were not frequent.

The storage practice had a very strong influence on parasitoid effectiveness. The rate of parasitism on maize stored as cob was very low, even at times with high host density, confirming the laboratory experiments (chapter 5). Both parasitoid species, *A. calandrae* and *L. distinguendus* were even more seldom on maize stored with the husk on. We attribute this low rate of parasitism to a possible spatial refugia for host larvae. Another pteromalid

parasitoid (*Pteromalus cerealellae*), parasitizing the Angoumois grain moth, *Sitotroga cerealella*, showed a much higher efficiency on shelled grain than on the cob, due to the difficulty for these ectoparasitoids of internally feeding host larvae to reach their hosts on grain stored on the cob (chapter 4). Parts of the maize kernels stored on the cob are not accessible to parasitoids as they are in shelled grain. They remain out of reach of the parasitoid, and release of parasitic wasps on maize stored as cob may not have the expected effect. The overall result however clearly shows that storage on the cob, with husk on, is the best storage practice to reduce damage by the maize weevil.

The absence of hemipteran predators can be explained by their preference for externally feeding larvae, such as secondary pests, than on internally developing insects (Brower and Press, 1992).

It could be expected that the numbers of secondary grain feeders increases only towards the end of the experiment, when maize was already heavily damaged. Storage with husk on may favour establishment of secondary pests, as indicated by their relatively higher numbers, possibly by providing a more protected and humid environment.

As mentioned above, some damage at the beginning of this experiment was due to rodents. This was most visible on maize stored as cob where grain was detached, and only the embryo was eaten from the kernels. Damage by rodents may be more important than by insects, (Boxall and Gilett, 1984), but it is more difficult to determine.

Conclusion

Parasitoids of the maize weevil arrive early in the storage season, and reduce the number of emerging weevils on shelled grain considerably. But very likely, they can exert some control only if the initial infestation is low, no new immigration of insects occurs, and maize is stored as shelled grain. Perhaps evaluation of different wasp biotypes may result in higher efficiency. Release of parasitoids are unlikely to be efficient in open storage structures, or when maize is stored on the cob, with or without husk. When conditions for the development of the maize weevil are very favorable, such as in the humid tropics, their control with natural enemies cannot be the only strategy of integrated pest management, additional measures are necessary to limit damage to stored maize.

Storage on the cob will reduce damage by maize weevils, in spite of the antagonistic effect on naturally occurring parasitoids. A good husk cover can provide temporary protection from insect infestation, but cobs with a deficient husk may harbor large numbers of insect pests that will spread and contaminate neighboring cobs.

Careful selection of maize cobs, a clean storage structure, and the elimination of residual insect population are additional means to delay heavy post harvest losses.

Acknowledgements: We thank Nasario Baleras Garcia and Crescencio Canto Genaro, for technical help, Sergio Almacende for help with the sampling and Silverio Garcia Lara for providing the climatic data. We are grateful to Jacqueline Moret for help with the statistics. The research was conducted at the laboratory of Entomology at the International Maize and Wheat Improvement Center (CIMMYT) and at its experimental station in Poza Rica (Veracruz).

References

- Adams, J. M. 1977.** The evaluation of losses in maize stored on a selection of small farms in Zambia, with particular reference to methodology. *Trop. Stored Prod. Inf.* 33, 19-24
- Anonymous. 1980.** On-farm maize drying and storage in the humid tropics. FAO Agricultural Services Bulletin No 40.
- Arbogast, R. T. , and M.A. Mullen, 1990.** Interaction of maize weevil (Coleoptera: Curculionidae) and parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) in a small bulk of stored corn. *J. Econ. Entomol.* 83 : 2462 – 2468
- Avertay, J.N., W.G. Meikle, C. Borgemeister, M. Camara, R.H. Markham, 1999.** Studies on predation of *Prostephanus truncatus* (Horn) (Col., Bostrichidae) and *Sitophilus zeamais* Mots. (Col., curculionidae) at different densities on maize by *Teretriosoma nigrescens* Lewis (Col., Histeroidae). *J. Appl. Entomol.* 123 : 265-271
- Baker, J. E. , and J. E. Throne. 1995.** Evaluation of a resistant parasitoid for biological control of rice weevils in insecticide-treated wheat. *J. Econ. Entomol.* 88: 1570 - 1579
- Baker, J. E., and D. K. Weaver. 1993.** Resistance in field strains of the parasitoid *Anisopteromalus calandrae* (Hymenoptera : Pteromalidae) and its host, *Sitophilus oryzae* (Coleoptera: Curculionidae), to malathion, chlorpyrifos-methyl, and pirimiphos-methyl. *Biol. Control* 3: 233-242
- Ball, J.C., Dahlsten, D.L. 1973.** Hymenopterous parasites of *Ips paraconfusus* (Coleoptera : Scolytidae) larvae and their contribution to mortality. I. Influence of host tree and tree diameter on parasitization. *Can. Entomol.* 105: 1453 – 1464
- Bellows T.S. Jr. 1985.** Effects of host and parasitoid age on search behaviour and oviposition rates in *Lariophagus distinguendus* Foerster (Hymenoptera: Pteromalidae). *Res. Popul. Ecol* 27 : 65 – 76
- Boeye, J., S. Burde, H. Keil, G.A. Laborius and F.A. Schulz. 1988.** The possibilities for biological integrated control of the larger grain borer (*Prostephanus truncatus* [Horn]) in Africa, pp 110-139. In G.G.M. Schulten & A.J. Toet (eds.) Workshop on the containment and control of the larger grain borer, Arusha, Tanzania, 16-21 May 1988.
- Borgemeister, C., C.Adda, B. Djomamou, P.Degbey, A.Agbaka, F.Djossou, W.G. Meikle, and R.H. Markham. 1994 .** The effect of maize cob selection and the impact of field infestation on stored maize losses by the larger grain borer (*Prostephanus truncatus* (Horn) Col., Bostrichidae) and associated storage pests. In E.Highley, E.J.

- Wright, H.J. Banks, & B.R. Champ (eds). Proceedings of the 6th international Working Conference on Stored product Protection, Canberra Australia Vol 2, 906-909
- Boxall, R.A., and R.Gillet. 1984.** Farm level storage losses in Eastern Nepal. Trop. Stored Prod. Information 42 : 20-25
- Brower, J. H. 1991.** Potential host range and performance of a reportedly monophagous parasitoid, *Pteromalus cerealellae* (Hymenoptera: Pteromalidae). Ent. News 102 : 231–235
- Brower J. H. and J.W. Press, 1992.** Suppression of residual populations of stored-product pests in empty corn bins by release of the predator *Xylocoris flavipes* (Reuter). Biological Control 2: 66-72
- Choi, W.I., T.J. Yoon, and M.I. Ryoo. 2001.** Host-size-dependent feeding behaviour and progeny sex ratio of *Anisopteromalus calandrae* (Hym., Pteromalidae). J. Appl. Ent. 125: 71-77
- Cline, L. D., J. W. Press, and B. K. Flatherty, 1985.** Suppression of the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae), inside and outside of burlap, woven polypropylene, and cotton bags by the parasitic wasp, *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). J. Econ. Entomol. 78: 835 – 838
- Dick, K. 1988.** A review of insect infestation of maize in farm storage in Africa with special reference to the ecology and control of *Prostephanus truncatus*. Overseas Development Natural Resources Institute Bulletin No 18.
- Dobie, P. 1977.** The contribution of the Tropical Science Production Center to the study of insect resistance in maize. Trop. Stored Prod. Inf. 34: 7-22
- Dobie, P., C.P.Haines, R.J. Hodges, P.F.Prevett and D.P. Rees. 1991.** Insects and Arachnids of tropical stored Products: Their Biology and Identification . National Resources Institute.
- Eden, W. G. 1952a.** Effect of husk cover on rice weevil damage in Alabama. J. Econ. Entomol. 45: 543-544
- Eden, W. U., 1952b.** Effect of kernel characteristics and components of husk cover on rice weevil damage to corn. J. Econ. Entomol. 45: 1084-1085
- Espinal, R., R.H. Markham and V.F. Wright. 1996.** Honduras-Summary of activities on the larger grain borer and storage pest status in meso-America. 109-124 .In Hill, G.& G. Farrell (eds.), Proceedings of the East and Central Africa storage pest management workshop, Naivasha, Kenya 14-18 April 1996. CAB Wallingford, UK.

- Farrell, G., M.G. Hill, F.L.O. Nang'ayo, and A. Stabrawa. 1996.** A review of investigations to improve pest management of stored maize in smallholder farms in Kenya. *Integrated Pest Management Rev.* 1 : 251-263
- Flinn, P.W., D.W. Hagstrum and W.H. McGaughey. 1996.** Suppression of beetles in stored wheat by augmentative releases of parasitic wasps. *Environ. Entomol.* 25 : 505-511
- Ghani, M. A. , and H. L. Sweetman, 1955.** Ecological studies on the granary weevil parasite, *Aplastomorpha calandreae* (Howard). *Biologia (Lahore)*1 :115 – 139
- Giles, P. H. , and F. Ashman, 1971.** A study of pre-harvest infestation of maize by *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in the Kenyan Highlands. *J. Stored Prod. Res.* 7: 69-83
- Helbig, J., 1998.** Ability of naturally occurring parasitoids to suppress the introduced pest *Prostephanus truncatus* (Horn) (Coleoptera, Bostrichidae) in traditional maize stores in Togo. *J. Stored Prod. Res.* 34 : 287-295
- Hong S.Y., and M.I. Ryoo. 1991.** Effect of temperature on the functional and numerical responses of *Lariophagus distinguendus* (Hymenoptera: Pteromalidae) to various densities of the host, *Sitophilus oryzae* (Coleoptera: Curculionidae). *J. Econ. Entom.* 84 : 837-840.
- Kossou, D. K., N. A. Bosque-Perez , and J. H. Mareck. 1992.** Effects of shelling maize cobs on the oviposition and development of *Sitophilus zeamais* (Motschulsky). *J. Stored Prod. Res.* 28: 187-192.
- Kossou, D. K., J. H. Mareck, and N. A. Bosque-Perez, 1993.** Comparison of improved and local maize varieties in the Republic of Benin with emphasis on susceptibility to *Sitophilus zeamais* Motschulsky. *J. Stored Prod. Res.* 29 : 333- 343.
- Leliveldt, B., G.A. Laborius, and F.A. Schulz. 1988.** The distribution of *Mattesia* sp. (Neogregarinida: Ophryocystidae), a pathogen of *Prostephanus truncatus* (Horn) (Coleoptera : Bostrichidae) in Togo. *J. Stored Prod. Res.* 24: 241-243.
- Longstaff B. C, 1981.** Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): A critical Review. *Prot. Ecol.* 2 : 83-130.
- Markham, R.H., N.A. Bosque-Perez, C. Borgemeister, and W.U. Meikle. 1994.** Developing pest management strategies for *Sitophilus zeamais* (Motschulsky) and *Prostephanus truncatus* (Horn) in the Tropics. *FAO Plant Protection Bulletin* 42: 97-116.
- Markham, R. H., F. Djossou, J.M. Hirabayashi, P. Novillo, V.F. Wright, R.M. Rios, F.J.**

- Trujillo, W. G. Meikle and C. Borgemeister 1994.** Biological control in the context of an integrated management for the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera, Bostrichidae) and associated storage pests. In E. Highley, E.J. Wright, H.J. Banks, & B.R. Champ (eds). Proceedings of the 6th international Working Conference on Stored Product Protection, Canberra Australia Vol 2 : 1106-1111
- McFarlane, J. A. 1988.** Pest management strategies for *Prostephanus truncatus* (Horn) as a pest of stored maize grain. Present status and prospects. Trop. Pest Man. 34 : 121-132
- Meikle, G. W., C. Adda, C. Azoma, C. Borgemeister, P. Degbey, B. Djomamou , and R. H. Markham, 1998.** The effects of maize variety on the density of *Prostephanus truncatus* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) in post - harvest stores in Benin Republic. J. Stored Prod. Res. 34 : 45-58.
- Meikle, W.G., R.H. Markham, N. Holst, B. Djomamou, H. Schneider, and K.A. Vowoter. 1998b.** Distribution and sampling of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in maize stores in Benin. J. Econ. Entomol. 91: 1366-1374
- Muhihu, S.K. 1985 .** Depth of infestation by *Sitotroga cerealella* (Oliver) into grain layers of wheat , maize and sorghum. Trop. Stored Prod. Information, 47: 34-37.
- Press, J. W. 1984.** Suppression of residual populations of the rice weevil, *Sitophilus oryzae*, by the parasitic wasp, *Anisopteromahis calandrae*. J. Georgia Ent. Soc. 19: 110- 113
- Price, P. W., C. B. Bouton, P. Gross, B. A. McPheron, J. N. Thompson, and A. E. Weis. 1980.** Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. Annual Research Ecological Systems 11: 41 – 43
- Rees, D.P., R. Rodriuez Rivera, and F.J. Herrero Rodriguez. 1990.** Observations of the ecology of *Teretriosoma nigrescens* Lewis (Col: Histeriooidea) and its prey *Prostephanus truncatus* (Horn) (Col: Boastrichidae) in the Yucatan peninsula, Mexico. Tropical Science 30 : 153-165.
- Richter, J., A. Biliwa, and S. Henning- Helbig, 1997.** Losses and pest infestation in different maize storage systems with particular emphasis on *Prostephanus truncatus* (Horn)(Col, Bostrichidae) in Togo. Anzeiger Fuer Schaedlingskunde, Pflanzenschutz, Umweltschutz 70 (6), 112-116
- Rios Ibarra, R. M., R. H. Markjam, P. Novillo Rameix , and V. F. Wright. 1992.** Ecology and biological control of the larger grain borer in Mexico and Honduras. In:

- Implementaion of and further Research on Biological Control of the Larger Grain Borer. J. Boeye, M. Wright and G.A. Laborius (eds) Proceedings of and FAO/GTZ Coordination Meeting. pp.123-136
- Ryoo, M. I., T. J. Yoon , and S. S. Shin. 1996.** Intra- and interspecific competition among two parasitoids of the rice weevil (Coleoptera: Curculionidae). Environ. Entomol. 25 : 1101 – 1108
- Scholz, D., C. Borgemeister, W. G. Meikle, R. H. Markham , and H. M. Poehling. 1997.** Infestation of maize by *Prostephanus truncatus* initiated by male- produced pheromone. Entomol. Exper. Et Applic. 83: 53-61
- Schulten, G. G. M. 1976.** Insects in stored maize ears. Abstracts on Tropical Agriculture. Review Article: 9
- Sharifi, S. and R.B. Mills. 1971.** Radiographic studies of *Sitophilus zeamais* Mots. in wheat kernels. J. Stored Prod. Res., 7, 195-206
- Shazali, M. E. H. and R.H. Smith. 1985.** Life history studies of internally feeding pests of stored sorghum : *Sitotroga cerealella* (Ol) and *Sitophilus oryzae* (L.). J. Stored Prod. Res.. 21 :171-178
- Smith, L., 1992.** Effect of temperature on life history characteristics of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). Environ. Entomol. 21: 877 –887
- Smith, L. 1993.** Effect of humidity on life characteristics of *Anisopteromalus calandrae* (Hymenoptera : Pteromalidae) parasitizing maize weevil (Coleoptera: Curculionidae) larvae in shelled corn. Environ. Entomol. 22 : 618 – 624
- Smith, L., 1994.** Temperature influences functional response of *Anisopteromalus calandrae* (Hymenoptera : Pteromalidae). Ann. Ent. Soc. Am. 87 : 849- 855.
- Smith, L., D. K. Weaver, and R. T. Arbogast 1995.** Suitability of the maize weevil and Angoumous grain moth as hosts for the parasitoids *Anisopteromalus calandrae* and *Pteromalus cerealella*. Ent. Exp. Appl. 76 :171-177
- Storey, C.L., D.B. Sauer, and D.Walker. 1983.** Insect populations in wheat, corn, and oats stored on the farm. J. Econ. Entomol. 76: 1323-1330
- Tigar, B. J., G. B. Key, M. B. Flores , and M. Vasquez. 1994.** Field and post-maturation infestation of maize by stored product pests in Mexico. J. Stored Prod. Res. 30 : 1-8
- Tigar, B. J., G. E. Osborne, G. F. Key, M. E. Flores-S. , and M. VasquezA. 1994.** Distribution and abundance of *Prostephanus truncatus* (Coleoptera: Bostrichidae) and its predator (*Tetramesa nigrescens* (Coleoptera: Histeridae) in Mexico. Bulletin of Entomological Research 84 : 555-565

- Urrelo, R. , and V. F. Wright 1989.** Development and behaviour of immature stages of the maize weevil (Coleoptera: Curculionidae) within kernels of resistant and susceptible maize. *Ann. Ent. Soc. Am.* 82: 712-716.
- Vowoter, K. A., N. A. Bosque-Perez, and J. N. Ayertey 1995.** Effect of maize variety and storage form on the development of the maize weevil *Sitophilus zeamais* Motschulsky. *J. Stored Prod. Res.* 31: 29-36.
- Wen, B. ,and J. H. Brower 1995.** Competition between *Anisopteromalus calandrae* and *Choetospila etegans* (Hymenoptera: Pteromalidae) at different parasitoid densities on immature rice weevils (Coleoptera: Curculionidae) in wheat. *Biol. Control*: 151 – 157.
- Wen, B., L. Smith, and J. H. Brower. 1994.** Competition between *Anisopteromalus calandrae* and *Choetospila elegans* at different parasitoid densities on immature maize weevil in corn. *Environ. Entomol.* 23: 367 –373.
- Wen, B., K. Weaver, and J. H. Brower 1995.** Size preference and sex ratio for *Pteromalus cerealellae* (Hymenoptera: Pteromalidae) parasitizing *Sitotroga cerealella* (Lepidoptera: Gelechiidae) in stored corn. *Environ. Entomol.* 24: 1160- 1166
- Weston, P. A., R. J. Barney , and X. S. Ge.1997.** Host-plant characteristics and environmental factors influencing flight activity of Angoumois grain moth (Lepidoptera, Gelechiidae) in the field. *Environ. Entomol.* 26: 229 – 233
- Wiseman, B. R., W. W. Mc Milan, and N. W. Widstrom, 1970.** Husk and kernel resistance among maize hybrids to an insect complex. *J. Econ. Entomol.* 63 : 1260-1262j

GENERAL CONCLUSIONS AND DISCUSSION

The grain storage ecosystem is a man-made ecosystem where the biotic community and its non-living environment constitute a functioning system of naturally occurring processes that man tries to control in order to preserve the stored grain for his own consumption and profit. The means of control are subject to socio-economic constraints that differ greatly among maize farmers around the world.

Man operates the field crop system that provides the grain, and manages the input of fertilizer, water and human labour to increase the productivity of the plant (Sinha, 1995). The plants are under artificial selection and diversity is reduced to maximize the yield of a specific food. Domestication is an evolutionary process operating under the influence of human activities. In addition to artificial selection by man, planting and harvesting crops also implies automatic selection procedures that will affect traits such as non shattering, size, number and dormancy of seed (Harlan, 1992). Man has selected for less chaff in cereals, but automatic selection pressures set up in the same direction, as dormancy is often controlled by inhibitors in the enclosing glumes. In maize the reduction of the fruitcase not only reduced dormancy, but also protection from seed predators and this protective function was only partially transferred to the husk leaves enclosing the maize cob.

Over the course of time, more than 3 dozen insect species, that have presumably evolved from free living ancestral species, have undergone sympatric speciation (3/4 Coleopteran and 1/4 Lepidopteran) by sharing food in a common habitat and being capable of growth and reproduction on desiccated and often nutritionally deficient food (Levinson and Levinson, 1995). The adaptive capacities of storage pest are shown in their increasing resistance to pesticides, but also to secondary metabolites that could act as resistance factors. In some species such as the genus *Sitophilus*, adaptation has been favoured by symbiotic

bacteria (Nardon, 1995; Delobel and Grenier, 1995; Grenier et al 1994). Symbiotic bacteria or yeast may supply some essential nutrients that increase reproduction and development of the pests (Wicker and Nardon, 1983), furthermore they may detoxify toxic plant substances or insecticides (Dowd, 1989). A protein contained in split peas is thought to be toxic to beetles of the genus *Sitophilus*, but some strains of *S. oryzae* are resistant to these toxins (Coombs et al., 1977; Holloway, 1986). This resistance involves only one recessive gene and illustrates how a minor genetic change can modify the adaptation to food (Grenier et al. 1997). The difficulties of selecting for grain resistance lay not only in the adaptive capacity of storage pests, but also in the fact that toxins or digestibility reducers accumulated in the seed are reduced by human selection if they are unsuitable for human or animal consumption. Selection for pleasant taste and cooking qualities may further narrow the spectrum of useful resistance traits. My findings that resistance factors in maize kernels do not affect all storage pests equally (chapter 1) presents another challenge, as a consistent reduction of post-harvest losses through grain resistance would require cross resistance against storage pests and the stability of this resistance under varying climatic conditions. Introduction of genetically engineered resistance factors may open new opportunities, but the nutritious quality for human consumption has to be maintained. The risk of resistance breakdown should be minimized through gene stacking and the use of insect resistance management strategies.

In chapter 2 it was shown that maize seed may benefit from attack by parasitic wasps in some cases, which implies that there can be evolutionary pressures on the plant that may favor the development of traits that facilitate host finding for parasitoids. Strong selection pressures exist only if seeds found by parasitoids are used for planting. The small emergence holes of parasitoids can easily be overseen and chances are high that such seeds are actually planted by the farmers. But has the time of coexistence been long enough to allow such features to evolve? How have parasitoids and predators adapted to the storage ecosystem? It

seems likely that oligophagous natural enemies such as *Teretriosoma nigrescens*, have adapted along with their prey or host from the original host-plant to the novel storage habitat. In the case of generalist parasitoids such as *Anisopteromalus calandrae* or *Lariophagus distinguendus* this could have happened as well, but this is not as likely. They may have arrived in the storage habitat along with their original host and then extended their host range. They also may have switched to storage pests from other hosts occurring close to or in the storage facility. For example, the corn ear worm, *Helicoverpa zea*, is known to infest maize ears in the field, but it finishes its development sometimes in the store (Schulten, 1976). Many other insect pests are known to feed on maturing corn and disappear once the grain has dried, for example sap beetles (Nitidulidae) (Rodriguez del Bosque et al., 1998) or the pink scavenger caterpillar, *Sathrobrotia rileyi* (Dicke, 1976).

Maize varietal differences were not found to have any impact on the effectiveness of the generalist parasitoid *Anisopteromalus calandrae* in eliminating the maize weevil, *Sitophilus zeamais*. But the specialist parasitoid *Pteromalus calandrae* showed a higher performance when its host, *Sitotroga cerealella* was in a resistant maize genotype than in a susceptible one (chapter 3). One current view of foraging strategies is that the more specialized parasitoids are, the more specific is the information used for successful foraging (Vet and Dicke, 1992). Generalist parasitoids likely rely on host-habitat related cues that are common to all maize varieties, and even to other seed. This could explain its low performance, as compared to the specialist. However this is compensated by the possibility to switch to other stored commodities, such as beans if hosts are scarce. This might be in agreement with the finding that parasitoids that had no experience with maize seed were able to detect infested maize (chapter 4).

In the olfactometer, *A. calandrae* showed no preference for any specific maize variety, and the analyses of volatiles emanating from these varieties failed to show any difference

among varieties (chapter 4). However, there was a tendency that a yellow hybrid maize was preferred and the difference in kernel structures, notably the ratio of floury and flint endosperm, suggest that differences are likely to exist. Another varietal aspect that may influence host finding is the diffusion of odours in the store. Maize has an average void volume (empty space between kernels) of 42.3% (Thompson and Isaacs, 1967). Obviously this amount is influenced by the shape and size of the kernels and differs therefore among varieties. Void volume influences the rate of passage of air or fumigants through the grain (Watson, 1992) and consequently also the diffusion of odours from infested grain. The diffusion of air and volatiles in stored grain is relatively slow and not influenced or disturbed by random events, as compared to a field where wind may disperse volatiles in different directions. Therefore prey or host related odours might be useful cues for parasitoids and predators in storage systems. Visual cues may have some importance in locating a store but the generally dark environment in which hosts are found make their use within the store unlikely. Storage practices were shown to significantly influence pest increase, either directly or through their parasitoids (chapter 5 & 6). Local conditions may not always allow the use of the most appropriate storage method, as some require more labor or more space than others. Moreover infestation by several species in the course of the storage season may lead to unexpected results.

The reduction of post-harvest losses through the use of resistant maize varieties was shown to be compatible with local parasitoid populations (chapter 3). The efficiency of the parasitoids of the maize weevil, however, was far too low to achieve an acceptable level of control. Augmented releases are unlikely to reverse this tendency. In small farm systems in developing countries, the impact of natural enemies cannot be manipulated by the farmer. Rearing of parasitoids at the farm level is no option, as it cannot be achieved by simple means due to the frequent occurrence of predatory mites of the genus *Pyemotes* that require careful

isolation of the rearing media. A natural enemy of the pupal and adult stage is obviously lacking. Moreover, the open storage structures allow immigration of new maize weevils from other stores.

The search for a more efficient parasitoid strain may be an option, as comparison of strains of the weevil parasitoid *Lariophagus distinguendus* have shown large differences in fecundity (Steidle et al., 2002)

Other control measures may not always be compatible with parasitoids and predators. The application of diatomaceous earth as grain protectant was shown to adversely affect the parasitoid *Lariophagus distinguendus*, and local populations of parasitoids or augmentative release of parasitoid would therefore have no additional effect (Perez-Mendoza et al, 1999). It is likely that other natural insecticides would pose similar problems as they are usually not specific to one insect species or group. The use of closed storage structures would impede the access of the natural enemies population to their preys in the store.

In conclusion, results from this thesis show that grain resistance is synergistic with the natural enemies of storage pests and the use of resistant maize combined with local parasitoid populations can considerably reduce post-harvest losses. It is, however, desirable that all storage pests are negatively affected by grain resistance. As yet, no such a cross-resistant maize genotype has been found. To avoid a shift in storage pests, selection programs should include testing resistant varieties to storage pests of minor importance. Resistance traits are unlikely to be found in wild relatives of maize such as teosinte, due to the dramatic structural changes that have affected the seed during the process of domestication. The most likely sources of resistant traits are landraces from tropical lowland, where insect pressure is high.

Kernel hardness frequently mentioned as resistance factor to the maize weevil, *Sitophilus zeamais*, had no negative impact on its parasitoid, *Anisotperomalus calandrae*. However the reproductive rate of the strain used for my experiments was very low, and may be insufficient to control a maize weevil population. In contrast, *Pteromalus cerealellae*, the parasitoid of the Angoumois grain moth, *Sitotroga cerealella*, was very efficient and may be a good candidate for biological control. Its performance was higher in a resistant maize variety so that the combined effect of grain resistance and natural enemy could considerably reduce post-harvest losses caused by the moth.

Parasitoids showed no preference for volatiles of any of six maize varieties in an olfactometer. Experienced parasitoids are able to locate even uninfested maize through olfactory cues and behavioral experiments are a useful means to verify that the attractiveness of improved maize varieties is maintained.

The appropriate storage method is not always compatible with the performance of parasitic wasps, as the hosts may be protected from their parasitoids when maize is stored on the cob. The quantitative impact of parasitoids and storage method has to be compared, in laboratory and field experiments, and control measures have to be adapted to the local pest population.

With the described methods post-harvest losses may still be at an unacceptable high level and additional measures will be needed. For each climate and socio-economic situation distinct combinations of control measures are appropriate. While the natural enemies may be a helpful means to reduce storage pests they may have to give way to more efficient control methods for the sake of food security in the third world.

OUTLOOK

New questions that arouse

Grain resistance

- Are there maize genotypes with cross-resistance to storage pests?
- Are there resistance factors in other seed crops that could be transferred to maize?

Cross-resistant maize genotypes, or varieties that have different resistance mechanisms are most likely to be found through large scale screening of maize accessions from tropical lowland areas where insect pressures is high. The tests should include the Angoumois grain moth, *Sitotroga cerealella*, the maize weevil, *Sitophilus zeamais*, and eventually the larger grain borer, *Prostephanus truncatus*.

Natural enemies

- How does maize genotype affect the penetration depth (and speed) of parasitoids, and the diffusion of volatiles in a bulk of grain?
- Do parasitoids of different geographical origin differ in their efficiency as control agents?
- Do specialist and generalist differ in the type of host location they use?

Larger maize quantities and lower host density can be used to evaluate the effect of empty space (void volume) which depends on kernel size and form. Parasitoid strains from other geographic locations may have a higher life time progeny and be more efficient.

Field studies

- How are the population dynamics of the pests and their natural enemies affected by grain resistance and storage method under field conditions?
- What is the outcome in the presence of more than one primary pest?

The ultimate answers of the synecology of storage insects can only be obtained by field studies as no laboratory experiment can imitate in a satisfactory way changing climatic conditions, migration of insects and pest outbreaks.

REFERENCES

- Arbogast, R. T. , and M. A. Mullen. 1990.** Interaction of Maize Weevil (Coleoptera: Curculionidae) and Parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) in a Small Bulk of Stored Corn. J. Econ Entomol. 83 : 2462 – 2468
- Anonymous. 1989.** Unidad postcosecha. Plan operativo de la fase 1990-1992. Tegugilpa. Honduras. Ministerio de Recursos Naturales, Cooperation Suiza de Desarrollo.
- Arnason, J. T., J. Gale, B. C. d. Beyssac, A. Sen, S. S. Miller, B. J. R. Philogène, J. D. H. Lambert, R. G. Fuicher, J. A. Serratos, and J. A. Mihm. 1992.** Role of phenolics in resistance of maize grain to the stored grain insects, *Prostephanus truncatus* (Horn) and *Sitophilus zeamais* (Motsch.). J. Stored Prod. Res. 28: 119-126
- Benrey, B., A. Callejas, L. Rios, K. Oyama, and R. F. Denno. 1998.** The effects of domestication of Brassica and Phaseolus on the interaction between phytophagous insects and parasitoids. Biol. Control, 11: 130-140
- Bergvinson, D.J. 2001.** Storage pest resistance in maize. In: Maize research Highlights 1999-2000. CIMMYT 6815. Technical bulletin . www.cimmyt.org. pp 32-39
- Botrell, D. G., P. Barbosa, and F. Gould. 1998.** Manipulating natural enemies by plant variety selection and modification - a realistic strategy. Ann. Rev. of Entomol. 43 : 347 – 367
- Brower J. H. and J.W. Press. 1990.** Interaction of *Bracon hebetor* (Hymenoptera: Braconidae) and *Trichogramma pretiosum* (Hymenoptera : Trichogrammatidae) in suppressing stored-product moth populations in small peanut stores. J.Econ. Entomol. 83: 1096-1101
- Buckland P.C. 1981.** The early dispersal of insect pests of stored products as indicated by archaeological records. J. Stored Prod. Res. 17: 1-12
- Coombs, C. W., C. J. Billings, and J. B. Porter. 1976.** The Effect of yellow split-peas (*Pisum sativum* L.) and other pulses on the production of *Sitophilus oryzae* (L.) (Col., Curculionidae) and the ability of other strains to breed thereon. J. Stored Prod. Res. 13, 53-5

- Cortesero, A. M., J. P. Monge , and J. Huignard, 1995.** Influence of two successive learning processes on the response of *Eupelmus vuilleti* (Hymenoptera, Eupelmidae) to volatile stimuli from host and host plant. J. Ins. Behaviour 8 : 751 – 762
- Delobel, B. , and A. M. Grenier. 1993.** Effect of non-cereal Food on Cereal Weevils and Tamarind pod Weevils (Coleoptera Curculinoidea). J. of st. Pr. Res. Vol 29, 7-14
- Dicke, F.F. 1977.** The most important corn insects. In Corn and Corn Improvement. G.F. Sprague (eds) Am Soc. of Agron., Madison, Wisconsin, USA. 501-590
- Dix, D. E., and J. N. All, 1985.** Invasion Patterns and Sex-Ratio Dynamics of the Maize Weevil Infesting Field Corn. J. Econ. Ent. 78 1072-1075
- Dobie, P., 1974.** The Laboratory Assessment of the Inherent Susceptibility of Maize Varieties to Post-Harvest Infestation by *Sitoph i/us zeamais* Motsch. J. Stored. Prod. Res. 10, 183-197
- Dobie, P.,C.P. Haines, R.J. Hodges, P.F.Prevett and D.P. Rees. 1991.** Insects and Arachnids of tropical stored Products: Their Biology and Identification . Nationale Ressources Institute
- Doebley, J., M. M. Goodman , and C. W. Stuber. 1985.** Isozyme variation in the races of maize from Mexico. Amer. J. of Bot. 72 , 629 – 639
- Doebley, J. 1990.** Molecular Evidence and the Evolution of Maize. Econ. Botany, 44 (3) , 6-27
- Dowd, P.F. 1989.** In situ production of hydrolytic detoxifying enzymes by simbiotic yeasts in the cigarette beetle (Coleoptra: Anobiidae). J. Econ. Entomol., 82, 396-400
- Eden, W. G. 1952.** Effect of Husk Cover an Rice Weevil Damage in Alabama. J. of Econ. Entomol. 45: 543-544
- FAO. 1980.** On-farm drying and storage in the humid tropics. FAO Agricultural Services Bulletin No 40. Rome, FAO. 60 pp.
- Fields P., and N.D.G. White. 2002.** Altenatives ro methyl bromide treatments for stored-product and quarantine insects. Annu. Rev. Entomol. 47: 331-359
- Galinat, W. C. 1977.** The Origin of Corn. in “Corn and Corn Improvement pp 3 - 27 (Sprague UF & Dudley JW) editors Madison Wisconsin, USA
- Ghani, M. A. , and H. L. Sweetman, 1955.** Ecological studies on the granary weevil parasite, *Aplastomorpha calandrae* (Howard). Biologia (Lahore)1 :115 – 139
- Giles, P. H. , and F. Ashman. 1971.** A Study of Pre-Harvest Infestation of maize by *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in the Kenyan Highlands. J. Stored Prod. Res. 7, 69-83

- Golob, P., J. Mwambula, V. Mhangoa, and F. Ngulube. 1982.** The use of locally available materials as protectants of maize grain against insect infestation during storage in Malawi. *J Stored Prod. Res.* 18 67-74
- Golob, P. , and K. M. Dick, 1992.** The Integration of Biological and Chemical Control Components of an Integrated Pest Management Strategy for the Larger Grain Borer in Africa. In: Implementation of and further Research on Biological Control of the Larger Grain Borer. Boeye J., M. Wright and G.A. Laborius (eds) 31-47.
- Grenier, A. M., C. Nardon , and P. Nardon. 1994.** The role of symbiotes in flight activity of *Sitophilus* weevils. *Entomol Exp. et Appl.* 70, 20 1-208
- Grenier, A. M., M. Mbaiguinam , and B. Delobel. 1997.** Genetical analysis of the ability of the rice weevil *Sitophilus oryzae* (Coleoptera, Curculionidae) to breed on split peas. *Heredity.* 79 , 15-23
- Guedes, R.N.C., Lima, L.O.G., Santos, J.P., and C.D. Cruz. 1995.** Resistance to DDT and pyrethroids in Brazilian populations of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). *J. Stored Prod. Res.* Vil 31, (2) 145-150
- Harlan, J.R.. 1992.** Domestication of Seed Crops. In, *Crops and Man* by J.R.Harlan. American Society of Agronomy, Inc. Crop Science Society of America Inc. Madison, Wisconsin, USA
- Heddi, A., F. Lefebvre, and P. Nardon, 1991.** The Influence of symbiosis on the respiratory control ratio and the ADP/O ratio in the adult Weevil *Sitophilus oryzae* (Col. Curculionidae). *Endocyt. Cell Res.* 8, 6 1-73
- Holloway, G. J. , and R. H. Smith. 1985.** Inheritance of the ability of *Sitophilus oryzae* L. (Col., Curculionidae) to feed and breed on yellow split-peas (*Pisum sativum*). *Bull. Entomol. Res.* 75, 367-375
- Holst, N. and W.G. Meikle. 2002.** The population dynamics of the biocontrol agent *Teretriosoma nigrescens*, its target *Prostephanus truncatus* and non-target *Sitophilus zeamais*, and the prospects for successful control in rural maize stores in humid West Africa. submitted
- Joubert, P.C. 1966.** Field infestation of stored product insect in South Africa. *J. stored Prod. Res.* 2, 159-161
- Kennedy, G.G., F. Gould, O.M.B. de Ponti, and R.E. Stinner, 1987.** Ecological, agricultural, genetic, and commercial considerations in the deployment of insect-resistant germplasm. *Environ. Entomol.* 16, 327-338
- Korunic, Z. 1998.** Diatomaceous earth, a group of natural insecticides. *J. Stored Prod. Res.*

- Kossou, D.K., N.A. Bosque-Perez, and J.H. Mareck. 1992.** Effects of shelling maize cobs on the oviposition and development of *Sitophilus zeamais* (Motschulsky). J. Stored Prod. Res. 28 : 187-192
- Levinson, A. , and H. Levinson, 1995.** Reflections on structure and function of pheromone glands in storage insect species. Anz. fur Schaedlingsk. Pflanzensch. Umweltsch. 68, 99-118
- Markham, R. H., N. A. Bosque-Perez, C. Borgemeister, and W. U. Meikle, 1994.** Developing Pest Management Strategies for *Sitophilus zeamais* (Motschulsky) and *Prostephanus truncatus* (Horn) in the Tropics. FAO Plant Protection Bulletin 42 (3) 97-116
- McClintock, B., T. A. Kato ,and A. Blumenschein, 1981.** Chromosome constitution of races of maize. Colegio de Postgraduados, Chapingo Mexico
- Meikle, W. G., N. Holst, D. Scholz , and R. H. Markham. 1998.** Simulation model of *Prostephanus truncatus* (Coleoptera:Bostrichidae) in rural maize stores in the Republic of Benin. Environ. Entomol. 27: 59-69.
- Nardon, P., 1995.** The role of symbiosis in adaptation and speciation. Bulletin de la Societe Zoologique de France-Evolution et Zoologie 120, 397-406
- Niber B.T. 1994.** The ability of powders and slurries from ten plant species to protect stored grain from attack by *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae). J. stored Prod. Res. 30 (4) 297-301
- Pena, M. M. A., A. L. Tejeda, and H. S. Arroyo. 1991.** Validacion de Polvos vegetales y minerales para el combate de *Sitophilus zeamais*, *Prostephanus truncatus* y *Rhyzopertha dominica* en maiz almacenado en Mexico. Agrociencia serie Protection vegetal Vol 2: 69 –87
- Perez-Mendoza, J, J.E. Baker,F.H. Arthur, P.W. Flinn. 1999.** Effects of protect-it on efficacy of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) parasitizing rice weevils (Coloptera: Curculionidae) in wheat. Environ. Entomol.. 28 , 529-534
- Price, P. W., C. B. Bouton, P. Gross, B. A. McPheron, J. N. Thompson, and A. E. Weis. 1980.** Interactions among three trophic levels : influence of plants on interactions between insect herbivores and natural enemies. Annual Research Ecological Systems 11, 41 – 43
- Rees, D. P. 1985.** Life history of *Teretriosoma nigrescens* Lewis(Coleoptera; Histeridae) and its ability to suppress populations of *Prostephanus truncatus* Horn (Coleoptera;

- Bostrichidae. J. Stored Prod. Res. 21, 115-118
- Rees, D. P. 1987.** Laboratory studies on predation by *Teretriosoma nigrescens* Lewis (Coleoptera, Histeridae) on *Prostephanus truncatus* Horn (Coleoptera, Bostrichidae). J. Stored Prod. Res. 23, 191-195
- Rees, D.P., R. Rodriuez Rivera, and F.J. Herrero Rodriguez. 1990.** Observations of the ecology of *Teretriosoma nigrescens* Lewis (Col: Histerioidae) and its prey *Prostephanus truncatus* (Horn) (Col: Boastrichidae) in the Yucatan peninsula, Mexico. Tropical Science 30 (20: 153-165
- Richter, J., A. Biliwa, J. Helbig , and S. Henning-Helbig, 1997.** Impact of *Tertriosoma nigrescens*(L) (Coleoptera, Histeridae) on *Prostephanus truncatus* (II) (Coleoptera, Bostrichidae) and losses in traditional maize stores in Southern Togo. J. Stored Prod. Res. 33,137-142
- Rodriguez-Del-Bosque, L. A., L.-M. J. , and P. F. Dowd, 1998.** Effect of ear wounding and cultural practices of abundance of *Carpophilus freemani* (Coleoptera: Nitidulidae) and other microlepidopterians in maize in northeastern Mexico. J. Econ. Entomol. 91, 796-801
- Schoeller, M., S.Prozell, A.G. Al-Kirshi, and Ch. Reichmuth. 1997.** Towards biological control as a major component of integrated pest management in stored product protection, J. Stored Prod. Res. 33 (1), 81-97
- Schulten, G. G. M. 1976.** Insects in stored maize ears. Abstracts on Tropical Agriculture. Review Article: 9
- Sinha, R.N. 1995.** The stored grain ecosystem. Pages 1-32 in : Stored-grain ecosystems, ed. D.S. Jayas, N.D.G. White and W.E. Muir. New York, NY: Marcel Dekker, Inc.
- Steidle, J.L.M., and Schoeller, M. 2002.** Fecundity and ability of the parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae) to find larvae of the granary weevil *Sitophilus granarius* (Coleoptera: curculionidae) in bulk grain. J. Stored Prod. Res. 38 (1): 43-53
- Smale, M., A. Aguirre, and M. Bellon, 1998.** Farmer management of maize diversity in the Central Valleys of Oaxaca, Mexico. Baseline Socioeconomic Survey CIMMYT/ INIFAP, 1998
- Smith, L., 1994.** Temperature influences functional response of *Anisopteris ma/us* (Hymenoptera : Pteromalidae). Ann. Entomol. Soc. Am. 87 (6), 849- 855
- Sprague, G.F. and S.A. Eberhart. 1977.** Corn breeding. In G.F. Sprague (ed) Corn and Corn Improvement. Amer. Soc. Agron.

- Steidle, J. L. M. , and M. Schoeller. 1997.** Olfactory host location and learning in the granary weevil parasitoid *Lariophagus distinguendus* (Hymenoptera : Pteromalidae). J. Insect Beh. 10 : 331 – 342
- Steidle, J.L.M., and J. Ruther. 2000.** Chemicals used for recognition by the granary weevil parasitoid *Lariophagus distinguendus*. J. Chem. Ecol. 26 (12) 2665-2675
- Thompson, R.A. and G.W. Isaacs. 1967.** Porosity determination of grains and seeds with an air comparison pycnometer. Trans. ASAE 10, 693-696
- Turlings, T.C.J. , and B. Benrey, 1998.** Effects of plant metabolites on behaviour and development of parasitic wasps. Ecoscience 5 (3) 321 –333
- Urrelo, R., V. F. Wright, R. B. Mills , and C. E. Wasson, 1989.** Screening for resistance against the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera :Curculioidea) in Peruvian maize accessions. Turrialba 39 : 9-17
- Vet, L. E. M. , and M. Dicke, 1992.** Ecology of infochemical use by natural enemies in a tritrophic context. Ann. Rev. Entom. 37, 141 –172
- Watson S.A. 1987.** In : Corn: Chemistry and Technology. S.A. Watson& P.E. Ramstad (eds) Am. Ass. Cereal Chem., St Paul
- Wellhausen, B. J., L. M. Roberts, and X. Hernandez. 1951.** Razas de maiz en Mexico. Folleto tecnico no 5. Secretaria de Agricultura y Ganaderia. Mexico DF
- Wellhausen, E. 1, F. A.O.. , and X. Hernandez. 1957.** Races of maize in Central America. Nat. Res. Council Publ. 511
- Weston, P. A., R. J. Barney , and X. S. Ge. 1997.** Host-plant characteristics and environmental factors influencing flight activity of Angoumois grain moth (Lepidoptera, Gelechiidae) in the field. Environ. Entomol. 26 : 229 – 233
- Wicker, C. and P. Nardon. 1983.** Differential vitamine requirements of symbiotic and aposymbiotic weevils, *Sitophilus oryzae*. In: Endocytobiology, vol 2pp 733-738 (De Gruyter et al., eds) Berlin

CURRICULUM VITAE

Anita Savidan-Niederer

Adress: 11, route de Nimes
30870 Clarensac, France

Date of birth: 08.12.1956

Nationality: Swiss & French

References: Dr. David Bergvinson, CIMMYT Mexico
Dr. Ted Turlings, University of Neuchatel, Switzerland

Languages: German : Mother tongue, French, English, Spanish, Portugese

Education: 1999-2002 : PhD University Neuchael/CIMMYT

1976-1981: Studies in Biology, Univercity of Neuchatel
1979/80 : Diploma work in Ivory Cost

1969-1975: Kantonschule Trogen AR: Maturity type C

1962-1968: Primary school in Speicher AR

Professional experience: 1997: CIMMYT (Mexico) Maize program: Entomology

1982: EMBRAPA (Brazil) Department of Animal health

1981: Cantonal Laboratory of Microbiology
St. Gallen Dep. Immuno Assays

Workshops: 2001: Exploitation of plant defence chemistry by insects
III cycle Romand en Sciences biologique
University of Neuchatel Sept 10-12

2000: Parasitoids, their Biology, Ecology and Application
IIIcycle Romand en Sciences biologique
University of Neuchatel Sept 25-27

2000: Interactions between plants and attacking
organisms Autumnschool Wageningen, Holland
Oct 18-22

1998 : Biotechnology applications to Plant Breeding
CIMMYT Headquaters, Mexico, Dec 9-18

Acknowledgements:

I would like to thank Prof. Martine Rahier, Drs. Ted Turlings, and David Bergvinson for their assistance and support during my thesis, and Drs. Philippe Kupfer and Franz Bigler for kindly accepting to be a member of the jury.

I am grateful for the help I received in Switzerland from Mary Fritzsche-Hoballah, Cristina Tamo, Thomas Degen, Jacqueline Moret, Cristina Faria and many others who assisted me during my stay in Neuchatel.

Muchas gracias a Silverio Garcia, Sergio Almacende, Nasario Balderas, John Woolston, Dr. Julien Berthaud y todo el equipo del programa de Entomologia del CTMMYT Mexico.

Un grand merci pour ma famille, mes parents, mon frère Daniel et mon man Yves pour leur soutien dont j'avais si besoin.