

HANY AHMED FOUAD HANAFY MAHMOUD

**RESPONSES OF *Sitotroga cerealella* TO SEMIOCHEMICALS
AND PHYSICAL CHARACTERISTICS OF CORN KERNELS**

Thesis submitted to the Universidade Federal de Viçosa, as partial fulfillment of the requirements of the Post-Graduate Program in Entomology, for obtaining the degree of *Doctor Scientiae*

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RESUMO

MAHMOUD, Hany Ahmed Fouad Hanafy, D. Sc., Universidade Federal de Viçosa, Junho de 2011. **Respostas de *Sitotroga cerealella* para semioquímicos e características físicas dos grãos de milho** Orientadora: Lêda Rita D'Antonino Faroni. Co-orientadores: Eraldo Rodrigues de Lima e Evaldo Ferreira Vilela

Características físicas e químicas da planta têm relação interespecífica com insetos, as quais podem ser utilizadas no Manejo Integrado de Pragas. Objetivou-se neste trabalho: i) Avaliar a resistência de cinco genótipos de milho (*Zea mays*) (UFVM100, UFVM200, UFVM100X200, AG1051 e DKB747) sobre *S. cerealella*; ii) Correlacionar as características físicas e químicas destes genótipos sobre o desenvolvimento e a sobrevivência de *Sitotroga*; iii) Avaliar o efeito de genótipos de milho na preferência para oviposição; iv) Acesso o papel dos voláteis de grãos de milho sobre o comportamento de vôo e comunicação sexual de *S. cerealella*; e vi) Determinar e identificar os compostos ativos dos voláteis dos grãos de milho. O período de desenvolvimento, de ovo a fase adulta, a emergência de adultos e a perda de massa dos grãos foram os parâmetros utilizados para avaliar a resistência desses genótipos. Para avaliação dos genótipos de milho sobre a atratividade da fêmea de *S. cerealella* em oviposição, foram utilizados cinco testes com chance de escolha e um sem chance de escolha. Em túnel de vento, foi testar a resposta dos machos utilizou-se feromônio e feromônio misturando com voláteis de grãos de milho. Foi avaliado também a resposta dos machos e fêmeas em voláteis de grãos de milho. Técnica de GC-EAD e GC-MS foi utilizada para determinar e identificar, respectivamente, os componentes voláteis dos grãos de milho induziu uma resposta no EAD utilizando antenas de macho e fêmea. A menor porcentagem de sobrevivência e menor perda de massa de grãos foram observadas com o genótipo AG1501. Existe correlação entre as características físico-químicas dos grãos de milho e parâmetros da suscetibilidade. A oviposição de *S. cerealella* foi semelhante entre os genótipos no teste sem chance de escolha. Nos testes com chance de escolha, o menor número de ovos foi observado em grãos de milho do genótipo AG1051. Quando se contrastou os genótipos em pares (UFVM100/outro genótipo), verificaram-se diferenças nos contrastes UFVM100/UFVM100X200 e UFVM100/AG1051, onde o número de ovos depositados foi maior nos genótipos UFVM100X200 e UFVM100, respectivamente. O maior número de ovos de *S. cerealella* foi observado em papel preto com menor

abertura (sanfonado com grampo) em relação àqueles papeis com maior abertura (sanfonado sem grampo). Obteve-se maior número de ovos no substrato artificial com menor abertura com o extrato UFVM100. A concentração 10 pg atraiu os machos para a fonte a mais do que as concentrações 1 pg e 100 pg. O tempo gasto em vôo foi maior para machos de *S. cerealella* na concentração de 100 pg. A mistura do feromônio com 15 ou 30 g de equivalente de voláteis de grãos de milho incrementou a quantidade de machos de *S. cerealella* que chegaram até a fonte, quando comparados apenas ao feromônio. A mistura do feromônio com 120 g de equivalente de voláteis de grãos de milho aumentou o tempo gasto para o início do vôo até a escolha de um lado ou até a fonte. Equivalente de voláteis de grãos de milho mostrou o mais atrativo no vôo de machos virgens e fêmeas acasaladas à fonte. Porém, machos acasalados e fêmeas virgens não foram atraídos pelos voláteis de grãos de milho. Antenas do macho e da fêmea responderam a quatro compostos de voláteis de grãos de milho. Três destes compostos foram identificados, os quais são: n-nonanal, n-decanal e acetona geranil. Portanto, *S. cerealella* têm preferência por algumas características dos grãos na seleção do seu hospedeiro para alimentação, oviposição e comunicação sexual. Assim, essas informações podem ser utilizadas como ferramenta em Programas de Manejo de Integrado Pragas de Grãos Armazenados e Programas de Melhoramento de Plantas.

ABSTRACT

MAHMOUD, Hany Ahmed Fouad Hanafy, D.Sc., **Universidade Federal de Viçosa**, June, 2011. **Responses of *Sitotroga cerealella* to Semiochemicals and Physical Characteristics of Corn Kernels**. Adviser: Lêda Rita D'Antonino Faroni. Co-Advisers: Eraldo Rodrigues de Lima and Evaldo Ferreira Vilela

Interaction relationships between physical and chemical characteristics of plants and insects have been found with great chance to use this interaction in Integrated Pest Management strategies. Therefore, our laboratory studies aimed at 1) Examine the extent to which corn genotypes (*Zea mays*) (UFVM100, UFVM200, UFVM100X200, AG1051 and DKB747) vary in their resistance to *S. cerealella*. 2) Evaluate the physical and chemical characteristics of these corn genotypes related to the variability of resistance. 3) Evaluate the effect of corn genotypes on the oviposition preference. 4) Access the role of corn kernel volatiles on the flight behavior and sexual communication of *S. cerealella*. 5) Determine and identify the active compounds of corn kernel volatiles. Development period, form eggs to adults, and progeny of emerging adults and kernel weight loss were taken as parameters for resistance test. For evaluate the corn genotypes on attractiveness of *S. cerealella* female in ovipositor were used five choice tests and one no choice test. Wind tunnel bioassay was done to test the respond of the males to pheromone and pheromone mix with corn kernel volatiles. On the other hand, the respond of the males and females to corn kernel volatiles was tested. GC-EAD and GC-MS techniques were to determine and identify, respectively, which components in corn kernel volatiles elicited an EAD response using male and female antennae. *S. cerealella* showed longer development period, shorter survival percent and lower kernel weight loss percent on AG1501 genotype. Correlation between physical-chemical characteristics of corn kernels and susceptibility preference parameter was observed. The result among the tested genotypes in no-choice test was not significant. In the choice tests, lower number of eggs was laid on whole kernels of AG1051 and greater number of eggs laid on corn extract of UFVM100. The number of eggs laid on whole kernels of UFVM100 was significant different compared with the number of eggs laid on UFVM100X200 and on AG1051 kernels in the two-choice test. Gravid female of *S. cerealella* laid significantly more eggs on black paper with narrow oviposition substrate than on black paper without narrow oviposition substrate. Black paper with 2 g-equivalent of

corn extract was elicited significantly greater enhanced oviposition compared with black paper alone. The 10 pg concentration at 10 pg min⁻¹ ratio did attract males to the source significantly more than 1 pg and 100 pg concentrations. The time-spent flight was longer in 100 pg concentration. Source contacts by males increased in the pheromone with 15 and 30 g-equivalent of corn kernel volatiles. The mean time-spent flight was decreased in presence of 15 g-equivalent of corn kernel volatiles with the pheromone compared with the pheromone alone. Equivalent of corn kernel volatiles were elicited attractant behavior of unmated males and mated females to landing near with the source. Mated males and virgin females were not attracted to corn kernel volatiles. Antennae of male and female responded to four active compounds of corn kernel volatiles. Three of these compounds were identified, n-nonanal, n-decanal and geranyl acetone. From our study conclude that, *S. cerealella* have preference to some kernel characteristics in the selection of its host for feeding, oviposition and sex communication. Thus, this information may be used as a tool in Integrated Management Programs for Stored Grain Pests and Plant Breeding Programs.

1. INTRODUCTION

Plants and insects have lived together for at least 100 million years in the same environments, and arisen between them kind of beneficial and deleterious interactions. To understand this interaction between insects and their host plants we must know about biochemistry, ecology, behavior, physiology and genetics (Stotz et al., 1999).

Corn is one of the most widely adapted crops in the world. About 70% of the global corn production is from four main countries in the world including United States of America, China, Brazil and Argentina (Ipni, 2008). In 2010/2011, the corn area was 12.68 million ha in Brazil, which produced 52.56 million ton. of kernels (Conab, 2011).

Angoumois Grain Moth (AGM), *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae), has been considered as one of major pests of agricultural stored products worldwide. The larvae of *S. cerealella* attack both in field or/and in storeroom a variety of kernels, among which; corn, sorghum, wheat, soybean, rice, paddy and products prepared from these kernels (Howlader & Matin, 1988; C nsoli & Filho, 1995; Trematerra & Gentile, 2002; Ashamo, 2010). The larva of this pest walks outside kernels about 24 h, and after this time, it makes hole in a kernel and stay in it until larval-pupa development is completed. This damage makes kernels more susceptible to secondary insect pests (Weston & Rattlingourd, 2000).

The preference of insects to a plant or to specific plant parts is result of the different compound produced by plant (Bernays & Chapman, 1994). Plants produce several secondary volatile compounds that are important in insect-plant interaction. These volatiles can be attractant or repellent to insects for feeding, mating and oviposition (McNeil & Delisle, 1989; Bashir et al., 2001; Coracini et al., 2004; Tasin

et al., 2006; Ukeh et al., 2010). However, insects may use these compounds to find their host, not only depends on the quantity of these compounds but additionally to quality of these compounds (Hoballah et al., 2002). Using these plant volatiles in integrated pest management programs to crop protection is one of a new and environmental safety approaches for control pests (Arab & Bento, 2006). Therefore, very little attention has been given related to physical and chemical characteristics from different genotypes of plants on feeding and oviposition behavior of insects and the role of host plant volatiles on sex communication of insects.

Insects cannot detect all plant volatiles, but it depends mainly on plant characteristics and sensory system of insect, which must be able to detect and encode the information received (Schoonhoven et al., 2005). One of the widely used methods to detect the behaviorally active chemical compounds in an insect antenna is electroantennography (EAD) technique (Blomquist & Vogt, 2003). Gas chromatography-electroantennography (GC-EAD) has been used to elucidate sensitive and specific systems of detection of odors from plants and insects (Zhang et al., 1999; Olsson et al., 2005b), obtained from headspace collection of a combination of insects or plants. Then these compounds are identified by gas chromatography-mass spectrometry (GC-MS).

The objective of this research was to: 1) Examine the extent to which corn genotypes vary in their resistance to *S. cerealella*. 2) Evaluate the physical and chemical characteristics of these corn genotypes related to the variability of resistance. 3) Evaluate the effect of corn genotypes on the oviposition preference. 4) Access the role of corn kernel volatiles on the flight behavior and sexual communication of *S. cerealella*. 5) Determine and identify the active compounds of corn kernel volatiles from the corn kernels.

2. LITERATURE REVIEW

2. 1. Kernel resistance from different genotypes to *Sitotroga cerealella*

Plant resistance to insects is a cultural method that has been neglected as an alternative control of *S. cerealella*. Several studies about resistance of different genotypes of grains to *S. cerealella* were published (Cônsoli & Filho, 1995; Ahmed et al., 2002; Khan et al., 2005; Ashamo & Khanna, 2006b; Shafique & Chaudry, 2007a,b; Ashamo 2010). *S. cerealella* reared on kernels of Shrunhen genotype has the developmental time longer than other tested corn genotypes (Cônsoli & Filho, 1995). Some genotype like HO4R 3075-3 was less susceptible giving to the larvae the longest developmental time, a decrease in adult emergence, and the lowest susceptibility index (SI) and weight loss when it compared with six kernels of corn genotypes (Ashamo & Khanna, 2006b). Among seven kernels of corn genotypes were evaluated for their comparative resistance to *S. cerealella*. Adult progeny and weight loss of kernels were significantly low in White Monsanto and Yellow Pioneer than other tested genotypes (Shafique & Chaudry, 2007a).

S. cerealella has been shown feeding preference to genotypes of others cereals like wheat (Ahmed et al., 2002; Khan et al., 2005), paddy (Ashamo 2010) and rice (Shafique & Chaudry, 2007b).

2. 2. Effect of physical-chemical characteristics on susceptibility of kernel genotypes to stored-product pests

Genotypes vary in susceptibility to infestation of *S. cerealella* and it is depends on their physical-chemical characteristics. Seed hardness directly affected on resistance of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in corn (Serratos et al., 1987;

Meikle et al., 1998). Significant correlation between kernel size of different corn genotypes and number of *S. cerealella* per kernel in a free-choice test was found (Villacis et al. 1972).

The corn kernels with moisture content above 31% were virtually non-infested with *S. cerealella* (Weston et al., 1993). Developmental time, weight loss and number of progeny produced by *S. cerealella* were correlated with the protein, sugar, and fat content of wheat kernels (Khan et al., 2005). The lowest survival from egg to adult was from *S. cerealella* reared on two genotypes, sugary (Su) and sugary-opaco₂ (SuO₂) and may be related to the high level of amylose of these genotypes (Cônsoi & Filho 1995).

2. 3. Effect of physical-chemical stimuli on oviposition

The mated females of herbivores are choice a host plant to lay their eggs that supports the acceptable conditions for protection and development of them and their progeny (Bernays & Chapman, 1994). This choice is determined by characteristics of plants, such as: physical-chemical characteristics (Awmack & Leather, 2002; Mannion et al., 2003; Meiners et al., 2005; Coley et al., 2006).

Physical stimuli are often important factors for insects to select their host (Renwick & Chew, 1994), but chemical stimuli are play the major role (Udayagiri & Mason, 1995). The effect of physical and chemical stimuli in moths during the host-selecting process has been reported (Rojas & Wyatt, 1999; Sambaraju & Phillips, 2008; Sidney et al., 2008).

Plant characteristics associated with ear appearance could be the main stimulatory factors for oviposition and feeding of *S. cerealella* moth, followed by

husk coverage of ear, under field condition (Butrón et al., 2008). The kernels traits may be important in the final stages of attraction to a plant by *S. cerealella*.

Several cues are used by insects to find and select its host. Insects can use volatile compounds released by their hosts, then landing and contact evaluation, and acceptance or rejection of the host (Renwick & Chew, 1994).

Gravid females of storage-product moths were had respond to plant odors to lay their eggs. However, oviposition preference of storage-product moths to grain odors has been showed in storage-product moths. For examples, *Ephestia cautella* Walker (Lepidoptera: Pyralidae) female was showed oviposition preference to wheat odor (Barrer & Jay, 1980). In a two-choice bioassay, gravid female of *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) was showed preference to lay its eggs in rearing diet based on corn meal (Phillips & Strand, 1994). Female of *P. interpunctella* was laid significantly more eggs in presence of chemical and physical stimuli of wheat compared with physical stimuli alone (Sambaraju & Phillips, 2008). Roasted almonds and hazelnuts extracts attracted gravid female of *P. interpunctella* for oviposition more than roasted cocoa bean extract (Hoppe, 1981). *P. interpunctella* female was laid significantly more eggs in dishes with wheat kernels treated with 17 types of oil compared with dished with wheat kernels only (Nansen & Phillips, 2003).

2. 4. Role of sex pheromone to management of *S. cerealella*

Trap with synthetic sex pheromone is one of the most safety methods for management of insect pests (Leal et al., 2006; Lima & McNeil, 2009, Witzgall, et al., 2010). The sex pheromone of AGM have been identified, the major components of which are (Z, E)-7,11-hexadecadien-1-ol acetate (or Z7,E11-16:Ac) (Vick et al., 1974). Synthetic sex pheromone of *S. cerealella* has been used to attract and monitor

S. cerealella males. A trap baited with sex pheromone of *S. cerealella* used for *S. cerealella* males during both pre-harvest in field and post-harvest storage (Vick et al., 1987; Barney & Weston, 1996; Weston et al., 1997; Trematerra & Gentile, 2002) or to mating disruption (Fadamiro & Baker, 2002).

2. 5. Role of plant volatiles on sex communication and oviposition behavior of moths

The use of semiochemicals for pest management and control is one of many new techniques to manage stored-product insects. This technique has been established as a viable alternative method, when problems are associated to the indiscriminate use of chemical control, like resistant insects to insecticide, toxic residues in food, environmental contamination and toxicity in non-target organisms (Hamacher et al., 2002; Saber et al., 2004; Pimentel et al., 2007).

Volatiles compounds from plants can enhance the response of insects to sex pheromones (Reddy & Guerrero, 2004). Additionally, mated females may use plant signals to find and select their host. Influence of host plants on sex communication and oviposition behavior has received very little attention. In recent years, several researchers have suggested that plant volatiles should modify sex pheromone caused behavior and that could possibly be used as a tool for enhancing the effect of synthetic sex pheromone moth or for disruption of mating (Light et al., 1993; Reddy & Guerrero, 2000; Yang et. al., 2004; Deng et al., 2004a,b; Kvedaras et al., 2007). Additionally, may possible using plant volatiles for attractants or repellents as insects (Cox, 2004; Arab & Bento, 2006).

Several storage-product moths have been responding to plant odours after mating. *E. cautella* female attracted to wheat more than alternative dried fruits

(Gothilf et al., 1993). Gravid female of *P. interpunctella* showed upwind flight and attractive to rearing diet as bait in wind tunnel (Phillips & Strand, 1994). Additionally, it showed high attraction to wheat kernels treated with walnut oil in wind tunnel too (Nansen & Phillips, 2003).

A 1:1 mixture of acetic acid and isoamyl alcohol (3-methylbutan-1-ol) captured both sexes of *Ephestia cautella* Zeller (Lepidoptera: Pyralidae) and *P. interpunctella* to traps in mills (Toth et al., 2002).

The relationships between host plant and herbivores are complex relationships in agroecosystems. Understanding this relationship is important for development Integrated Pest Management (IPM) strategies.

2. 6. Use of GC-EAD for detection of volatile compounds

The EAG technique has been used insects of stored products to identify pheromone components and grain odors (Vick et al., 1974; White et al., 1989; Olsson et al., 2005b). Chemical volatiles compounds, which elicit an EAD response, can be identified using gas chromatography-mass spectrometry (GC-MS). The EAD technique has been used for determination of active volatile compounds from oat attractive the both sexes of *Oryzaephilus surinamensis* L. (Coleoptera: Cucujidae) (White et al., 1989). One compound, Hexanoic acid, isolated from pods of the carob tree, *Ceratonia siliqua* L. (Leguminaceae) was the most attractive volatile compound to *O. surinamensis* (Stubbs et al., 1985). Two compounds, phenylacetaldehyde and nonanal, were consistently eliciting responses in both sexes of *E. cautella* and *P. interpunctella* (Olsson et al., 2005b).

3. MATERIALS AND METHODS

3. 1. Insects

The insects of *Sitotroga cerealella* were obtained from stock cultures in Laboratory of Integrated Management of Grain-Pests, Departamento de Engenharia Agrícola (DEA) at the Universidade Federal de Viçosa (UFV). They were reared on wheat kernels, at 28 ± 1 °C, $65\pm 5\%$ relative humidity (rh) and 12:12 photoperiod in glass jars.

3. 2. Crops

The kernels of five corn genotypes (UFVM100, UFVM200, UFVM100X200, AG1051 and DKB747) were planted and collected in experimental farm of UFV without using any insecticides. All kernels were stored in environmental-controlled chamber under 5 °C until experiments starts.

3. 3. Development and survivorship experiment

Five samples of 50 g of kernels of each corn genotype were infested with 30 eggs 24 h age. The eggs were obtained by transferring *S. cerealella* adults to a plastic container (350 mL) with piece of gauze for ventilation. A piece of black paper was folded by Ellington method (Ellington, 1930) to create a narrow oviposition substrate.

Corn samples with eggs were putted in plastic containers (350 mL) covered at the top with piece of gauze for ventilation. They were placed in environmental-controlled chamber at 28 ± 1 °C and $65\pm 5\%$ relative humidity. Development period, number of emerging adults and weight loss of kernels were tacked as parameters in this experiment.

3. 4. Surface chemicals collection from corn kernels

Odor from surface corn kernels was extracted using 15 g of whole corn kernels of each genotype and placed in 50 mL Erlenmeyer flask with 15 mL of hexane 97% (Aldrich/Sigma Chemical Co., USA). Then the flask transferred to environmental-controlled chamber at 24 ± 1 °C, $65\pm 5\%$ rh, and the kernels were changed every 2 h at 8 h, with 60 g a total quantity of kernels were used. After this period, the extract was filtered by filter paper (Whatman No.1) in 50 mL Erlenmeyer flask. Finally, we prepared stock solutions of 4 g-equivalent corn mL^{-1} concentrations. The stock solutions were stored in freezer at -19 °C until being experiments.

3. 5. Oviposition preference experiments

To study impact of physical and chemical stimuli of corn kernels on ovipositional preference of *S. cerallela*, six different experiments were used, no-choice test and five choice tests.

3. 5. 1. Oviposition preference on different kernels of corn genotypes

Adults of *S. cerallela* were obtained before mating by transfer rearing culture of *S. cerallela* in column of PVC closed with mesh screen lids from one side and placed it in cage (45 cm x 45 cm x 45 cm).

The first instar larvae were collected by shaking the PVC column. The larvae were placed in gelatin capsules with crushed wheat at their pupal stage. The pupae were separated by sex and each sex was transferred to cage (45 cm x 45 cm x 45 cm), which was placed on environmental-controlled chamber (28 ± 1 °C, $65\pm 5\%$ rh and L12:D12 photoperiod). The pupae were checked daily until adult emergence.

Pair (male and female) with 2-3 days age was transferred carefully to a 120 mL plastic container with 10 g of kernels of each corn genotype, in the same environmental-controlled chamber, for 48 h. After this time the number of eggs in each container was counted. Fifteen containers were observed for each genotype.

Multiple-choice test using five glasses Petri dish (5 cm diameter) with 10 g of whole kernels of each genotype were placed in a plastic box, 22 cm wide × 32 cm long × 12 cm high (7.5 liters), and placed in environmental-controlled chamber with the same condition. One pair of *S. cerealella* adults (male and female) with 2-3 days age was transferred carefully to the plastic box and left to oviposit for 48 h. After this time, the number of eggs in each container was counted. Twenty-five replicates were placed in environmental-controlled chamber, than the first twenty pair started mating were used for this test, and in all replicates Petri dishes were placed in boxes rotate to prevent position effects.

In two-choice test used the same method described above; just we selected UFVM100 genotype to be more susceptible to *S. cerealella* to compare it with each other genotypes for preference oviposition. Twenty replicates were placed in environmental-controlled chamber and the first fifteen pair started mating were used for this test.

3. 5. 2. Effect of physical stimuli on *S. cerealella* Oviposition

In two-choice test, 20 cm of a piece of black paper was folded eight times and stapled in one side to create a narrow oviposition substrate, and placed in one tube (3.5 cm diameter and 8 cm higher), and 20 cm of a piece of black paper was folded eight times without stapling, and placed in other tube. Then, the two tubes were

placed in a 22 cm × 32 cm × 12 cm plastic box in the environmental-controlled chamber as described above in the paragraph 3.5.1.

One pair of *S. cerealella* adults (male and female) was transferred carefully to plastic box, and after 48 h, the eggs in each tube were counted. A total of twenty boxes were placed in environmental-controlled chamber and the first fifteen pair started mating were observed for this test.

3. 5. 3. Effect of odor emissions from kernels on oviposition preference of *Sitotroga cerealella*

Extract of UFVM100 kernels with hexane was tested in two-choice oviposition test. 3.5 cm diameter tubes with black paper applied with 0.5 mL of extract (2 g-equivalent of kernels), and 0.5 ml of hexane was used a control. Twenty cm of a piece of black paper was folded eight times and stapled in one side to create a narrow oviposition substrate. Two tubes were placed in a 22 cm × 32 cm × 12 cm plastic box in the environmental-controlled chamber as described above in the paragraph 3.5.1.

One pair of *S. cerealella* adults (male and female) was transferred carefully to plastic box, and after 48 h, the eggs in each tube were counted. Multiple-choice test was used to study the effect of different chemical stimuli from different genotypes of corn on ovipositional response of *S. cerealella* female. We used the same method described above just placed all five extracts in the same box.

All experiments were replicated twenty times, than placed in environmental-controlled chamber and the first fifteen pair started mating were observed for this test. In all replicates, the tubes were putted in box rotate to prevent position effects.

3. 6. Physical-chemical characteristics of tested corn genotypes

Fifteen kernels of each corn genotype were used to determine the kernel size by using caliper rule. Ten replicates of one hundred kernels were randomly collected from each genotype and weighed using an electronic balance to determine the hundred-kernel weight. A set of fifteen kernels was randomly collected from each genotype to evaluate the kernel hardness. Hardness of kernel was expressed as the break force (Newtons, N) required to break the kernels at a maximum deflection of 1 mm was taken as a measure of kernel hardness using a TA.HD texturometer (Stable Micro Systems).

Fifteen kernels of each corn genotypes were used to determine the kernel length, kernel width and kernel depth. Ten replicates of ten grams of kernels were randomly collected from each genotype to count the number of Kernels/10 g, crevice number/10 g and crevice %. The color of corn kernel was measured by using a colorimeter (CR-400, Konica Minolta, Japan) with the color scores L^* , a^* and b^* . The values of L^* , a^* and b^* demonstrate darkness-lightness, greenness-redness and blueness-yellowness, respectively. The grayness color is intermediate scale for values.

The moisture content of kernels was determined according to ASAE methods (ASAE 2000). Fat content was determined according to AOCS (2009), method Am 5-04. Ash and protein contents were determined according to the method described by Silva & Queiroz (2002). Carbohydrate content was calculated by the empirical formula: Carbohydrate content = $100 - \Sigma (\text{Ash \%} + \text{Protein \%} + \text{Fat \%})$.

Results from percentages of ash, protein and fat were calculated in dry material of kernels.

Table 1. Agronomic traits of corn tested genotypes*

Parameters	Genotypes	UFVM100	UFVM200	UFVM100X200	AG1051	DKB747
Conventional/Transgenic Type	Conventional Variety	Conventional Variety	-	-	Conventional double-cross hybrid	Conventional double-cross hybrid
Cycle	-	-	-	-	Semi-premature	premature
Degree-Day or Days	-	-	-	-	950 °C	845 °C
Planting time	Off season	Off season	-	-	Early/off season/ Normal/ later	Off season/ Normal/ Later
Use	Grains/Silage/ Green corns	Grains	-	-	Grains/Silage/ Green corns	Grains/Silage
Kernel color	Yellow/ orange	Orange	-	-	Yellow	Orange
Density (1000 plants / ha)	50-55	50-55	-	-	45-50	55-65
Texture of the grain	Soft	Hard	-	-	Soft	Hard
lodging resistance	High	High	-	-	High	High
Ear length (m)	1-1.15	1.10	-	-	1.6	1.2
Plant length (m)	2-2.30	2.20	-	-	2.6	2.2
Technology level	Medium/Low	Medium	-	-	High/Medium	High/Medium
Adaptation region in Brazil	SE/NE	SE	-	-	SUL,CO, SE, NE, RO	SUL,CO, SE, NE, RO, AC
Company	UFV	UFV	-	-	SEMENTES AGROCERES	Dekalb

*From: Cnpms, 2011

3. 7. Culture rearing for wind tunnel bioassay

The insects of *S. cerealella* were obtained from stock cultures in laboratory of semiochemicals and behavior, Department of Animal Biology, UFV. They were reared on wheat kernels, at 28 ± 1 °C, $65\pm 5\%$ rh and 12:12 photoperiod in glass jars. Culture rearing of *S. cerealella* was transferred in column of PVC closed with mesh screen lids from one side and placed it in cage (45 cm x 45 cm x 45 cm), than collected the larvae first instar by shaking the PVC column. The larvae were placed in gelatin capsules with crushed wheat at their pupal stage. The pupae were separated by sex and each sex was placed in glass jars on different two environmental-controlled chambers (24 ± 1 °C, $65\pm 5\%$ rh and L12:D12 photoperiod). The pupae were checked daily until adult emergence. We made this method to confirm that all males were no received any female sex pheromone before start experiment.

3. 8. Volatile collection

Volatiles from whole corn kernels were collected using 600 g of UFVM100 genotype that was placed in 2 liters glass flask, with two glass tubes. In one tube was putted charcoal-filtered ad in other tube was putted over a 50 mg Super Q trap that was held between rubbery hose and glass tube. The air was drawn by rate of 300 mL min^{-1} , and was passed for 3 days. After this time, the trap was washed with $300 \mu\text{L}$ of hexane 97% (Aldrich/Sigma Chemical Co., USA) in vials with an elongated tip, and the sample was concentrated to $50 \mu\text{L}$.

To prepare a stock solution of 600 g-equivalent corn kernel volatiles mL^{-1} , $50 \mu\text{L}$ of sample was diluted with $950 \mu\text{L}$ of ethanol 99.5% (Aldrich/Sigma Chemical Co., USA) in 2 mL glass flask.

3. 9. Solution preparation

One μL of synthetic sex pheromone of *S. cerealella* female (Z7, E11-16: Ac) (Trécé Inc. Adair, USA) was mixed with 1000 μL of hexane 97% (Aldrich/Sigma Chemical Co., USA) to prepare a stock solution $1 \mu\text{g } \mu\text{L}^{-1}$. Then, stock solution was diluted to 1, 10 and 100 picogram (pg) using ethanol 99.5% (Aldrich/Sigma Chemical Co., USA) in 2 ml glass flask.

To prepare solution from *S. cerealella* pheromone with kernel corn volatiles, 100 μL of pheromone solution (100 pg) was mixed with 25, 50, 100 and 200 μL of stock volatiles solution and completed with 875, 850, 800 and 700 μL of ethanol 99.5%, respectively, at 1 mL to prepare four-mixing solution; 10 pg of pheromone with 15, 30, 60 and 120 g-equivalent of corn kernel volatiles, respectively.

A stock solution of 600 g-equivalent corn kernel volatiles was used to prepare four solutions of corn kernel volatiles; 15, 30, 60 and 120 g-equivalent corn kernel volatiles mL^{-1} . 25, 50, 100 and 200 μL of stock volatiles solution and completed with 875, 850, 800 and 700 μL of ethanol 99.5%, respectively. All solutions were stored at $-19\text{ }^{\circ}\text{C}$ until start experiments.

3. 10. Wind tunnel bioassays

The experiments with wind tunnel were done at the Laboratory of Semiochemicals, UFV in a (300 cm long x 100 cm wide x 100 cm high) using a piezoelectric sprayer (Figure 1). Wind tunnel conditions were maintained at $23\pm 2\text{ }^{\circ}\text{C}$ and $60\pm 10\%$ rh. The wind was produced by fan, which was filtered using active charcoal. Distance between dilution source and the insect was fastened in 1.80 m. Two hours before the end of the photophase, insect individually was placed in glass

tube (15 x 3 cm) covered with piece of gauze in both side, and transferred to wind tunnel room for adaptation.

In the first experiment, virgin males responding were recorded to pheromone solutions (1, 10 and 100 pg), and in the second experiment they were recorded to 10 pg of pheromone alone and mixed with kernel volatile solutions; 15, 30, 60 and 120 g-equivalent. In the third experiment, virgin and mated males and females responding were recorded to corn kernel volatile solutions (15, 30, 60 and 120 g-equivalent). Ethanol was used to control in all experiments. Dilutions were injected using a microinjection pump (CMA/100), with $10 \mu\text{L min}^{-1}$ rate into ultrasonic nozzle system (Figure 2). Vibration of the nozzle at ultrasonic frequency was ca. 120 kHz. The glass tube and the metallic nozzle were placed on a stand 30 cm high.

All experiments were conducted using 2-3 days old virgin males. Experiments were started during the last 1 h of photophase. For each dilution, 40 insects were tested individually in four different days, and each individual was given 2 min to respond. The following behavioral responses were recorded; numbers of insects were activated from rest (wing-fanning and walking in the tube), flight, oriented flight toward the source at 120 cm, contact with source and time-spent flight (the time was the male tacked it from start flight at select one side or the source).



Figure 1: A wind tunnel with piezoelectric sprayer was used in this experiment; A. wind tunnel, B. microinjection pump (CMA/100) and C. ultrasonic generator.



Figure 2: The ultrasonic nozzle system used in the wind tunnel and the flying response of a male *Sitotroga cerealella*.

3. 11. Gas chromatography-electroantennography detection

Sample corn kernel volatiles from UFVM100 genotype was sealed in glass capillaries and stored at -19 °C until start gas chromatography analysis. A model used of GC-EAD was a Shimadzu GC-2010 and a Syntech electroantennography system (Hilversum, The Netherlands). The GC was equipped with a DB-5 column (30 m length x 0.25 mm diameter, film thickness 0.25 µm) and interfaced with an flame ionization detector (FID) were used with temperature of 270 °C. Nitrogen was used as carrier gas. Column oven temperature was held at 70 °C initially for 2 min, and then increase to 210 °C at a rate of 6 °C min⁻¹, and then increase to 270 °C at a rate of 15 °C min⁻¹. The temperature of the gas chromatography (GC) injector was maintained at 250 °C. Injection of 1µL of sample corn kernel volatiles, split ratio at 5, column flow was at 1.41 mL min⁻¹, total flow 11.5 mL min⁻¹, linear velocity 28 cm sec⁻¹, sampling time 1 min.

Recordings were done both from male and female antennae with 3 days old. An insect antenna was mounted between two stainless steel electrodes. Contact with the ends of the antenna was made with an electrically conductive gel (Parker laboratories, INC, Fairfield, New Jersey, USA).

Twenty individuals used for each sex. Compounds that elicited consistent responses (in at least ten recordings on male and female antennae) are recorded. To record potentials from the antenna used Syntech GC-EAD32 software (version 4.6).

3.12. Gas chromatography-mass spectrometer analysis

A model of gas chromatography-mass spectrometer GCMS-QP5050A (Shimadzu, Kyoto, Japan), equipped with a DB-5 column (30 m length x 0.25 mm diameter, film thickness 0.25 μm) and interfaced with an ion trap detector were used. Helium was used as carrier gas. Column oven temperature was held at 70 $^{\circ}\text{C}$ initially for 2 min, and then increase to 210 $^{\circ}\text{C}$ at a rate of 6 $^{\circ}\text{C min}^{-1}$, and then increase to 270 $^{\circ}\text{C}$ at a rate of 15 $^{\circ}\text{C min}^{-1}$. The temperature of the gas chromatography (GC) injector was maintained at 250 $^{\circ}\text{C}$. Injection of 2 μL of the sample corn kernel volatiles, split ratio at 2, column flow 1.41 mL min^{-1} , total flow 6.8 mL min^{-1} , linear velocity 43.4 cm sec^{-1} . The identity of antennal active components was assigned by comparison of its mass spectrum with either reference data from the equipment database (Wiley 330,000), from the literature (Adams, 2007) and comparison with arithmetic Kovates indices (KI), and on comparison of retention times with synthetic compounds.

3. 13. Chemicals

The synthetic compound, decanal was purchased from Fluka (Switzerland) and other chemicals (nonanal and geranyl acetone) were sourced from Max Planck (Germany).

3. 14. Statistical analysis

Generalized Linear Modeling (GLM) with appropriated distribution was used. Data from choice oviposition test were subjected to binomial analysis model. Data from susceptibility test, no-choice oviposition test and wind tunnel tests were subjected to Poisson analysis model, if necessary, we corrected to sub-dispersion with quasi-binomial or quasi-Poisson, respectively. The analysis was performed and followed by residual analyses to confirm the error distribution and the suitability of the model.

Moreover, the data from time spent flight and physical-chemical characteristics of corn genotypes were subjected to analysis of variance (ANOVA), and correlation analysis was analyzed by Spearman rank correlation using R program (R Development Core Team, 2010). For all analyses we performed to contrast analyses between categories of the treatments.

4. RESULTS AND DISCUSSION

4. 1. Development and survivorship experiment

The data from development and survivorship experiment showed that significant differences were found in development period, percentage of survival and percentage of kernel weight loss among tested genotypes (Table 2). The lengthened egg to adult developmental period was found in AG1051 kernels (39.33 days) ($F=4.20$; $P=0.012$), and did not found significant difference among other genotypes.

The results regarding survival % and kernel weight loss % were almost of the same. Lower survival % was in UFVM100X200 and AG1051 with 28.67 and 28.00%, respectively ($F=6.92$; $P=0.001$). Similarly, kernel weight loss % was significantly lower in UFVM100X200 and AG1051 than on the other genotypes ($F=10.77$; $P<0.0001$).

Table 2: Developmental period (days), survival (%) and grain weights loss (%) (Mean \pm SE) of *Sitotroga cerealella* was reared on five corn genotypes

Parameters	Development	Survival %	Grain weight loss %
Genotypes	period (days)		
UFVM 100	34.03 \pm 0.26 b	52.00 \pm 2.26 a	10.46 \pm 1.4 a
UFVM 200	38.16 \pm 1.50 b	41.33 \pm 4.78 a	7.52 \pm 0.98 b
UFVM 100X200	37.83 \pm 1.49 b	28.67 \pm 3.09 b	4.35 \pm 0.47 c
AG1051	39.33 \pm 0.82 a	28.00 \pm 4.29 b	3.92 \pm 0.62 c
DKB747	35.70 \pm 0.53 b	55.33 \pm 5.37 a	7.18 \pm 0.38 c

Means followed by the same letter(s), within the same column, are insignificantly different at 5% level of probability.

4. 2. Physical-chemical characteristics of kernels for development and survivorship experiment

Physical and chemical characteristics of kernels of corn genotypes tested are shown in Table 3. Kernel size (mm), hundred-kernel weight (g), moisture % and ash

% parameters were significantly higher in kernels of DKB747 genotype than other tested genotypes. The greater kernel hardness (N) was found in kernels of UFVM200 genotype. Moisture % of AG1051 kernel was significantly lower than in the other genotypes ($F=102.28$; $P<0.0001$), but it had higher percentage of carbohydrate compared with the other genotypes ($F=23.51$; $P<0.0001$). Kernels of UFVM100 genotype had greater percentage of protein compared with other genotypes ($F=8.51$; $P=0.003$).

4. 3. Correlation between physical-chemical characteristics of kernels and development and survivorship parameters

In correlation test that presented in Table 4 we found the moisture %, protein %, carbohydrates %, kernel hardness (N), and kernel weight were an important factors determining the susceptibility of corn kernels to infestation with *S. cereallela*. Negative correlation was found between development period and protein %, moisture % and kernel weight, and positive correlation between it and kernel hardness (N) and carbohydrate %. On the other hand, positive correlation was found between survival % and protein %, moisture % and Ash %, and negative correlation between it and carbohydrate % was found. Positively correlated was found between kernel weight loss % and protein %, and negative correlated was found between it and carbohydrate concentrate.

Table 3: Physical and chemical characteristics of kernels of corn genotypes

Parameters	Kernel (mm)	Size Hundred- kernel weight (g)	Kernel hardness (N)	Moisture % Wet Basis (WB)	Ash %	Protein %	Fat %	Carbohydrate %
UFVM 100	247.43±8.81b	29.2±0.28b	347.25±43b	14.5±0.18b	1.9±0.02b	11.5±0.36a	5.0±0.03 c	81.6±0.31c
UFVM 200	219.42±14.04b	25.6±0.64c	379.28±41a	13.9±0.03c	2.1±0.07b	11.2±0.26a	5.7±0.05a	81.0±0.13c
UFVM 100X200	240.26±7.47b	27.8±0.46b	377.56±37b	14.7±0.02b	2.1±0.02 b	10.9±0.22a	5.3±0.04b	81.7±0.28c
AG1051	246.67±11.35b	28.6±0.83b	348.12±42b	13.5±0.03d	1.6±0.02b	9.8±0.04b	4.9±0.03c	83.7±0.01a
DKB747	255.99±7.88a	31.2±0.45a	334.70±40b	15.7±0.08a	2.3±0.30a	10.5±0.07b	5.1±0.09c	82.1±0.13b

Means followed by the same letter(s), within the same column, are insignificantly different at 5% level of probability.

Table 4: Correlation coefficient (r) between susceptibility preference parameter and physical-chemical characteristics of corn genotypes

Parameters	Development period (days)	Survival %	Kernel weight loss %
Development period (days)	1.00		
Survival %	-0.85	1.00	
Kernel weight loss %	-0.85	0.84	1.00
Kernel size	-0.43	0.30	-0.01
100 Kernel weight (g)	-0.53	0.49	0.12
Kernel hardness	0.47	-0.55	-0.25
Moisture%	-0.60	0.63	0.25
Ash %	-0.40	0.58	0.27
Protein %	-0.64	0.47	0.78
Fat %	0.22	-0.001	0.13
Carbohydrates %	0.47	-0.48	-0.63

Results from development and survivorship experiment shows a significantly difference among the tested corn genotypes (Table 2). The mean developmental period from egg to adult of *S. cerealella* when reared on the tested corn genotypes was between 34.03 and 39.33 days. This is similar to that reported by Consoli & Filho (1995), they found that the mean developmental period from egg to adult reared on corn was between 5 and 6 weeks. However, the period was shorter on sorghum (Shazali & Smith, 1985; Ashamo & Khanna, 2006a) and rice, *Oryza sativa* L. (Cogburn et al., 1989).

The higher survival % was found in DKB747 genotype (55.33 %) (Table 2). In other study, a percent survival of *S. cerealella* reared on different corn genotypes were between 46.5 and 64.5% (Cônsoi & Filho, 1995). On paddy varieties was from 42.0 to 68.7% (Ashamo, 2010). A survival from egg to adult in our study was lowest

compared with data from Shazali & Smith (1985), found that a survival % of *S. cerealella* was (77.1%) when reared on sorghum. A highest weight kernel loss % was in UFVM100 kernels (10.46%). Ashamo & Khanna (2006b) found that the highest weight loss of grains was 8.7% when *S. cerealella* reared on corn varieties.

Several investigators have been found correlation between the physical and chemical characteristics of kernels and their ability to attacking by pests. Susceptibility preference in cereals to *S. cerealella* has been correlation between different parameters.

Positive and negative correlation was showed in Table 4. The lowest survival from egg to adult was between *S. cerealella* reared on two genotypes, sugary (Su) and sugary-opaco₂ (SuO₂) and may be related to the high level of amylose of these genotypes (Cônsoli & Filho, 1995). In wheat, negatively correlated was found between present weight loss and fat and carbohydrate concentrations, and positive correlated was found between present weight loss and protein concentration (Khan et al., 2005). Other report demonstrated that the development period of *S. cerealella* was depending on the maturity of corn kernels (El-Sebai, 2006). Kernel Hardness was play important role to resistance plant against several stored product insects. In this study, positive correlation was found between kernel hardness and development period, and negative correlation was found between it and percentage of survival and kernel weight loss (Table 4). The resistance of different maize varieties to *P. truncatus* and *S. zeamais* during storage was affected by kernel hardness characteristic (Meikle et al., 1998).

4. 4. Oviposition preference using whole corn kernels

In no-choice experiment was observed that non significant difference was found among the numbers of eggs laid on tested genotypes ($F=1.11$; $P=0.36$). *S. cerealella* female presented the same preference level on tested genotypes (Figure 3A).

Ovipositional response of *S. cerealella* female among five corn genotypes in choice test is presented in Figure 3B. Higher mean number of eggs was laid on UFVM100X200 genotype. But insignificant difference was found among UFVM100, UFVM200, UFVM100X200 and DKB747. On the other hand, no eggs were found on kernels of AG1051 genotype in all replicas ($F=6.48$; $P=0.0001$).

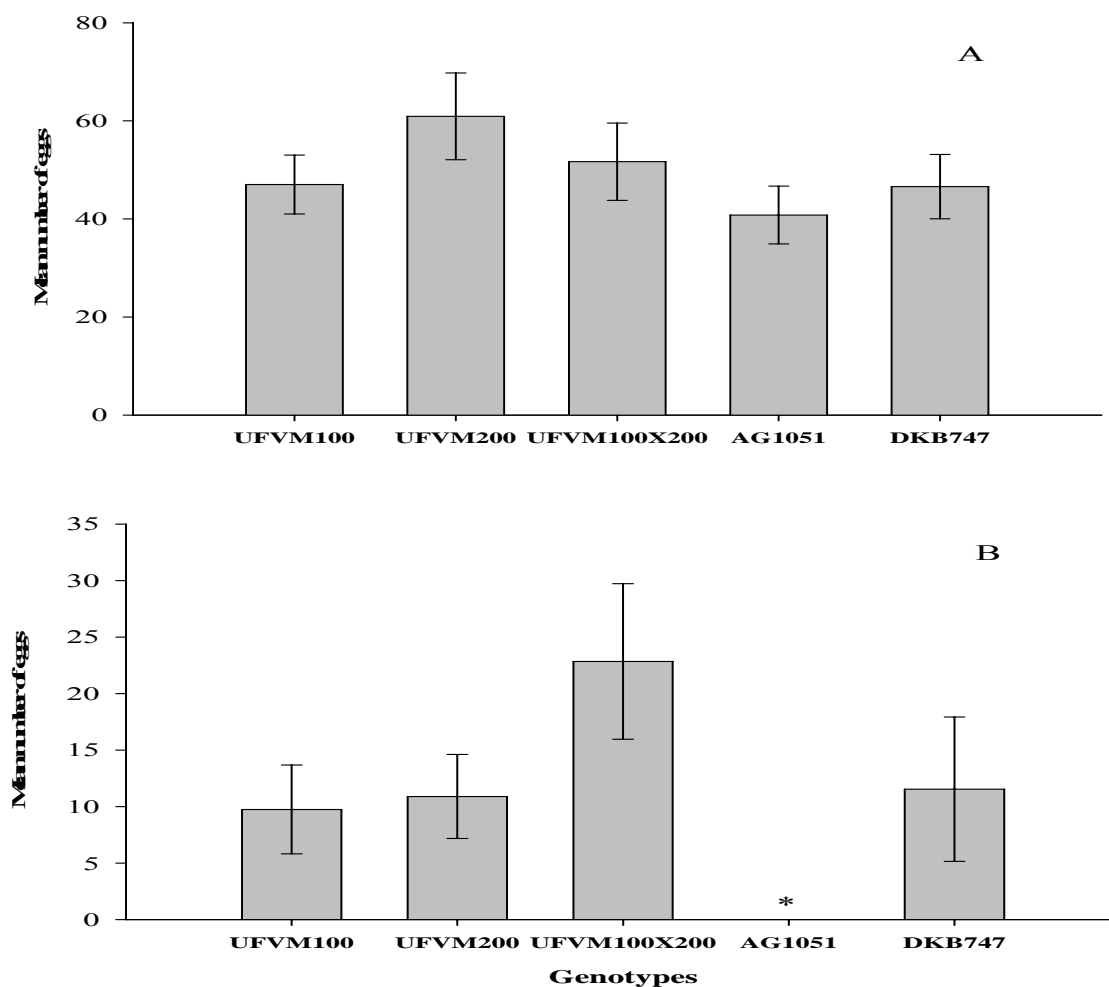


Figure 3: Mean number of eggs laid by *Sitotroga cerealella* female on five corn genotypes: A. No-choice ($P < 0.05$; $n=15$) and B. Choice tests ($P < 0.05$; $n=20$).

An ovipositional response of *S. cerealella* female comparison between the genotype UFVM100 with other genotypes is presented in Figure 4. The obtained results demonstrate that the preference of *S. cerealella* female to oviposition was significantly greater in UFVM100X200 kernels. Approximately 2.58-fold more eggs were laid on UFVM100X200 kernels than on UFVM100 kernels. On the other hand, UFVM100 kernels received approximately 2.26-fold eggs more than AG1051 kernels.

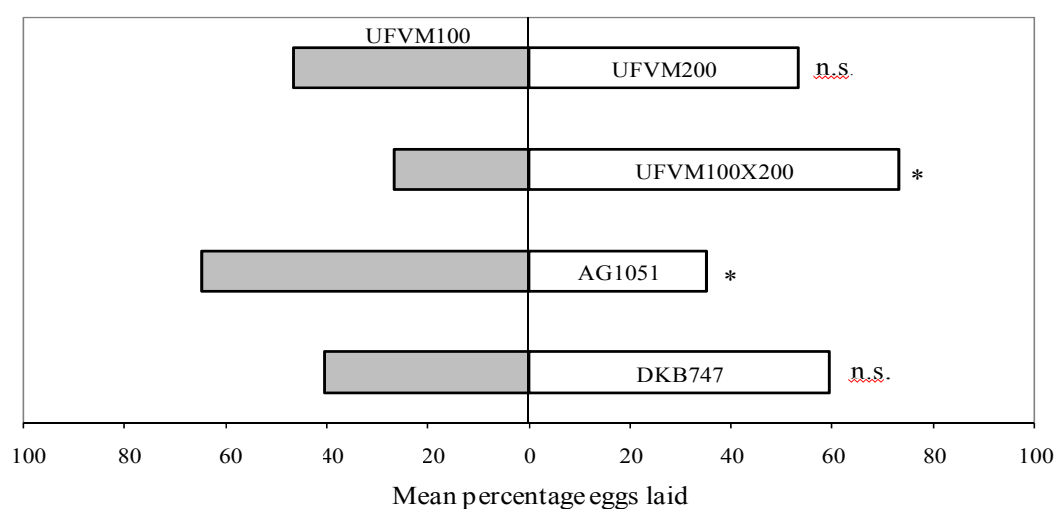


Figure 4: Mean number of eggs laid by *Sitotroga cerealella* female on two genotypes (choice tests, $P < 0.05$; $n = 15$).

4. 5.1. Oviposition preference to substrate form

The number of eggs laid on black paper with a narrow substrate was significantly greater compared with black paper without a narrow substrate (Figure 5). Female of *S. cerealella* laid eggs on black paper with a narrow substrate 34.75-fold more than on black paper without a narrow substrate in a choice test ($F = 757.84$; $P < 0.0001$).

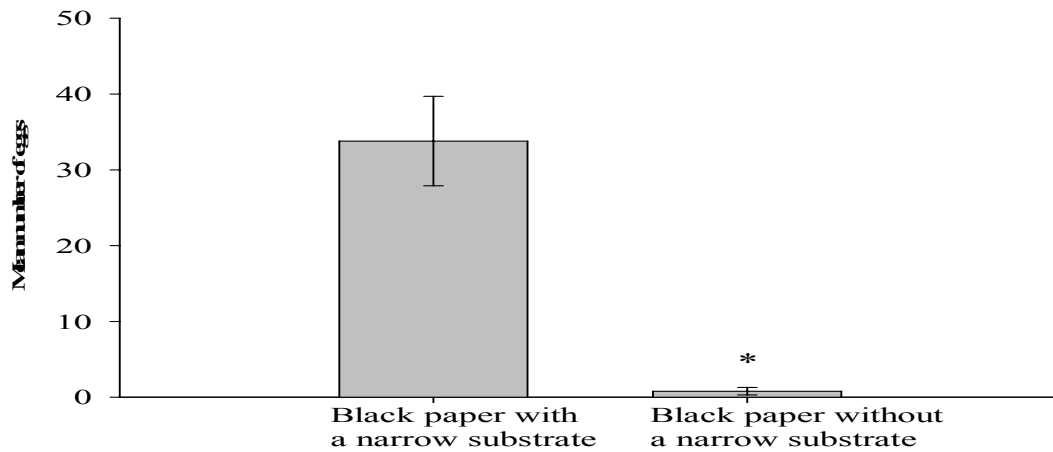


Figure 5: Mean number of eggs laid by *Sitotroga cerealella* female on black paper with a narrow substrate in one side or with black paper without a narrow substrate (choice test, $P < 0.05$; $n = 15$).

4. 5. 2. Oviposition preference using corn extract

The comparison between impacts of physical stimuli alone or together with chemical stimuli is presented in Figure 6. Association of chemical stimuli with physical stimuli significantly enhanced oviposition preference of *S. cerealella*. Black paper treated with 0.5 ml of corn extract (2 g-equivalent of whole kernels) received significantly more eggs than their respective control (black paper treated with hexane only) in a choice test ($F = 18.96$; $P = 0.0002$).

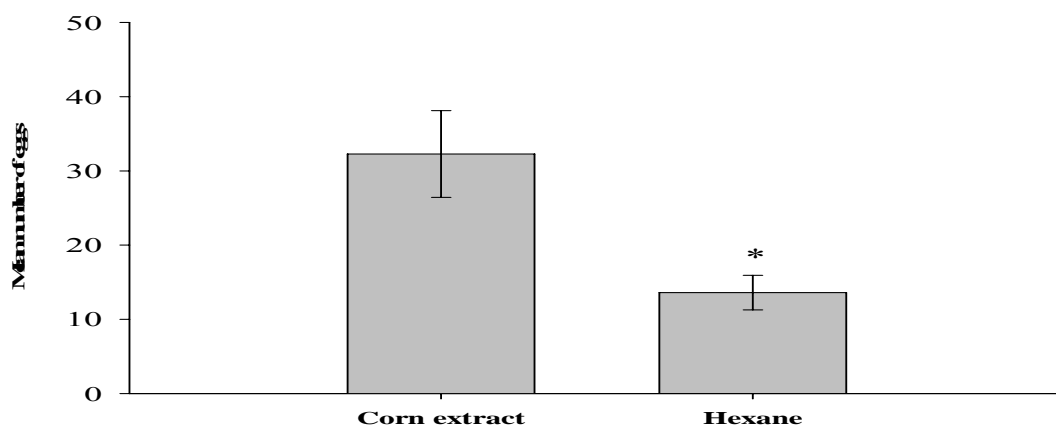


Figure 6: Mean number of eggs laid by *Sitotroga cerealella* female on black paper treated with hexane only (physical stimuli only) or black paper treated with 2 g-equivalent corn extract (UFVM100) in 0.5 mL of hexane (physical-chemical stimuli) (choice test, $P < 0.05$; $n = 15$).

A comparison between extracts from all tested kernel corn genotypes are presented in Figure 7. Black paper treated with extract from UFVM100 whole kernel received significantly greater number of eggs (21.73) compared with black paper treated with extracts derived from other corn genotypes ($F=2.61$; $P=0.04$).

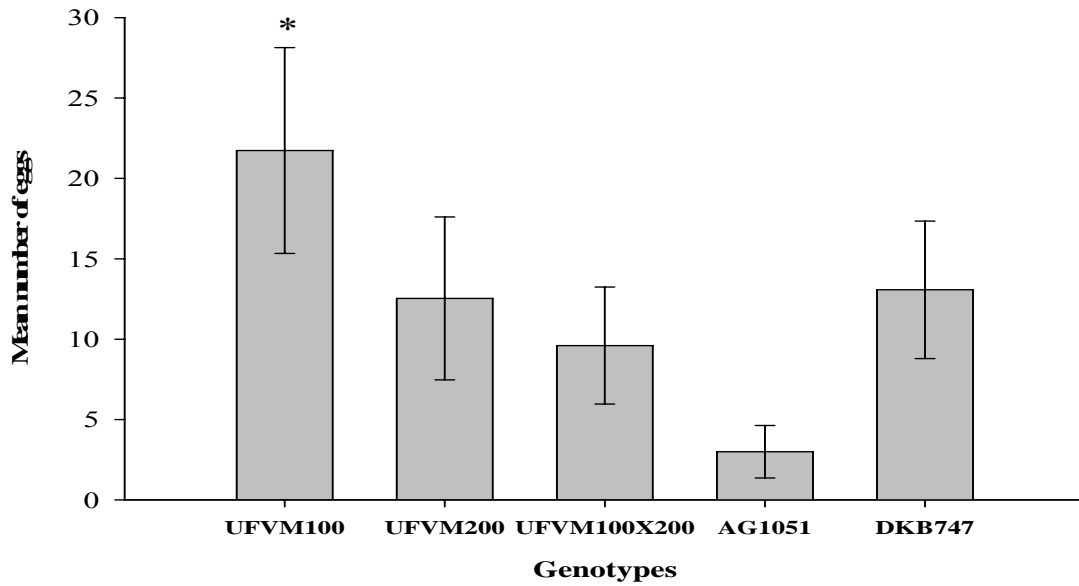


Figure 7: Mean number of eggs laid by *Sitotroga cerealella* female on black paper treated with 2 g-equivalent corn extract of five corn genotypes (choice test, $P<0.05$; $n=15$).

4. 6. Physical characteristics of kernels for oviposition experiments

Physical characteristics of kernels are presented in Table 5. AG1051 kernel had higher degrees of color scores and kernel length, but it had lower kernel depth and crevice % compared with the other tested genotypes.

Table 5: Characteristics of kernels of corn genotype

Parameters	Genotypes	UFVM100	UFVM200	UFVM100X200	AG1051	DKB747
Color scores*						
L		62.34±1.39b	64.46±0.47b	64.48±1.45b	68.08±1.24a	62.53±0.90b
a		12.50±0.80b	13.63±0.84b	12.28±1.05b	15.27±1.03a	11.18±1.16b
b		43.68±2.24b	40.51±2.43b	41.42±1.88b	49.0±1.96a	38.42±3.43b
Kernel length (mm)		10.72±0.21b	10.77±0.28b	11.16±0.22b	11.82±0.37a	10.10±0.26b
Kernel width (mm)		9.13±0.13b	8.69±0.25b	9.11±0.12b	9.33±0.19b	9.50±0.17a
Kernel depth (mm)		4.84±0.16a	4.44±0.15b	4.51±0.08c	4.27±0.11b	5.14±0.16a
Number of kernels/10g		32.9±0.74c	37.1±0.81a	34.5±1.1b	34.0±1.02c	31.1±0.5c
Number of kernels have crevice/10g		13.6±0.81a	14.8±1.07a	14.6±0.48a	10.2±0.84b	11.7±1.24b
Crevice %		41.16±1.88a	40.01±2.89a	42.78±2.13a	29.8±2.04b	37.8±4.14a

* Color scores: L= lightness of sample, the + values for a and b indicate higher degrees of redness and yellowness, respectively.

Means followed by the same letter (s), within the same row, are insignificantly different.

5. 7. Correlation between physical stimuli of kernels and oviposition preference

In correlation test that presented in Table 6 showed that the color scores (a and b) and crevice % were an important factors determining the preference of *S. cerealella* gravid female to corn kernels. Negative correlation was found between preference of *S. cerealella* gravid female and color scores (a and b) of kernel, and strongly positive correlation between it and crevice %.

Table 6: Correlation coefficient (r) between oviposition preference and physical characteristics of corn genotypes

Parameters	Oviposition preference
Oviposition preference	1.00
Color scores	
L	-0.48
a	-0.57
b	-0.67
Kernel length (mm)	-0.38
Kernel width (mm)	-0.22
Kernel depth (mm)	0.24
Number of kernels/10g	-0.08
Number of kernels have crevice/10g	0.75
Crevice %	0.86

The gravid females of herbivores are choice a host plant to lay their eggs that supports the acceptable conditions for protection and development of them and their progeny. This choice is determined by characteristics of plants, such as physical-chemical characteristics (Awmack & Leather, 2002; Mannion et al., 2003; Meiners et al., 2005; Coley et al., 2006).

Our laboratory studies demonstrated that there is a difference between oviposition preferences of *S. cerealella* females to kernels from different corn genotypes. The oviposition preference experiments with whole corn kernels showed

that *S. cerealella* female had less oviposition preference in no-choice test (Figure 3A). Whereas, gravid female of *S. cerealella* had not preferred to lay its eggs on whole kernels of AG1051 when it allowed selecting among the tested genotypes (Figure 3B). In two-choice test it preferred to lay its eggs on UFVM100X200 kernels (Figure 4). On the other hand, *S. cerealella* female laid more eggs on black paper treated with corn extract than black paper treated with hexane (Figure 6). Additionally, it had selected among corn extracts when it presented in multi-choice test, female laid more eggs in response to UFVM100 genotype extract compared with the other genotype extracts (Figure 7).

Different oviposition preference responds to *S. cerealella* female has been observed when it was tested on whole corn genotypes or on corn extracts in the choice tests. This might be due to that the attraction from a distance might involve chemical or physical stimuli, or both (Bernays & Chapman, 1994). The chemical stimuli associated with physical stimuli are important in the final stages of attraction for oviposition of gravid female moths to a host (e.g., Ramaswamy, 1988; Renwick & Chew, 1994; Foster et al., 1997). Weston & Rattinour (1999) found that chemical stimuli associated with physical stimuli of grains are important factors for *S. cerealella* oviposition, but physical stimuli, the straw on the kernel tip, which stimulates, primarily the presence of crevices, as receptors for eggs, were more important.

In the choice tests, the female did not preferred to laid eggs on genotypes with little crevice (Figure 3B). Additionally, we observed, in no-choice test, that females laid eggs in kernel crevice when it presented. But in genotypes this has little crevice, the female laid eggs on surface of kernels. However, in some species, the females, if

approach the end of their life and not have choice, they laid all their eggs (Roitberg et al., 1999).

Preference for physical stimuli to oviposition site has been reported for other moth in Gelechiidae family (Sidney et al., 2008), found that *Anarsia lineatella* Zeller (Lepidoptera: Gelechiidae) female prefer laid its eggs on leaves with hairs. However, this preference may due to the hairs allow the female to keep it on the leaf during oviposition, and its eggs are able to hold on the hairs (Bernays & Chapman, 1994). In this work, the female was preferably laid its eggs under kernel crevice when it present. This preference may due to the crevice keep a female on the kernel during oviposition too, and/or to protect its eggs against natural enemies (Thompson & Pellmyr, 1991; Tagawa et al., 2008).

When physical stimuli were a presented alone (black paper treated with hexane) a low number of eggs laid by female compared when both physical and chemical stimuli a presented together (black paper treated with corn extract) (Figure 6). Other species of stored-product moths were had the same behavior. For example *P. interpunctella* laid more number of eggs on glass beads treated with wheat extract (physical and chemical stimuli) than glass beads treated with hexane alone (physical stimuli) (Sambaraju & Phillips, 2008). But, when physical stimuli of corn genotypes become equal (Figure 7), the preference difference of *S. cerealella* female to tested genotypes was resulting to the different among chemical stimuli of corn extracts. These results agree with those reported for other stored-products moth, *E. cautella* female, where Petri dishes treated with steam distillation extracts of four diets (wheat-based rearing diet, plain chocolate, a nut-containing chocolate product and a rum-flavored chocolate product) proportion of eggs laid was significantly higher in the Petri dish with extract from rum-flavored chocolate than other diet extracts (Olsson et

al., 2005a). *P. interpunctella* females laid significantly more eggs in dishes with 17 types of oil-treated wheat compared with dishes with wheat kernels only, with high preference for Sesame oil-treated wheat compared with dishes with wheat kernels only (Nansen & Phillips, 2003)

Corn tested genotypes may have different of quantity and qualitative composition of volatiles that influence on the oviposition behavior of *S. cerealella*. The genetic variability in herbivore-induced plant signals and about quantity and qualitative emissions of the volatiles blend depend on the corn genotype may make different response by insects. Thus, large differences in total volatile emission, quantity and qualitative compounds, among different corn genotypes have been recorded (Hougen, 1971; Gouiguené et al., 2001; Degen et al., 2004).

However, the high preference of *S. cerealella* females to extract of UFVM 100 and the low preference of *S. cerealella* females to AG1051 whole kernels and extract indicates that might have different ratio of stimulatory physicals and chemicals and/or deferent concentration of odors than other tested genotypes, and/or presence of inhibitory chemicals in AG1051 genotype. Other studies were demonstrated that the activity of odor emissions in attraction of females in family Gelechiidae. Odors from quinoa, *Chenopodium quinoa* (Willd.) showed that the females of *Eurysacca melanocampta* Meyrick (Lepidoptera: Gelechiidae), is a main insect pest of quinoa crop, were more attractive to the Blanca de Junin variety than to Amarilla de Marangani variety in choice bioassay (Costa et al., 2009).

Correlation between physical characteristics of kernels and oviposition behavior was found (Table 6). Female was preferably lay more eggs on kernels have high crevice %. This correlation me explain why the female was laid its eggs on black paper with a narrow substrate more than black paper without a narrow substrate

(Figure 5), additionally, the female was laid its eggs on kernels have dark yellow and red color. Color may be importance factor in the final stages of attraction to select oviposition host (Bernays & Chapman, 1994).

4. 8. Flight responses of male to different concentrations of synthetic sex pheromone

Male behavior in wind tunnel bioassay is presented in Figure 8. The number of males activating from rest increased in all concentrations (1, 10 and 100 pg concentrations) than in ethanol. However, orientation flight to source % and contact with the source % was decreased with the high (100 pg) and low (1 pg) concentrations compared with middle concentration (10 pg) ($F=25.74$; $P<0.0001$).

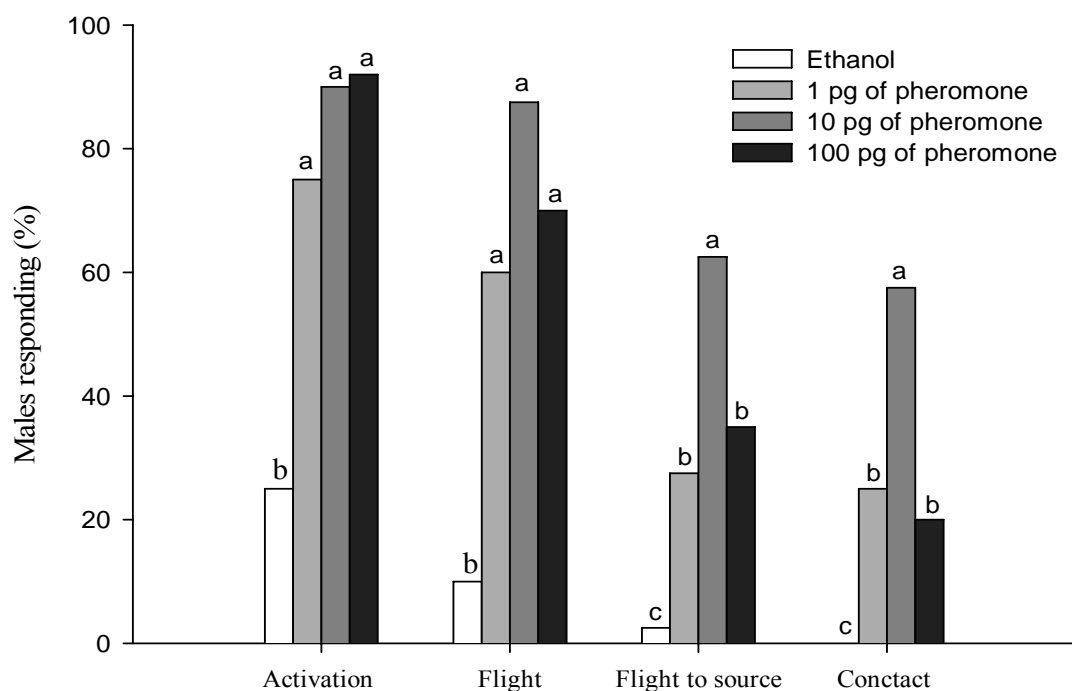


Figure 8: Behavioral responses of *Sitotroga cerealella* male in flight tunnel bioassay to different concentrations of synthetic sex pheromone. Bars with the same letter(s), are non significantly different.

4. 9. Flight time to reach the source of sex pheromone

The result in Figure 9 shows that the males of *S. cerealella* spent significantly more time flight at 100 pg of synthetic pheromone than with 1 and 10 pg of pheromone in wind tunnel bioassay ($F=7.44$; $P=0.01$).

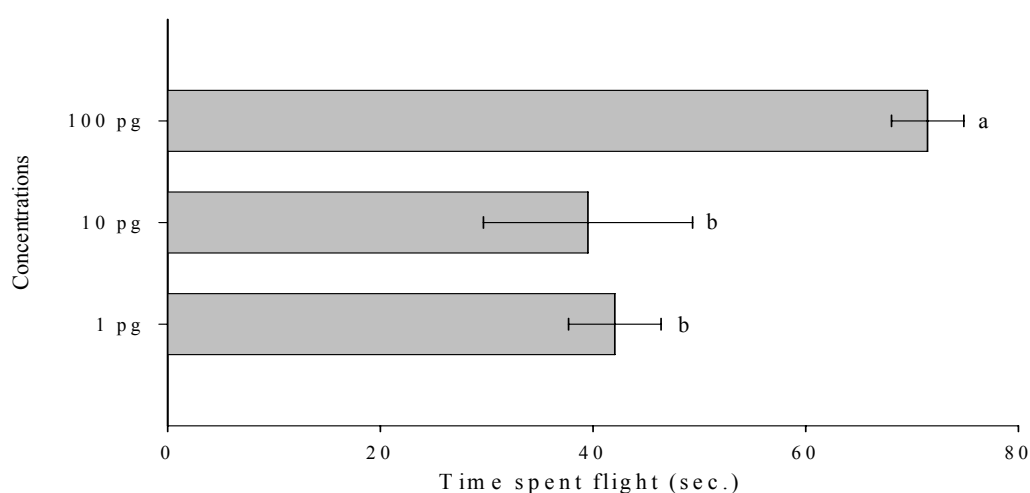


Figure 9: Mean time spent by *Sitotroga cerealella* male to synthetic sex pheromone in flight tunnel bioassay. Bars with the same letter(s), are non significantly different.

4. 10. Flight responses of sex pheromone mixed with different concentrations of corn kernel volatiles

To know the effect of plant volatile mixtures on *S. cerealella* pheromone, the response of a mixture of corn kernel volatile solutions 15, 30, 60 and 120 g-equivalent with AGM pheromone was tested. Blends of AGM pheromone with 15 and 30 g-equivalent corn kernel volatiles significantly enhanced the male behavioral to contact with source ($F=84.39$; $P<0.0001$). In response to these blends, 75% and 70% of the males were contacted with the source compared with 60% for AGM pheromone alone (Figure 10). No significant difference in the response to flight and upwind orientation to the source for AGM pheromone alone and mixed with these four different quantities of kernel volatiles was recorded.

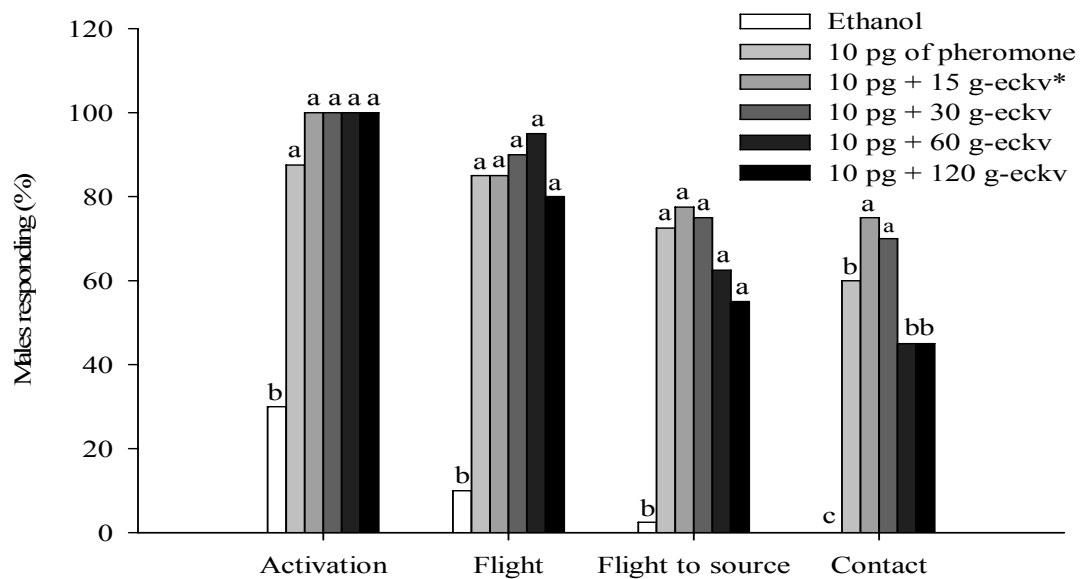


Figure 10: Behavioral responses of *Sitotroga cerealella* male in flight tunnel bioassay to synthetic sex pheromone alone (10 pg) and in mixtures with different quantities of corn kernel volatiles [15, 30, 60 and 120 g-equivalent of corn kernel volatiles (eckv)]. Bars with the same letter(s), are non significantly different.

*eckv = equivalent of corn kernel volatiles.

4. 11. Flight time spent of behavioral responses of male to sex pheromone mixed with corn kernels volatiles

Influence of plant volatiles on flight time of *S. cerealella* male is recorded (Figure 11). The mean flight time from activation to select any side or contact with source in comparison with the pheromone alone and with different quantities of corn kernel volatiles (15, 30, 60 and 120 g-equivalent of corn kernel volatiles). Result showed that corn kernel volatiles also influenced flight time. Generally, the longer time of flight was showed for males in mixed of 10 pg of AGM pheromone with 120 g-equivalent of corn kernel volatiles ($F=7.67$; $P=0.001$).

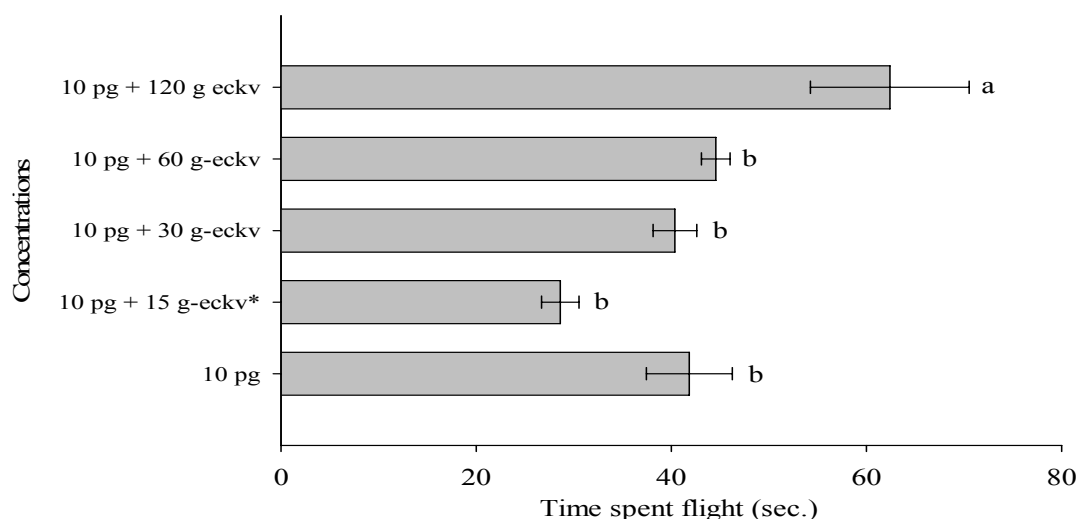


Figure 11: Mean time spent by *Sitotroga cerealella* male to synthetic sex pheromone alone and mixed with different quantity of kernel corn volatiles in flight tunnel bioassay. Bars with the same letter(s), are non significantly different.

*eckv = equivalent of corn kernel volatiles.

4. 12. Flight responses to different concentrations of corn kernels volatiles in male and female of *Sitotroga cerealella*

The responses of the two sexes of *S. cerealella* in flight tunnel experiment with different concentrations of corn kernel volatiles are presented in Table 7. Mated males and virgin females of *S. cerealella* were not showed any response to corn kernel volatiles. There was no significant difference between the concentrations of volatiles and ethanol in responses of mated males and virgin females to corn kernel volatiles. The percentage of males contacting the source was significantly higher in 60 g-equivalent of corn kernel volatiles compared with other concentrations and ethanol (Table 7). Ten percent of males were flight, 2.5% flew upwind toward the source, and 2.5% successfully landed. Mated females showed a significantly greater response to corn kernel volatiles ($F=6.35$; $P=0.003$). No significant difference was detected in responses of mated female to 60 and 120 g-equivalent of corn kernel volatiles. A 120 g-equivalent of corn kernel volatiles elicited upwind flights in 30% of the females

tested and 7.5% approached the source (Table 7). A maximum landing of mated females (10%) was obtained by using a 60 g-equivalent of corn kernel volatiles. Fewer females were flight with lower concentration of corn kernel volatiles, and females did not flight to the source or landing.

Ethanol at the release rate of $10 \mu\text{l min}^{-1}$, no had effect on behavior of males or females of *S. cerealella* in wind tunnel bioassay.

Table 7: Behavioral responses of *Sitotroga cerealella* adults in wind tunnel to different concentrations of volatiles collected from corn kernels

Parameters	Virgin males			Mated males			Virgin females			Mated females		
	Flight %	Flight to source %	Landing %	Flight %	Flight to source %	Landing %	Flight %	Flight to source %	Landing %	Flight %	Flight to source %	Landing %
Ethanol	7.5 a	0 a	0 b	7.5 a	0 a	0 a	5 a	0 a	0 a	5 b	0 b	0 b
15 g-eckv*	7.5 a	2.5 a	0 b	7.5 a	0 a	0 a	5 a	0 a	0 a	7.5 b	0 b	0 b
30 g-eckv	12.5 a	5 a	0 b	7.5 a	2.5 a	0 a	7.5 a	2.5 a	0 a	20 b	12.5 a	2.5 b
60 g-eckv	10 a	2.5 a	2.5 a	7.5 a	0 a	0 a	7.5 a	2.5 a	0 a	25 a	17.5 a	10 a
120 g-eckv	7.5 a	0 a	0 b	10 a	2.5 a	0 a	7.5 a	2.5 a	0 a	30 a	17.5 a	7.5 a

Means followed by the same letter(s), within the same column, are non significantly different.

*eckv = equivalent of corn kernel volatiles.

4. 13. Gas chromatography– electroantennography detection for the volatiles compounds

The GC-EAD results were demonstrated in Figure 11. We recorded GC-EAD responses of *S. cereallela* male and female antennae to aeration of corn kernels. Compounds that elicited consistent responses for both sexes' antennae are marked with numbers in Figure 12. GC-EAG analysis of the volatiles from corn kernels, using male and female antennae, detected a total of four active components for male and female together. Three compounds were recorded in both male and female antennae.

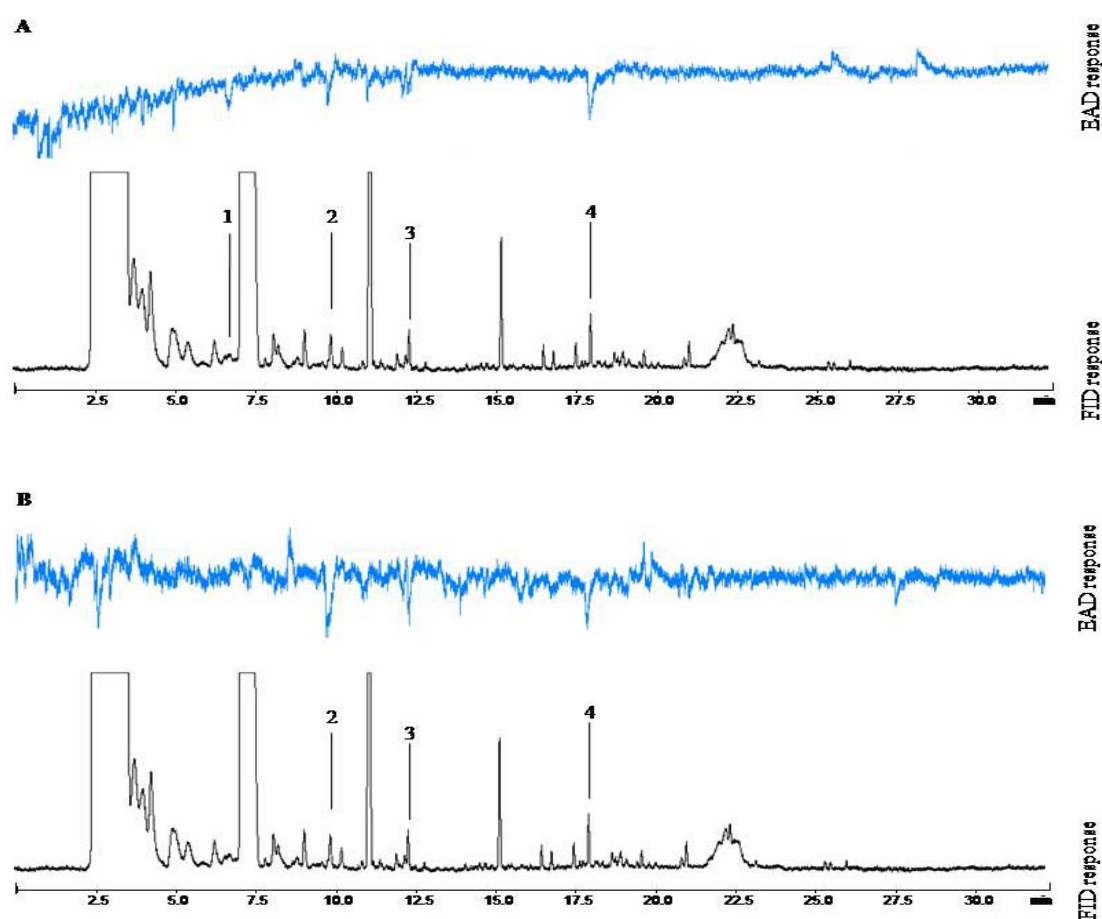


Figure 12: GC-EAD responses of: (A) female and (B) male antennae of *Sitotroga cereallela* to aeration of corn kernels of UFVM100 genotype. EAD active compounds are marked by numbers according to their retention time.

4. 14. Identification of active compounds

Several compounds were showed from volatiles collected by aeration of corn kernels, but the three active compounds that had responding by both male and female antennae were identified (Table 8). These compound were, nonanal, decanal and geranyl acetone [(*E*)-6,10-dimethyl-5,9-undecadien-2-one]. One more compound was unidentified that had responding by female antenna only.

Table 8: Volatile compounds identified in aeration collected from whole corn kernels of UFVM100 genotype

Peak no.	Compound	Class-functional groups	Retention time (min)	Kovates indices ¹ (KI)	Kovates indices ² (KI)
1	Unidentified	-	-	-	-
2	Nonanal	Aliphatic-aldehyde	7.201	1096	1096
3	Decanal	Aliphatic-aldehyde	9.533	1198	1197
4	Geranyl acetone	Terpene-ketone	15.20	1442	1450

¹Kovates indices form the sample corn kernel volatiles.

²Kovates indices from the synthetic compounds.

The result from the wind tunnel bioassay showed that the highest percentage of the males contacted with the source was in 10 pg than in 1 and 100 pg (Figure 8). Several investigators found that the optimal males response was differences by different of pheromone release rates (Hillbur et al., 2000; Witzgall et al., 2005; Schmidt-Büsser et al., 2009).

We observed that, the males in high concentration of pheromone (100 pg) showed in flight arrestment behavior. Normally, the density of pheromone is more near the source than near the receptor. This means that males can encounter high quantity of high concentration near of source, consequently, decrease numbers of

males contact with source. This behavior probably occurs due to in high concentrations of pheromone causing excitation of olfactory receptor neurons and reducing the male's capable to detect pheromone levels in the air (Schmidt-Büsser et al., 2009). This behavior may explains why the males taken more time flight to select on side in case of the high concentration of pheromone (100 pg) and in case of 10 pg of pheromone mix with the high concentration of corn volatiles (Figure 9 and 11).

Synthetic sex pheromone of *S. cerealella* with corn kernel volatiles were increased the percentage of *S. cerealella* males made contact with the source (Figure 10). The role of volatiles compounds was showed clearly in the final step for male finds its female. We observed that, the most individuals' males that made upwind orientation to source (>120 cm) are made contact with the source. The role of physical characteristics may unimportant to affect in flight activity for *S. cerealella*, because the flight activity of the males are under low light condition (Weston et al., 1997; Kanaujia & Sidhu, 2009).

In several studies, when the two types of semiochemicals are presented in the same traps, volatiles from host plant could enhance attractively of sex pheromones. Studies with *S. cerealella* showed that a number of *S. cerealella* males captured in traps with sex pheromone and corn extract or cracked corn kernels was significantly different more than a number of *S. cerealella* males captured in those traps with pheromone alone (Stockel & Boidron, 1981; Ukeh et al., 2008). More strongly attracted of *S. cerealella* males found by the mixture of Z7, E 11-16: Ac + Z7, E 11-16: Ald (9:1) than Z7, E 11-16: Ac alone (Ando et al., 1985).

The flight activity of *S. cerealella* males is increased in cornfield more than in sunflower field (Weston et al., 1997), and this activity of males may due to corn-specific volatiles. On other moths, volatiles from host plant were enhancing

attractively of sex pheromones too. For example, sex pheromone of *Cydia pomonella* L. (Lepidoptera: Tortricidae) and *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) with green-leaf volatiles were captured significantly more males in traps than traps with sex pheromone alone (Light et al., 1993). Enhance responses of *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae) males to sex pheromone with the green leaf volatile were more than pheromone or plant volatiles alone (Dickens et al., 1993). Males of *Plutella Xylostella* L. (Lepidoptera: Plutellidae) were responding a mixture of odors from whole plant of *Brassica oleracea* subsp. *capitata* L. with sex pheromone significant more than pheromone alone (Pivnick et al., 1994).

Additionally, a mixture of some individual synthetic host plant volatiles with sex pheromone enhanced attractant behavior of *Eupoecilia ambiguella* Hb. (Lepidoptera, Tortricidae) (Schmidt-Büsser et al., 2009) and *P. xylostella* (Dai et al., 2008) moths more than single plant volatiles added to pheromone. In contrast, single individual synthetic host plant volatile enhanced attractiveness of pheromone more than whole host plant volatiles (Reddy & Guerrero, 2000).

The ratio between sex pheromone and plant volatile are important too. The mixture of 10 pg of pheromone with 15 and 30 g-equivalent of corn kernel volatiles were attracted more males than pheromone alone to the source (Figure 10). Blends of *Cydia pomonella* L. (Lepidoptera: Tortricidae) pheromone with plant volatiles of apple in a 1:100 ratio were attracted more males than 1:1 or 1:10000 blends (Yang et al., 2004). Pheromone with synthetic host plant volatiles at a 1:10000 ratio were attracted significantly more *E. ambiguella* males to source than 1:1000 ratio (Schmidt-Büsser et al., 2009). The results were found in this work agree with other works that an importance of appropriate ratios between pheromone and plant volatiles.

Some of host plant volatiles are important olfactory stimuli to activate sex pheromone communication on moths. Male may use these olfactory stimuli with female sex pheromone to optimize the chance of find mate location (Landolt & Phillips, 1997; Bruce et al., 2005). Interactions between host plant volatiles and pheromones in the olfactory receptor neurons of insects have been reported (e.g. Ochieng et al., 2002; Broome et al., 2006; Sandoz, 2006; Carlsson et al., 2007; Namiki et al., 2008). Additionally, many plant families are producing structurally identical or similar as pheromones of many insects. Similar of 87% of volatile organic compounds were produced in 96 plant families and 87 insect families (Schiestl, 2010).

The flight tunnel bioassay demonstrated that, mated *S. cerealella* female was stimulated to find ovipositional host by corn kernel volatiles. A result showed that different preference respond of *S. cerealella* female to select ovipositional host when was tested in flight tunnel (Table 7). Generally, the first phase is search when females select their host for oviposition, orientation and then encounter (Renwick & Chew, 1994). The olfactory antennal system of insect performs to receive information of quality and quantity (concentrations, ratios) of odors, and translates this information to behavioral decisions (Carlsson & Hansson, 2003; Schoonhoven et al., 2005). Activity of plant volatiles in attraction of females in family Gelechiidae has been reported in other studies. Volatiles from intact potato tubers were attractive to mated females of *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) in olfactometer bioassay (Arab et al., 2007). Pink bollworm moth, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) was respond to volatiles from cotton flowers in wind tunnel bioassay (Wiesenborn & Baker, 1990).

From GC-EAD and GC-MS experiments, four active compounds (Figure 12), one unidentified and three were identified (nonanal, decanal and geranyl acetone)

found in corn kernel volatiles for both sexes (Table 8). Responses to some of these compounds were small in the baseline of some EAD recording, but they were consistent. Other investigators were found these compounds in different part of corn plant, principally, in ear, husk, silk and kernels (Buttery et al., 1978; Hibbard, et al. 1997; Solé et al., 2010)

From other stored product moths, males and females, of *E. cautella* were responded to twelve electrophysiologically active compounds with GC-EAD. Additionally, both sexes of *P. interpunctella* responded to eight compounds, with greater responses to phenylacetaldehyde and nonanal in both species and sexes (Olsson et al., 2005b).

The ability of both sexes to detect the compounds of volatile presented is probably due to their similar habitat. Therefore, they use of the same cues to locate their host for survive, reproduction and for their progeny. Females of *S. cerealella* were responded to one compound of volatiles more than males. From several insects, females have been reported greater responses exhibited to compounds of plant volatiles compared with males (Ramachandran et al., 1990; Raguso et al., 1996; Zhang et al., 1999). These greater responses of female to compounds of corn kernel volatiles probably due to the female antennae in lepidopterans are have with a large number of olfactory receptor cells that are sensitive to plant volatiles (Raguso et al., 1996).

The overlap of electrophysiologically active compounds between both sexes was showed in tree compounds. This implies that there is a key compound produced by corn kernel. These compounds may an important alone for male and female of *S. cerealella* to find their host.

5. CONCLUSIONS

From the obtained results in this work concluded that, in development and survivorship experiment, the susceptibility of corn genotypes to *Sitotroga cerealella* was dependent on physical-chemical characteristics of kernels. Modifications in physical-chemical characteristics of kernels may an important factor in integrated pest management strategies for *S. cerealella* and in insect resistant genotype programs.

From the results obtained in oviposition experiments, conclude that *S. cerealella* gravid females are using several sensory strategies to select their ovipositional host. It showed response to characteristics of kernels to oviposition behavior in choice test, but when hadn't chance for choice they didn't any preference to tested genotypes. Additionally, showed response to chemical stimuli associated with physical stimuli to oviposition more than physical stimuli alone. Considering the number of eggs lay on the kernels or artificial oviposition substrate with or without corn extracts was result of difference among genotypes that affected on oviposition preference of *S. cerealella* female. This difference among genotypes tested was due to physical-chemical stimuli that favored oviposition preference. However, further studies will be need to establish exactly which compounds of odors are responsible for the observed differences in attractiveness among corn genotypes and if such differences can be used to enhance attract or repellent *S. cerealella* females in integrated pest management strategies and/or in insect resistant genotype programs.

The current research in wind tunnel bioassay focuses on the role of corn kernel volatiles on attractiveness of male and female of *S. cerealella*. The males were showed responding to synergistic sex pheromone with corn kernel volatiles. Additionally, virgin males and mated females were showed responding to corn kernel volatiles. Enhancement of insect pheromone and attracting or disruption of insects by

plant volatiles is very important to decrease the costs of integrated management programs of insect pests.

The result from GC-EAD and GC-MS focuses on determining and identification of relevant compounds with responds of males and females of *S. cerealella*. Four compounds were elicited responds of both male and female antennae.

In future, more studies needs to test these compounds individually and mix for enhance activity flight of males and females of *S. cerealella* to improvement ways of integrated pest management (IPM) to attraction, repellent or mating disruption of this insect.

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