

# **Induced plant volatiles and their effect on *Spodoptera littoralis* choice of host plant**

- oviposition on damaged or undamaged cotton or maize



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## 1. Summary

Insects are the main pests of many crops despite many different control measures. There is a need for a better understanding of the factors that affect the relationship between insects and plants. It is important to investigate how insects find and choose their host plants. One factor affecting the relationship between insects and plants is plant-derived chemical cues. Many plants release increased levels of volatiles after insect herbivory. How the so-called induced volatiles affect the herbivore when it is about to oviposit is not well known. Avoidance of plants that release herbivore-induced plant volatiles in relation to oviposition have been found in some Lepidoptera species. Anderson & Alborn (1999) showed that females of *Spodoptera littoralis* choose to lay eggs on undamaged cotton plants, in two-choice test with damaged plants. In these bioassays the females showed that they were able to discriminate between damaged and undamaged cotton plants, until 10 days after the herbivory was terminated.

This study has investigated how the induced defence, in cotton and maize, respectively affect the pattern of oviposition in *S. littoralis*. Herbivory by *S. littoralis* larvae induce changes in female decision of oviposition on cotton plants. My bioassays in cotton showed that *S. littoralis* is affected by the induced defence: the females prefer to oviposit on undamaged plants over damaged plants. My bioassays in the maize variety Pactol showed partly the same pattern as in the cotton, while in the variety Delprim there was no response from the females to larval feeding.

From the literature it is known that if herbivory by *S. littoralis* on maize is ceased, the emissions of the induced plant volatiles drop rapidly already 10 hours after the stop and the emissions of plant volatiles from damaged maize plants are reduced to almost zero until day 3 after the damage occurred. In comparison, the induced defence in cotton lasts longer than in maize.

*S. littoralis* larvae faeces only made a difference in the bioassay with the ongoing damage on cotton, in the sense that the females oviposited more on the side with undamaged plants compared to the side with the ongoing damage. In the bioassays with the ceased herbivory, with the damaged leaves still left on the plants, there was no impact on choices of the females.

In a bioassay with the dummy paper plants, the females did not oviposit. In this case, the females lacked at least one of the cues needed for oviposition on a plant, such as the odour and the contact with the texture of the leaves. It was obvious that the dummies did not fool *S. littoralis* of being a host plant good enough to oviposit on.

It seems like the decision by the female on how many eggs to oviposit could be controlled at two levels. *S. littoralis* can control both the number of egg batches as well as the number of eggs per egg batch in their choice between different oviposition places; this was shown in the case with the maize variety Pactol.

## 2. Sammanfattning

Insekter är den främsta orsaken till skador på många grödor trots många olika försök att kontrollera dem. Det finns behov av en bättre förståelse av de faktorer som påverkar relationerna mellan insekter och växter. Det är viktigt att undersöka hur insekter söker, finner och väljer värdväxter. En faktor som påverkar relationerna mellan växter och insekter är växternas innehåll av kemiska föreningar. Många växter avger ökade nivåer av växtdofter efter ett insektsangrepp. Hur de så kallade inducerade växtdofterna påverkar herbivoren när den ska till och lägga ägg är inte välkänt, men har studerats i några fall, men flera studier behöver göras. Man har funnit att flera arter av fjärilar undviker växter som avger växtdofter inducerade av herbivorer i samband med äggläggning. Anderson & Alborn visade 1999 att honor av arten *Spodoptera littoralis* (Egyptiskt bomullsfly) väljer att lägga ägg på oskadade bomullsplanter i ett test med oskadade respektive skadade planter. I samma försök visade författarna även att honorna kan skilja mellan oskadade respektive skadade bomullsplanter upp till 10 dagar efter att skadan på bomullsplantorna upphört.

I denna studien har jag undersökt hur inducerat försvar i bomull respektive majs planter påverkar äggläggningsmönstret hos *S. littoralis*. Ett angrepp av *S. littoralis* larver inducerar förändringar hos honornas beslut i samband med äggläggning på bomull. Det inducerade försvaret påverkar honan så att hon väljer att lägga ägg på oskadade planter istället för på de skadade plantorna. Mina försök med majssorten Pactol visade delvis på samma mönster som med bomull. I försöken med majssorten Delprim fann jag däremot ingen respons hos honorna.

Det är känt från litteraturen att om ett angrepp från *S. littoralis* larver på majs avbryts så sjunker mängden avgivna plantdofter redan 10 timmar efter angreppet avbrutits. Efter 3 dagar har de inducerade växtdofterna nästan helt försvunnit. Det inducerade försvaret varar längre hos bomull än hos majs.

Exkrement från *S. littoralis* larver hade bara effekt på valen hos *S. littoralis* honorna i försöket med pågående skador på bomull, honorna lade mer ägg på den sidan av buren där de oskadade plantorna stod uppställda och undvek sidan med den pågående skadan. I försöken där angreppen hade avslutats, med de skadade bladen fortfarande kvarlämnade på plantan, hade exkrementen ingen effekt på honornas val.

I försöken med planter av papp lade honorna inga ägg. Troligen saknade honorna många av de egenskaper som stimulerar dem till att lägga ägg på en växt. T.ex. saknades lukten helt från plantan, även om den fanns i omgivningen samt att även bladens textur saknades. Pappplantorna lurade inte *S. littoralis* honorna på ett sådant sätt att de betraktades som ett objekt bra nog att lägga ägg på.

Det verkar som beslutet av honorna om hur många ägg de lägger kan kontrolleras på två sätt. *S. littoralis* honorna kan styra både antalet ägg per äggsamling (åtminstone i fallet majssorten Pactol) och antalet äggsamlingar under äggläggningen.

### 3. Introduction

Insects have been and still are the main pests of most crops despite many different control measures. Thereby there is a need for a better understanding of the factors that affect the relationship between insects and plants (Schoonhoven et al., 2005). For example, it is important to investigate how insects find and choose their host plants.

One important factor affecting the relationship between insects and plants is plant derived chemical cues (Schultz, 1988). These may even be the most important cues in many insect plant interactions. Many plants release increased levels of plant volatiles after insect herbivory. Induced volatiles are also important cues for the natural enemies of the pests in their search for their victims (Turlings et al., 1990, Dicke et al., 2003). Recruiting parasitoids and predators that attack the herbivore, by the use of plant volatiles, may be beneficial to the plant (Hoballah & Turlings, 2001). How the induced volatiles affect the herbivore when it is about to oviposit is not well known, but has been studied in some cases. Avoidance of plants that release herbivore-induced plant volatiles in relation to oviposition have been found in several Lepidoptera species (Schurr & Holdaway, 1970, Landolt 1993, Anderson & Alborn, 1999, DeMoraes et al., 2001, Kessler & Baldwin, 2001, Jönsson, 2005).

#### 3.1 Choice of host plant

The selection of a host plant by the female is often crucial for a successful development of her offspring. Chemical cues are an important part for the female during the decisions regarding ovipositing on a host plant or not. The female first orientate towards the plants. Already before landing on the plant she has the possibility to choose between the odour of different plants. After landing on the plant the female has the possibility to assess the plant physically and get in contact with stimulants and deterrents. The female then finally choose if she is ready to oviposit or not (Renwick, 1989). For polyphagous insects the acceptance of a site of oviposition seems directed by general green leaf stimuli and lack of deterrence (Schöni et al., 1987). The choice of the females of oviposition site reflects the suitability of the plant for the larvae (Papaj & Prokopy, 1989).

Many plant species, among them cotton and maize, respond to insect herbivory by releasing an increasing (compared to unattacked plants) amount of volatile compounds (Paré & Tumlinson, 1996). The release of volatiles results in that the attacked plants become more attractive to predators (Paré et al., 1998). The presence of indirect defence plant volatiles in plants have in total been reported in more than 23 plant species and 13 families and in relation to a number of attacking herbivores (Dicke, 1999, Dicke & van Loon 2000, Kessler & Baldwin, 2002).

Females of *Ostrinia nubilalis* (Hbn.) were shown to be repelled to oviposit on maize due to herbivore induced plant volatiles, while undamaged maize plants were attractive to the female for oviposition (Schurr & Holdaway, 1970). *Heliothis virescens* (Fabr.) is another example of herbivores that are deterred from oviposition on *Nicotiana tabacum* (L.) when the plants are fed on by conspecific larvae. The amount of emitted volatiles was lower after the feeding herbivores were removed but were still enough to get a behavioural reaction from the ovipositing females during the first and second night. In this bioassay it was also shown that the plants emit one collection of volatiles during daytime and another blend during night time, with different level of deterrence (DeMoraes et al., 2001).

*N. attenuata* (Torr.) uses both direct and indirect defence and deter oviposition by *Manduca quinquemaculata* (Haworth) (Kessler & Baldwin, 2001). In *Trichoplusia ni* (Hbn.) the

reaction to herbivore induced volatiles from cotton was attraction when then female were flying in a windtunnel, while the females oviposited on undamaged cotton plants instead (Landolt, 1993).

The polyphagous Comma butterfly, *Polygonia c-album* (L.) chooses to oviposit on the host with the better quality, over a low ranked host. The clutches were bigger in number of eggs, however the number of clutches did not differ (Bergström et al., 2006). *Spodoptera exigua* (Hbn.) choose to oviposit on a less good plant over a better host, for the larvae. In some cases the female oviposition preferences do not correspond to the performance of the larvae on the same plant (Wiklund, 1981, Berdegué et al., 1998). Recognition of cues from plants as a result from a previous damage can be a part of this.

It is known that the behaviour of the moth *Spodoptera littoralis* (Boisd.) is affected by plant odours; both the volatiles fractions of flowers and leaves from cotton are attractive to both sexes of the moth (Salama et al., 1984). Receptor neurons on the antennae of *S. littoralis* females have been shown to respond to plant odours with both high selectivity and sensitivity (Anderson et al., 1995a) and they make it possible for the female to differ between damaged and undamaged plants using volatile compounds (Jönsson & Anderson, 1999). Some of the receptor neurons of the females in *S. littoralis* have the sensitivity to sense as small amounts as 1pg (Jönsson & Anderson, 1999). Anderson & Alborn (1999) showed that females of *S. littoralis* choose to lay eggs on undamaged cotton plants, in two-choice test with damaged plants, if the plants have 8-10 true leaves. While if the cotton plants have only 3-4 true leaves the females choose the wounded plants over the undamaged plants. In these bioassays the females showed that they were able to discriminate between damaged and undamaged cotton plants, up to 10 days after the herbivory was terminated.

### **3.2 Plant defence against herbivory**

Plants can have both a constitutive and induced defence. A constitutive defence is active all the time and maintain a constant level regardless injury or not, while the induced defence is latent and is only activated when the plant is attacked (Karban & Baldwin, 1997).

It is favourable to invest in an induced defence, over a constitutive defence, when the attack of the herbivores are unpredictable and when the herbivore may not kill the plant. Induced defence also has the advantage that it does not continuously reveal the position of the plant, as constitutive defence does, so that it is less apparent for herbivores (Zangerl, 2003).

Plant leaves normally release only diminutive quantities of volatile chemicals such as monoterpenes and sesquiterpenes (Turlings et al., 1990, Turlings & Tumlinson, 1992). In contrast, a mixture of compounds in all plants is released from the surface of the plant when they are mechanically damaged. The chemicals that are released upon mechanical damage are so-called constitutive chemical reserve of the plant; the chemicals making up the constitutive defence chemicals are a combination of saturated and unsaturated six-carbon alcohols, aldehydes and esters (Paré & Tumlinson, 1999). Mixtures of different compounds act as host plant signals (Städler, 1986). A collection of volatiles from a plant may consist of hundreds of different chemical components (Raguso, 2004). Some volatiles seem to be unique for a plant family, while others are more common and found in plant species from many different and unrelated plant families (Knudsen et al., 1993).

The blend of released induced volatiles can be complex and often it differs both qualitatively and quantitatively from blends that are released constitutively. The release of volatiles from a

damaged plant may be released either locally from only the place of herbivory or systemically from undamaged parts of the plant away from the damaged part. The different blends that are released can differ depending on the herbivore and the type of herbivory (Stout, 2007).

Abiotic factors may affect the emission of inducible plant volatiles both in a qualitatively and quantitatively way, but few investigations have put effort in investigating their effect on an inducible defence (Dicke & van Poecke, 2002). Abiotic factors that affect the release of induced plant volatiles are among others air- and soil humidity, fertilization, light intensity, light cycle, and temperature (Gouinguéné & Turlings, 2002).

One major reason for plants to release volatiles is to defend the plant. The release of volatiles can work directly by deterring the herbivores from the plant through the release of the constitutive or induced plant volatiles (Pichersky & Gerzhenzon, 2002). They can also have an indirect defence effect by alarming parasites and predators and thereby deterring the herbivore from the plant. Bernasconi et al., (1998) suggested that herbivores may be deterred of induced plant volatiles due to three reasons:

- 1) “the plant may have initiated the production of toxic compounds
- 2) potential competitors are present in the plant
- 3) the plant is attractive to predators and parasitoids”.

Two types of responses in the plants with known induced defence have been shown so far:

- The first group produces a blend of plant volatiles that is dominated by *de novo* produced compounds that intact or mechanically damaged plants do not emit. This group consist of e.g. cucumber, gerbera, lima bean and maize (Dicke & van Poecke, 2002).
- The second group produces a blend of volatiles that is qualitatively similar to the blend of intact or mechanically damaged plants emit. In comparison to the first group, the rate of emission of induced plant volatiles is higher and it continues longer after termination of the damage. There is a *de novo* biosynthesis of volatiles also in this group of plants. Cabbage, cotton, potato and tomato are some of the species in this group. In both groups the induced plant volatiles are released systemically (Dicke & van Poecke, 2002).

Multiple mechanical damages by human hand can not alone completely mimic the response of an herbivore attack, but mechanically injury in combination with caterpillar regurgitant can do this to a high degree. Herbivory by caterpillars, *Anticarsia gemmatalis* (Hbn.), *Helicoverpa zea* (Bodd.), *Spodoptera frugiperda* (Smith), *S. littoralis* and *T. ni* are known to induce releases of volatiles in maize and the release of volatiles seem to be a general response in maize when attacked by phytophagous insects (Turlings et al., 1993).

Specific elicitor of caterpillars enhances the amount of several systemically released volatiles. The systemically released volatile compounds seem to be regulated by at least two different mechanisms, one mechanical part and one part that are induced by an oral elicitor from the herbivore. It was shown that in cotton a certain amount of damage is needed on the lower leaves to get a response in the plant and to get a following systemic release of volatiles. This indicates that cotton plants may tolerate certain levels of herbivory before the induced systemic defence is activated. In some cases induced volatiles appeared to be triggered by the specific elicitor volicitin (a fatty-acid derivative, N-(17-hydroxylinoyl)-L-glutamine), from the oral secretion of *S. exigua* larvae, while the systemic release of other compounds appears to be triggered by the mechanical wounding of the leaves alone (Röse & Tumlinson, 2005). This also shows the active role of cotton plants in responding systemically to an elicitor in



caterpillar oral secretion (Paré & Tumlinson, 1997). Volicitin seems to be a characteristic compound of many noctuid species; it also seems that the proportion of volicitin and volicitin-related compounds in the regurgitant of different noctuid species differs down to species level (Mori, 2003).

### 3.3 Larval frass

Many phytophagous insects have been shown to be deterred from oviposition by larval frass due to relatively non-volatile chemicals emitted from the frass. In the case of cabbage looper, *T. ni* the effect of the larval frass lasted for 3 days after removal of the larvae (Renwick & Radke, 1980). *S. frugiperda*, a polyphagous moth, is deterred from oviposition when it gets in contact with larval frass when the larvae have been feed with maize leaves (Williams et al., 1986). *Spodoptera eridania* (Cramer) and *S. exigua* are also deterred by larval frass, but in this cases plant related volatiles seemed to have a more deterring effect than the larval frass itself (Mitchell & Heath, 1985).

Females of *S. littoralis* have been shown to be deterred by conspecific larval frass, when the larvae feed on perennial cotton, *Gossypium barbadense* (L.), plant leaves (Hilker, 1985). A mixture of six compounds identified from the frass was found to have a deterring effect, similar to the one from the frass itself. However, it was also shown that if one compound of the mixture of compounds was lost, then the deterrent effect was also lost (Anderson et al., 1993). The activity of the larval frass of *S. littoralis* was shown to retain its activity for only two days when applied to cotton leaves, while on the third days of trials, the effect of deterrence was lost (Hilker & Klein, 1989).

### 3.4 Parasitoids

It has been proved that parasitic wasps can find an herbivore-damaged plant with the help of the systemically induced and released volatiles. This system, with a parasitoid finding of an herbivore with the help of volatiles has been proved both in cotton, maize and tobacco. All three plants produce a herbivore-specific blend of volatiles (the amount of the chemicals and the number of chemicals vary) in response to the species of herbivore that are feeding on the plant, and different parasitoids can then find their herbivores (Paré & Tumlinson, 1999).

The parasitoid *Cotesia marginiventris* (Cresson), a parasitoid on *S. exigua*, is attracted by the volatiles from maize plants and can find their host and try to parasitize on it. However, the mechanically wounded plant do not emit the same kind of volatiles and the parasitoid is not attracted to the plant, but if the mechanically wound is spiced up with some volicitin then the plant's response is almost identical to the attack of the herbivore and the parasitoid can once again find the plant and search for the caterpillar (Turlings et al., 1990). *C. marginiventris* seems attracted to both separate substances of the induced volatiles that are released from the damaged maize plants (Schnee et al., 2006), but also the full blend, this last mentioned attraction occur without prior training of *C. marginiventris*.

Cotton and maize plants under attack of an herbivore attract more parasitic wasps than unattacked (Paré et al., 1998). On *S. littoralis* the parasitoid *Microplitis rufiventris* (Kok.) have proved to be the most common and effective larval parasitoid in cotton fields in Egypt (Hegazi et al., 1977, Hafez et al., 1976).

*M. rufiventris* can accomplish up to 100% of parasitism on *S. littoralis* (Ibrahim, 1987), but is not as easily pleased as *C. marginiventris*, it responds only to fresh odour and only after training and *M. rufiventris* may respond to other cues such as the faeces of the larvae of

*S. littoralis* (Hoballah and Turlings, 2005). *M. rufiventris* is found prevalent on cotton in Egypt but also respond to 25 different compounds from, a variety of maize, Delprim, that was induced to release plant volatiles after herbivory by *S. littoralis* (Gouinguéné et al., 2005).

### **3.5 Species included in the study**

#### **3.5.1 Egyptian cotton leafworm**

Willcocks described already in 1922 that *Prodenia litura* F. (= *S. littoralis* (Boisduval) (*Lepidoptera: Noctuidae*)) is a difficult pest on cotton in Egypt and in many other countries. *S. littoralis* is a highly polyphagous insect and is spread over almost the whole of Africa, parts of Middle East and south of Europe (Hafez & Hassan, 1969). It has at least 112 species in 44 plant families as host plants either as food or as plants that are accepted for oviposition (Moussa et al., 1960b). In Egypt *S. littoralis* is considered being a major economic pest of both cotton and maize in Egypt (Brown & Dewhurst, 1975, Hegazi & Schopf, 1984; Hosny et al., 1986). Cotton provide enough nutrients for the larvae to survive and develop well on it (Moussa et al., 1960b) and *S. littoralis* may have up to 7 generations in a year in Egypt (Bishara, 1934).

*S. littoralis* have been proved to be able to develop resistance to a number of pesticides and different methods to control the pest need to be developed (Brader, 1979).

The female of *S. littoralis* live for 2-7 days during summertime, 3-11 days during spring and autumn and 10-22 days during winter and the lifespan of the male is shorter than the one of the female in all the seasons (Avidov & Harpaz, 1969). Oviposition may take place on the night following emergence from the pupae (Willcocks & Bahgat, 1937) but often also on the third night. Wiesmann (1952) found that oviposition often occur during the same night as the female copulated. However, other authors claim that most adults may wait 2 to 11 days before they mate and that the largest rate of copulation takes place the night between the second and the third day of their life (El-Sayes & Kaschef, 1977).

*S. littoralis* seems to prefer to oviposit in irrigated areas (Madkour & Hosny, 1973) and the raised humidity seems to be the reason for this (Moussa et al., 1963). Two factors affecting the distribution of the eggs on the plant, the height and the orientation of the plant to south, north or east/west (El-Saadany & Abdel Fattah, 1976). However, *S. littoralis* oviposit on almost any vertical object with a height of 20-200 cm above the soil surface, but they also oviposit on lower plants and wet soil nearby plants (Abul-Nasr et al., 1972). Eggs are laid all over the cotton plant by *S. littoralis* but a majority of the eggs are oviposited up to a height of 40-45 cm on the plant, then the numbers lowers again when the total height of the plant is about 60 cm (Bishara, 1934). The spacing between different varieties of cotton plants does not seem to affect the density of egg-masses laid by the females (Madkour & Hosny, 1973). Studies on *S. littoralis* have shown that it may oviposit egg batches with a range of 20 to 1000 eggs per egg batch (El-Saadany & Abdel Fattah, 1976) and most often the egg batches are laid on the underside of the cotton leaves (Bishara, 1934). Females that have access to honeywater lay more eggs, the hatching rate of the eggs and longevity of the female was better than with females that have access to sucrose with water and they lay more eggs than females that have no access to either of this (Moussa et al., 1960a). The fecundity of *S. littoralis* is affected by the different host plants that the larvae are feed with (Dimetry, 1972).

Depending on the larval diet *S. littoralis* develops at different rates. In a study of a *S. exigua*, it was shown that it had a slower development on a low nitrogen based food, while on high nitrogen level food the development was the opposite. The deficit of dietary protein was the main reason for the differences in the test. In the test an artificial diet with maize as a main ingredient were the one that the larvae developed most slowly on (Bloem & Duffy, 1990). Alfalfa seems to be a relatively good host plant in relation to other host plants to *S. littoralis*. This was shown in a study where development as larvae, the weight of the pupae, the longevity and fecundity in relation to the other host plants was tested. Seemingly the constituents of calcium, magnesium and potassium in the tested plants affect the development of *S. littoralis*, also the level of nitrogen in the plant to some part affect the herbivore (Abdel-Fattah et al., 1977). The development of *S. littoralis* larvae as well as the reproductive potential of the adults is affected by temperature. A small difference between 25 and 30°C gave differences in the number of eggs laid by the females (Sidibé & Laugé, 1977).

### 3.5.2 Cotton

Annual cotton, *Gossypium hirsutum* (L.), is the most important cash-crop in Egypt and account for a large part of the exports and is a matter of great importance to the people of Egypt. The growing of cotton is related to other crops in growing schedules such as alfalfa, maize, rice, sorghum and wheat (Clapham, 1980).

The response of the plant to an herbivore feeding on a plant is three-fold in cotton. First the breakage of leaves causes the release of the constitutive compounds, often called green leaf volatiles. This takes place shortly after the herbivore starts to feed on the plant. Secondly, a group of terpenes, hexenyl acetate, indole and linalool, are also released, in a diurnal cycle. These compounds are released at higher quantities during the day than during the night. The induced defence of the plant is produced *de novo* in the plant at attack and is not stored in the plant (Paré & Tumlinson, 1999). Thirdly, leaves far from the feeding place of the herbivore release the same group of induced volatile substances after an herbivore attack (Turlings & Tumlinson, 1992). This systemic release of volatiles occurs both in cotton and maize, and many of the compounds released are the same in the both species (Paré & Tumlinson, 1999).

The induced plant volatiles emitted from the damaged cotton plants indicate that the defence of the plant is activated. The plants also may be more toxic or have a lower nutritional value. The cues from the damaged plants are also used by *S. littoralis* to escape competitors and predators. (Turlings & Wäckers, 2004).

Induced volatiles are systemically emitted from undamaged cotton plant leaves after an attack of an herbivore, and not only from the local site of damage. After 2 to 3 days of continuous damage an emission of volatiles from upper undamaged leaves was detected (Röse et al., 1996). After 16 to 19 hours of feeding by larvae of *H. zea* a mix of 22 compounds were detected to be released by the attacked plant that was not, or in less amounts, being detected from an undamaged plant or from a plant with a fresh damage. The fresh damage only induced a release of 9 compounds as a comparison. This shows the difference between the constitutive and the inducible emission of volatiles in cotton (McCall et al., 1994). Among the induced volatiles emitted by cotton plants (variety Delta Pineland 90) there are several substances, mainly the cyclic terpenes, which ceases already on day 3 after the herbivory have stopped. Some components differ in the release during the photophase and the scotophase, during the scotophase there is a ten-fold lower emission of

volatiles. There is also a difference between cotton and maize, where maize only seems to commit to defence when the plant is under attack (Loughrin et al., 1994). In the light of Jönsson & Andersons article from 1999 on the high sensitivity of the *S. littoralis* females it seems clear that even with very low amounts of induced plant volatiles emitted during the scotophase by the cotton plants the levels could be more than enough to affect the behaviour of the females during the oviposition. Jönsson (2005) showed that herbivory induced plant volatiles have an effect on *S. littoralis* in the sense of which cotton plant they select for oviposition, damaged or undamaged ones.

### 3.5.3 Maize

Maize, *Zea mays* (L.), is one of the most important crops for both humans and animals in Egypt. *S. littoralis* attacks maize and it is desirable to establish management systems also in maize for this insect to minimize the use of insecticides (Zeinab et al., 1991).

*S. littoralis* may attack maize and in some occasions do harm to the plants by setting back the growth, but generally the plant is not killed and from fieldtests it has been pointed out that infestation of maize by *S. littoralis* is low (Zeinab et al., 1991). Often *S. littoralis* do not oviposit on maize; instead big larvae migrate from adjacent field to maize fields, some leaves are tasted and left, but sometimes whole field are attacked (Bishara, 1934). *S. littoralis* is considered as a minor pest on maize in Egypt, Israel, Libya and Sudan (Willcocks, 1922, Avidov & Harpaz, 1969, Schmutterer, 1969, Lal & Naji, 1990).

*S. littoralis* have difficulties to sustain a living on plants of maize; in some research on maize they do not even survive (Moussa et al., 1960b, Prasad & Bhattacharya, 1975, Zoebelein, 1977). In a recent study on *S. littoralis* and maize it was shown that the insect survive on maize, but at a low rate (Fiscian, 1995). *S. littoralis* have problems to survive on maize because the plant does not supply sufficient amounts of nutrients to support growth (Aboul Nasr et al., 1975). One more reason for *S. littoralis* to avoid maize compared to other crops is that *S. littoralis* is more often parasitized when found on maize compared to other crops (Bishara, 1934).

Maize plants have been shown to release different amount of volatiles in response to different herbivores. In the case with *H. virescens* and *H. zea* it was shown that a specialised parasitoid of *H. virescens* can use herbivore induced plant volatiles from cotton, maize or tobacco to distinguish between the two herbivores (De Moraes et al., 1998). Maize plants that are damaged by *S. exigua* emit a blend of many substances which is dominated by two types of terpenes (Turlings et al., 1991). In maize the production of the induced volatiles occur within a few hours and the *de novo* produced plant volatiles are released from the whole plant (Turlings & Tumlinson, 1992). There are similar emissions from maize plants of the same variety, but a variation between species and varieties (Turlings et al., 1995). The total amount of induced plant volatiles among different maize cultivars differs and there are also qualitative differences among the maize cultivars. In maize it have been shown that regardless of the instar of *S. littoralis* maize releases induced plant volatiles, at the same amount of damage. However both the number of larvae and the level of damage make a difference in the amount of induced volatiles that are released (Gouinguéné et al., 2003).

If the damage by *S. littoralis* on maize stops, the emissions of the induced plant volatiles drop rapidly and stop within hours. Already 10 hours after a stop in the herbivory the emission of volatiles drops remarkably steep. Different cultivars of maize have been shown to differ considerably in the release of volatiles of different kinds (Turlings et al., 1998).

It has been shown how the emissions of plant volatiles from damaged maize plants are reduced to almost zero until day 3 after the damage occurred. The induced plant volatile emissions from cotton last longer than from maize (Turlings et al., 1995). The relaxation in plants, when they stop to emit induced plant volatiles, is not well studied and more studies need to be done (Metlen et al., 2009).

Pactol and Delprim are two varieties of maize. Pactol do not differ from Delprim in the total amount of released induced plant volatiles, but qualitatively, in that it does not release  $\beta$ -caryophyllene (Gouinguéné et al., 2001).

### **3.6 Aims**

My first aim was to investigate if volatiles from ongoing damage on cotton would deter oviposition in *S. littoralis* females.

My second aim was to investigate if the induced volatiles from damaged cotton plants would be deterrent to *S. littoralis* females even without contact to plants.

My third aim was to investigate if cotton or maize plants would still deter oviposition in *S. littoralis* after the removal of feeding larvae.

My fourth aim was to investigate if systemic changes in volatile emission from cotton or maize plants would still effect oviposition even after removing both the damaged leaves and feeding *S. littoralis* larvae.

## 4. Material and methods

### 4.1 Plants

Cotton plants, (variety Delta Pineland 90) and maize plants, (varieties Delprim and Pactol) were individually grown from seeds in 1.5 litre respectively 1 litre pots and kept in a greenhouse chamber at  $25\pm 5^{\circ}\text{C}$  and  $70\pm 10\%$  relative humidity (r.h.). In addition to natural light; 16 hours of artificial light (high pressure sodium, 400 watt) was used from October until the 15<sup>th</sup> of March. After 15<sup>th</sup> of March light was set to be on between 06.00 until 09.00 and 16.00 until 22.00. Non-flowering plants with 7-10 true leaves were used in all experiments.

### 4.2 Insects

*S. littoralis* (figure 4.1) were obtained from a laboratory rearing that has been reared for several years on a semi-artificial diet (Hinks & Byers, 1976), based on potatoes instead of beans. Rearing climate for all the stages of the insects was  $25^{\circ}\text{C}$ , 65 r.h. and L16:D8 photoperiod. The sex of the pupae was determined and the pupae were kept in separate chambers until used in the experiments. Moths collected in the wild have been introduced into the culture annually during the last eight years.



Figure 4.1 *Spodoptera littoralis* on cotton leaf

### 4.3 Oviposition bioassays

Two-choice cages bioassays were used to test the effect of odours on ovipositing *S. littoralis* females. The bioassays were performed in greenhouse chambers under  $20\pm 10^{\circ}\text{C}$ , and  $50\pm 20\%$  r.h. In addition to natural light, 16 hours of artificial light (high pressure sodium, 400 watt) were used during all the experiments. The insects were transferred to the cages as pupae, generally six of each gender, and were allowed to hatch normally among the plants.



The pupae and the plants were transferred to the cages on the same day. The moths were then allowed to emerge inside the cages, since experiments have shown that moths that have been transferred to the cage as adults deposit less eggs (Anderson & Alborn, 1999). No additional food, such as honey water, was provided in the cages for the moths.

#### 4.4 Bioassays

##### 4.4.1 Damaged plants inside the cage, terminated damage

##### 4.4.1.1 Damaged plants inside the cage: No removal of damaged leaves

In this bioassay the *S. littoralis* were tested in a two-choice oviposition experiment with pairs of non-infested cotton or corn plants (of one of the varieties of the maize) (figure 4.2). The plants were placed inside one of the ends in the plastic mesh covered cage (height: 80 cm, length: 120 cm and width: 60 cm), and in the other end of the cage a pair of damaged plants from the same species and variety were placed. The plants were placed to have as little contact as possible with the mesh. The distance between the two pairs of plants was approximately 70 cm.

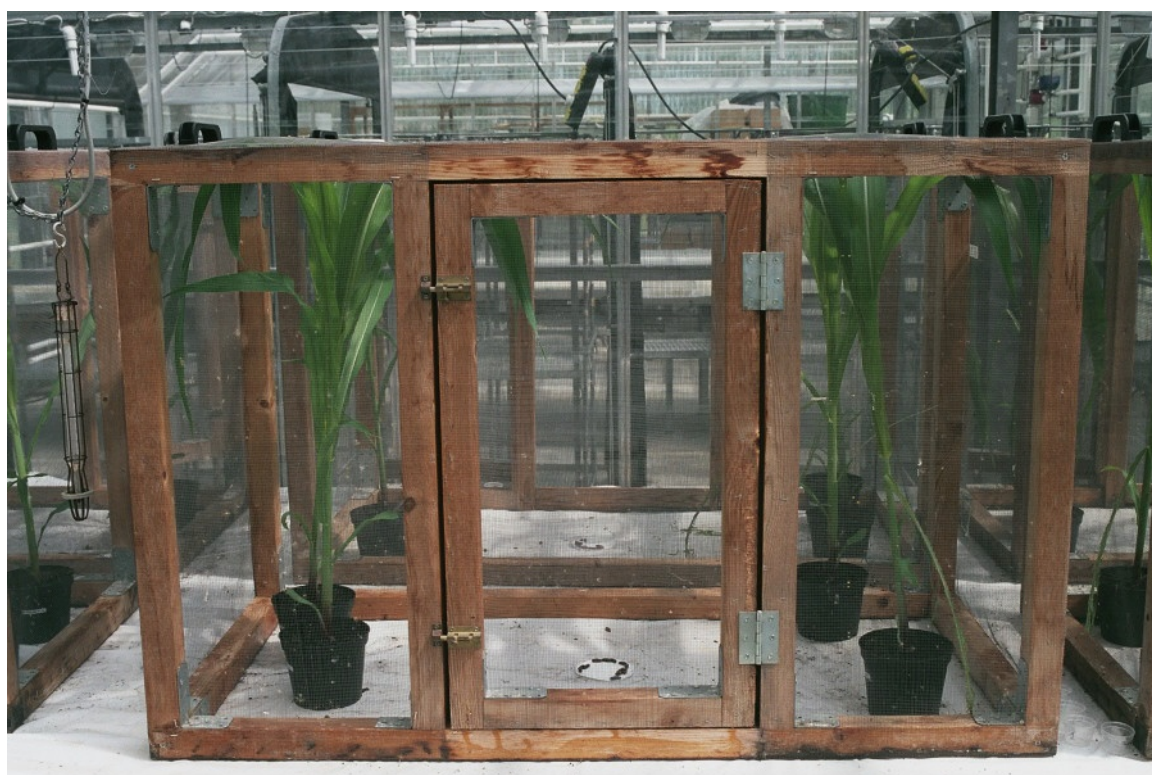


Figure 4.2 Oviposition bioassay, damaged plants inside the cage, terminated damage

The pupae were introduced to the centre of the cage in a Petri dish. In this set-up 2 leaves were damaged on the plants. To damage the leaves of the plants separately, four 3rd to 4th instar larvae were used per leaf. The larvae were placed in plastic freezing bags (2 or 3 litres; depending on the size of the leaf) for 2-4 days. The larvae were allowed to feed approximately 50-70% of the leaf during the period of damage.

How long time the larvae were allowed to damage the leaves were regulated solely by how fast they consumed the leaf, if they were fast in feeding on the leaves, they were removed after 2 days but if they were eating more slowly they were allowed to feed up to 4 days. On cotton, true leaf 2 and 3 were consistently damaged. On the corn varieties either true leaf 2

and 3 or 3 and 4 were damaged. The damaged leaves were left on the plants after the damage. The plants were placed directly on the working tables inside the cage. On the other side of the cage two undamaged plants were placed, with no leaves removed.

#### 4.4.1.2 Damaged plants inside the cage: Removal of the damaged leaves

In the second version of this bioassays the leaves were damaged in the same way as described in part 4.4.1.1 above, but the damaged leaves on the plants were removed after the damage and the corresponding leaves on the two undamaged plants on the other side of the cage were also removed. The removal of the damaged and undamaged leaves was done just before the pupae were placed in the cages.

#### *4.4.2 Ongoing damage/undamaged plants outside the cage, undamaged plants inside the cage*

In the third type of bioassays, four undamaged plants were placed, inside the cage, two in each end, in the same way as in the above experimental set-up. Outside the cage, two damaged cotton were placed at one side and on the other side two undamaged plants were placed (figure 4.3).



**Figure 4.3 Ongoing damage/undamaged plants outside the cage, undamaged plants inside the cage, with recipients of water in between**

To infest the whole plants ten 3rd to 4th instar larvae were introduced per plant. The larvae were placed from the top to the bottom of each plant. The infestation was started on the same day as the pupae and plants were placed inside the cage. If any larvae were found missing the following days new larvae were introduced the first two days after starting of the damage. The aim was to maintain an ongoing damage outside the cage. The plants on both sides outside of the cage were placed on supports in recipients containing water (to avoid eventual



different visual effects) to minimize larval dispersion. In addition a one meter high, half circular screen of white cardboard paper was placed on the outside of the plants on each side of the cage to equalize the visual impression from inside the cage and to reduce the larvae escapes and air movement around the plants. This set-up was only performed with cotton plants.

#### *4.4.3 Ongoing damage/undamaged plants outside the cage, dummy plants inside the cage*

This experiment was done to test *S. littoralis* sense of a host plant lacking many of the cues inducing oviposition behaviour. In this fourth type of bioassays the same set-up as described in part 4.4.2 above was used, except that paper dummy plants with green leaves were used inside the oviposition cages. The difference compared to bioassay number three was that inside the cage there were four paper dummies shaped as cotton plants instead of four real plants (figure 4.4). The dummy plants had a height of 40 cm, with four paper leaves on each side. This bioassay was only done with cotton plants.



**Figure 4.4 Dummy plant**

### **4.5 Recording of the oviposition**

In earlier oviposition bioassays it has been shown that 90 percent of the eggs are laid by the *S. littoralis* female during the first 5 days (Anderson et al., 1995b). The females were allowed to oviposit for four nights, counting started at the first day at which an egg batch was laid in any cage of the set-ups and the experiment was terminated on the fifth day.

Egg batches deposited on any of the plants were included in the statistical tests, i.e. no kind of minimum size of egg batches was applied. Only in the case with the dummy plants all eggs found within a cage were included in the results. The recording of eggs was done each consecutive morning, with a few exceptions, of the experiment. The egg batches were recorded separately while the weight of eggs oviposited on the plants or other surfaces in the cage were recorded control side versus test side and on cage basis. The oviposited eggs on the plants and non-plants parts were kept separated. The egg batches were weighted separately. The number of eggs per batch was determined using a previously prepared “weight vs. number” standard curve, as done by Sadek et al. (2010). Regarding the bioassay with the dummy plants the eggs were counted as being placed on the control or the test side by dividing the cage in two equal sized parts and eggs on sites other than on the dummy plants.

The positions of the damaged and non-damaged plants were shifted between each bioassay and set-up to avoid eventual positional effects. Some cages were discarded due to different disturbances such as hatched larvae, fungi, mites or trips on the plants. All other experiments were included in the statistical analyses.

#### **4.6 Statistics**

The number of egg batches as well as the weight of the eggs was summed up into two sets of data: control and test side, from each bioassay. The treatments were compared by a non-parametric test, Wilcoxon signed rank test, with  $\alpha$  set at 0.05. In the two different bioassays 4.4.1.1 and 4.4.1.2 mentioned above, plants with the damaged plants inside the cage, the data was also pooled and analyzed in all the three plants varieties.

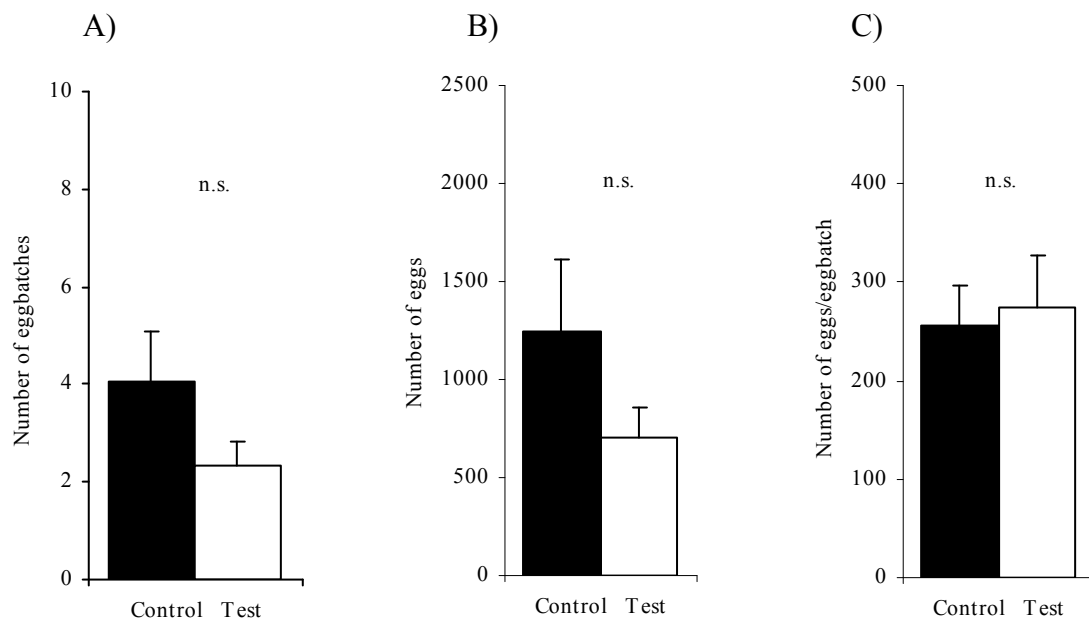
## 5. Results

### 5.1 Cotton

In parts 5.1.1 to 5.1.4 only the number of egg batches and eggs deposited by the *S. littoralis* on the cotton plants are presented. In part 5.1.5 I counted the eggs on the dummy plants and on the experimental cage.

#### 5.1.1 Damaged plants inside the cage: No removal of damaged leaves, terminated damage

In the two-choice experiment comparing damaged and undamaged cotton plants no significant difference between the control and test treatment was found, either regarding the number of egg batches [control ranging from 0 to 14 and damaged plants, ranging from 0 to 6,  $p=0.133$ ] or the mean number of eggs on the plants [control ranging from 0 to 4470 and damaged plants ranging from 0 to 1986,  $p=0.851$ ]. Furthermore, the mean number of eggs per egg batch were similar between the treatments [control ranging from 0 to 489 and damaged plants ranging from 0 to 545,  $p=0.187$ ] (figure 5.1).

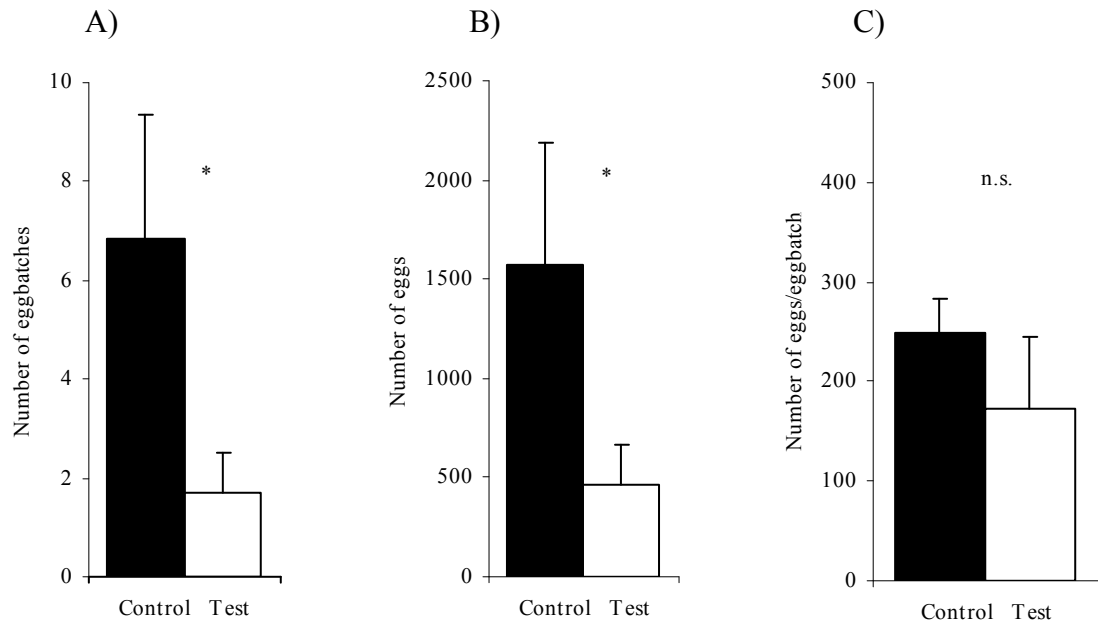


**Figure 5.1 Two-choice experiment comparing damaged (test) and undamaged cotton plants (control) inside the cage I: No removal of damaged leaves**

Mean numbers of A) egg batches and B) eggs laid on undamaged control plants and damaged plants respectively and C) eggs per egg batch. Error bars indicate SEM,  $n=15$ , Wilcoxon signed rank test.

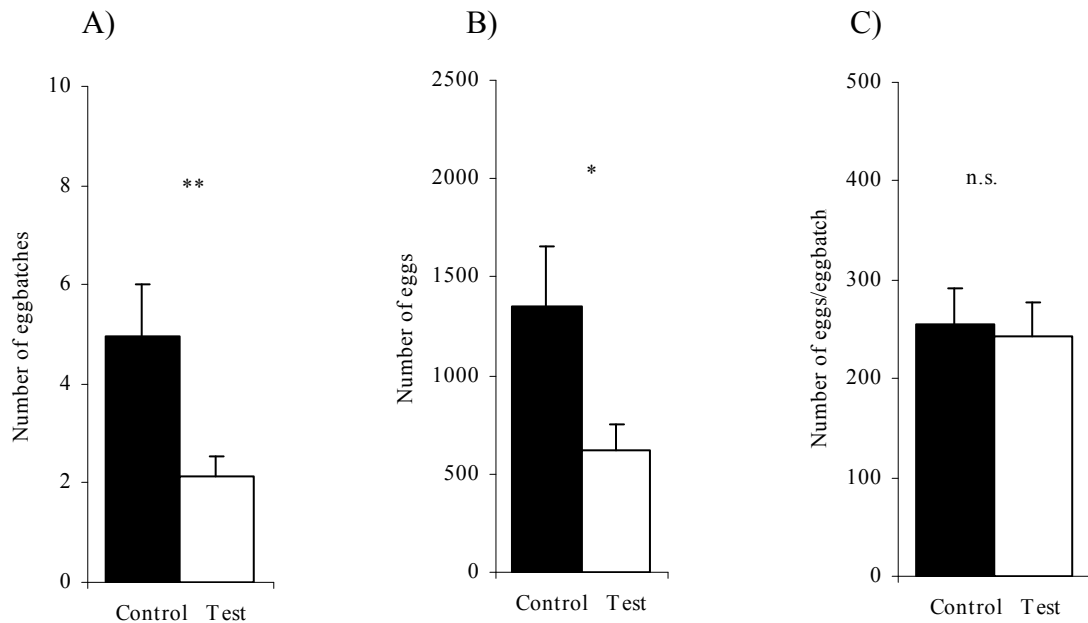
#### 5.1.2 Damaged plants inside the cage: Removal of the damaged leaves, terminated damage

In the two-choice experiment comparing damaged and undamaged cotton plants, with the damaged leaves removed from the test and control plants a significantly lower number of egg batches [control ranging 2-21, test ranging 0-5,  $p=0.036$ ] and eggs on the plants [control 306-5048, test 0-1102,  $p=0.022$ ] was found on the damaged plants (figure 5.2). The number of eggs per egg batch was not significantly different [ $p=0.272$ ].



**Figure 5.2 Two-choice experiment comparing damaged (test) and undamaged (control) cotton plants inside the cage II: Removal of the damaged leaves**

Mean numbers of A) egg batches and B) eggs laid on undamaged control plants and damaged plants respectively and C) eggs per egg batch. Error bars indicate SEM, n=7, Wilcoxon signed rank test.



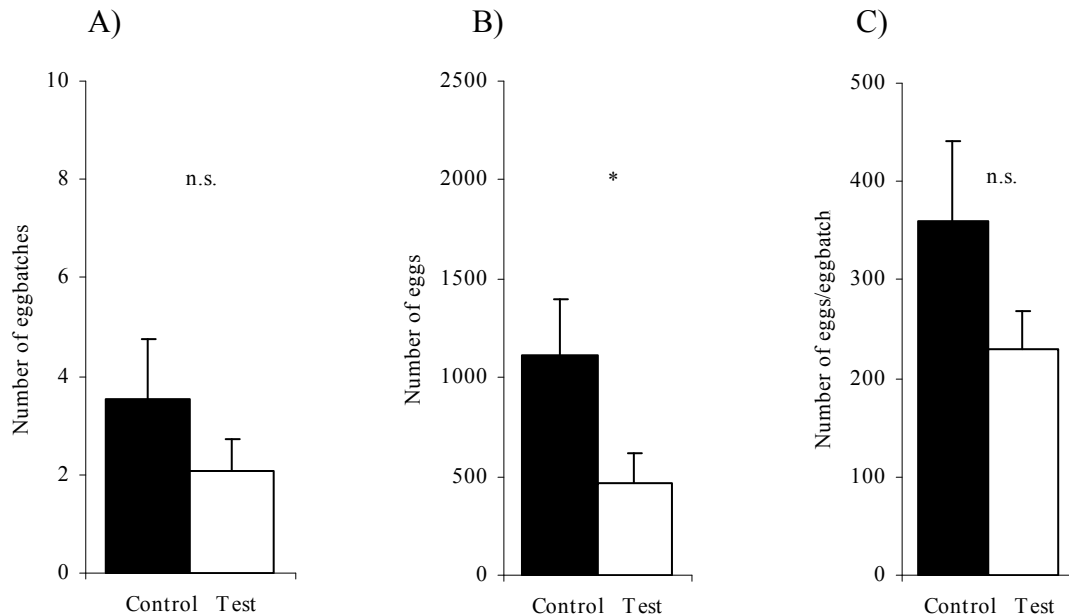
**Figure 5.3 Two-choice experiment comparing damaged (test) and undamaged (control) cotton plants inside the cage: Pooled results**

Mean numbers of A) egg batches and B) eggs laid on undamaged control plants and damaged plants respectively and C) eggs per egg batch. Error bars indicate SEM, n=22, Wilcoxon signed rank test.

Pooling the data from 5.1.1 and 5.1.2, gave a significant difference between the control and test side, both regarding the number of egg batches [ $p=0.006$ ] and the number of eggs deposited on the plants [ $p=0.014$ ] (figure 5.3). However the number of eggs per egg batch was not significant [ $p=0.702$ ].

### 5.1.3 Ongoing damage/undamaged plants outside the cage, undamaged plants inside the cage

In this bioassay, less number of eggs were laid on the undamaged plant close to the damaged plant (figure 5.4) [control, ranging 0-3261, test ranging 0-1824,  $p=0.014$ ]. The mean number of egg batches [control, 0-13, test 0-7,  $p=0.286$ ] and the number of eggs per egg batch [control 0- 1003, test 0-505,  $p=0.230$ ] did not differ.

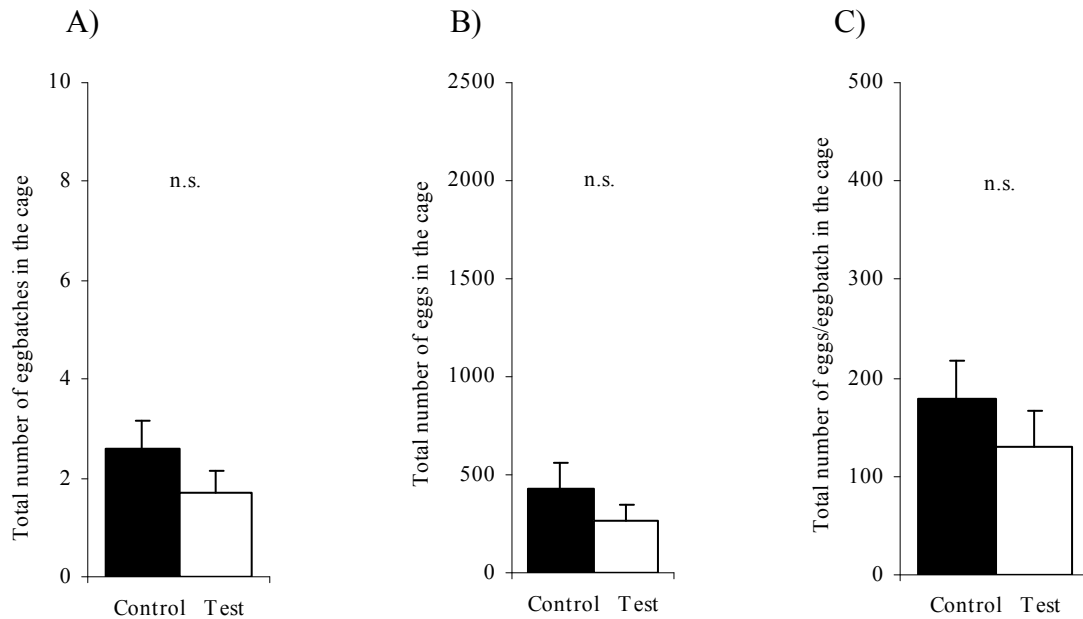


**Figure 5.4 Two-choice experiment comparing ongoing damage (test) and undamaged (control) cotton plants outside the cage and with undamaged plants inside the cage**

Mean numbers of A) egg batches and B) eggs laid on undamaged control plants and damaged plants respectively and C) eggs per egg batch. Error bars indicate SEM,  $n=11$ , Wilcoxon signed rank test.

### 5.1.4 Ongoing damage/undamaged plants outside the cage, dummy plants inside the cage

Replacing the real cotton plants inside the cage with dummy plants and damaging the cotton plants on one side of the cage on the outside of the cage ended with the result that not a single egg batch was laid on the plants. Counting the total number of eggs and egg batches laid on the cage gave a non-significant trend of fewer eggs on control side (figure 5.5).



**Figure 5.5 Two-choice experiment comparing damaged (test) and undamaged (control) cotton plants outside the cage and with dummy plants inside the cage, total eggs per cage**

Mean numbers of A) egg batches and B) eggs laid on undamaged and damaged side respectively and C) eggs per egg batch, all total number of eggs per cage, including walls etc. Error bars indicate SEM, n=10, Wilcoxon signed rank test.

## 5.2 Maize, Delprim

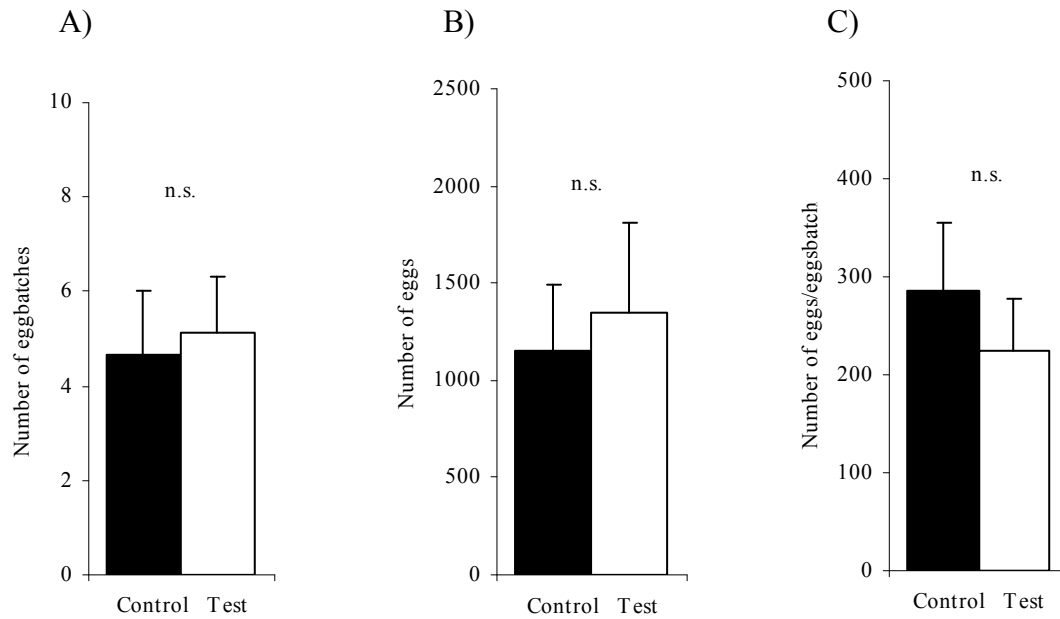
### 5.2.1 Damaged plants inside the cage: No removal of damaged leaves, terminated damage

In the two-choice experiment comparing damaged and undamaged maize plants no differences were found (figure 5.6) between the control and test treatment either in the number of egg batches [control 0-13, test 0-12,  $p=0.343$ ], the number of eggs on the plants [control 0-2697, test 0-3759,  $p=0.477$ ] or the number of eggs per egg batch [control 0-633, test 0-498,  $p=0.813$ ].

### 5.2.2 Damaged plants inside the cage: Removal of the damaged leaves, terminated damage

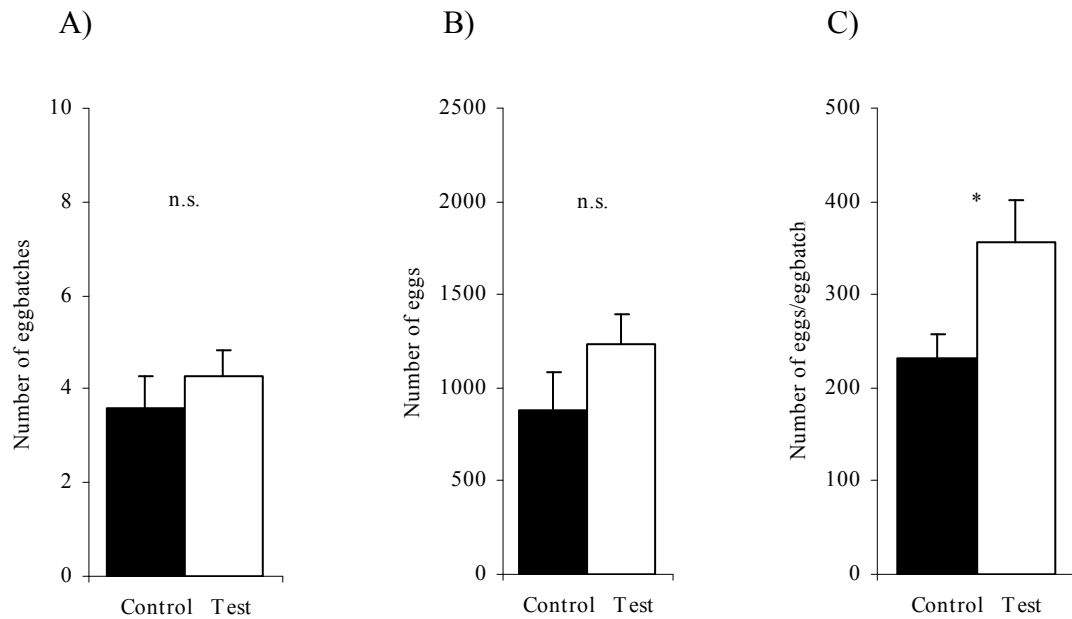
Comparing damaged and undamaged maize plants, with the damaged leaves removed from the test and the control plant resulted in no difference in the number of egg batches or the number of eggs was found (figure 5.7) [control 1-10, test 1-7,  $p=0.272$ ] [control 51-2721, test 209-2489,  $p=0.149$ ]. However, the number of eggs per egg batch was significantly lower on the damaged plants [control 51-351, test 77-477,  $p=0.033$ ].

Pooling the above the two groups of results gave no significant differences between the control and test side for any of the parameters (figure 5.8).



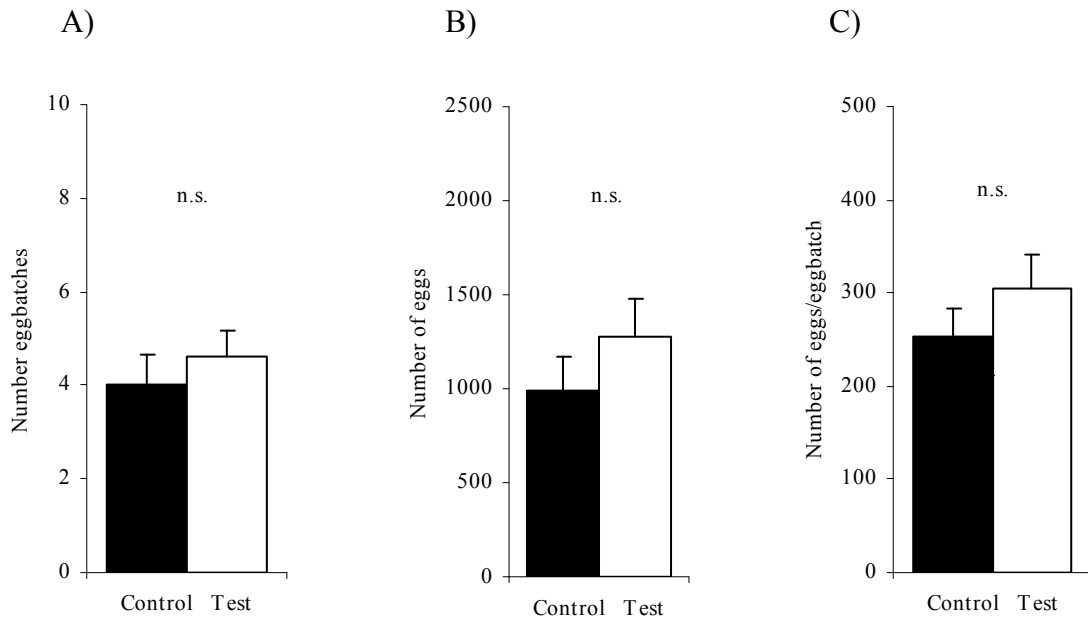
**Figure 5.6 Two-choice experiment comparing damaged (test) and undamaged (control) Delprim plants inside the cage I: No removal of damaged leaves**

Mean numbers of A) egg batches and B) eggs laid on undamaged control plants and damaged plants respectively and C) eggs per egg batch. Error bars indicate SEM, n=9, Wilcoxon signed rank test.



**Figure 5.7 Two-choice experiment comparing damaged (test) and undamaged (control) Delprim plants inside the cage II: Removal of the damaged leaves**

Mean numbers of A) egg batches and B) eggs laid on undamaged control plants and damaged plants respectively and C) eggs per egg batch. Error bars indicate SEM, n=14, Wilcoxon signed rank test.



**Figure 5.8 Two-choice experiment comparing damaged (test) and undamaged (control) Delprim plants inside the cage: Pooled results**

Mean numbers of A) egg batches and B) eggs laid on undamaged control plants and damaged plants respectively and C) eggs per egg batch. Error bars indicate SEM,  $n=23$ , Wilcoxon signed rank test.

### 5.3 Maize, Pactol

#### 5.3.1 Damaged plants inside the cage: No removal of damaged leaves, terminated damage

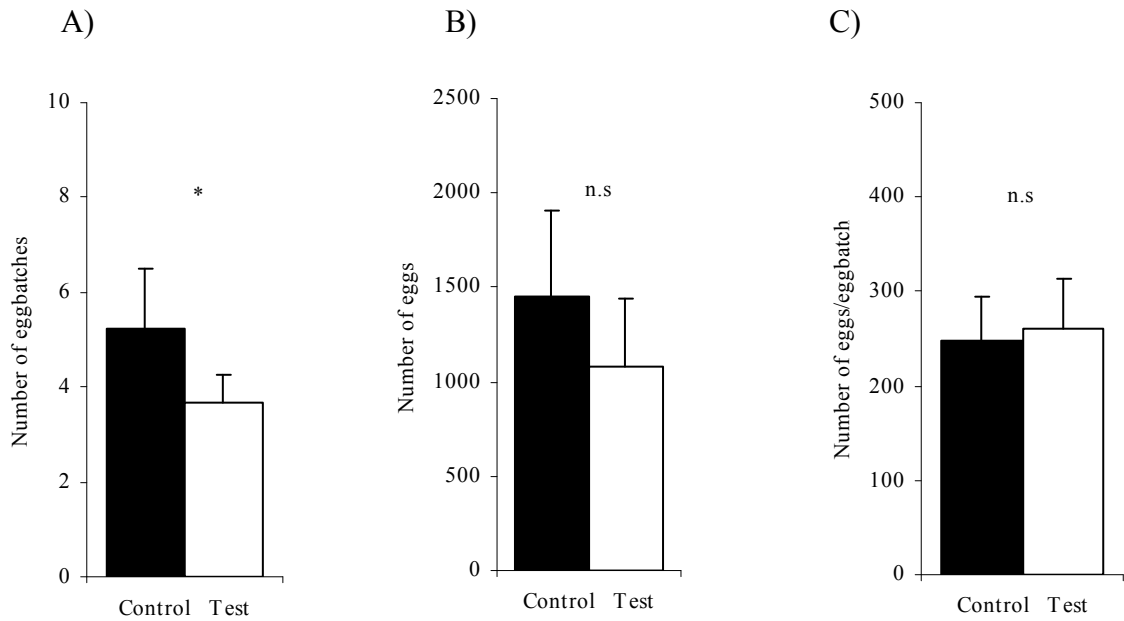
In the two-choice experiment comparing damaged and undamaged Pactol maize plants there was a significant difference between the control and test treatment regarding the number of egg batches [control, 0-13 test 1-7,  $p=0.032$ ] (figure 5.9). The number of eggs on the plants [control 0-4223, test 73-4177,  $p=0.920$ ]. The number of eggs per egg batch had the same trend [control 0-518, test 73-724,  $p=0.969$ ] and did not differ.

#### 5.3.2 Damaged plants inside the cage: Removal of the damaged leaves, terminated damage

Comparing damaged and undamaged Pactol plants, with the damaged leaves removed from the test and the corresponding undamaged leaves on the control plant, a difference in the number of eggs per egg batch was found [control 205-493, test 0-326,  $p=0.025$ ] (figure 5.10). No difference was found in the number of egg batches [control 2-13, test 0-10,  $p=0.477$ ] and the number of eggs on the plants [control 580-4732, test 0-2961,  $p=0.065$ ] between the treatments.

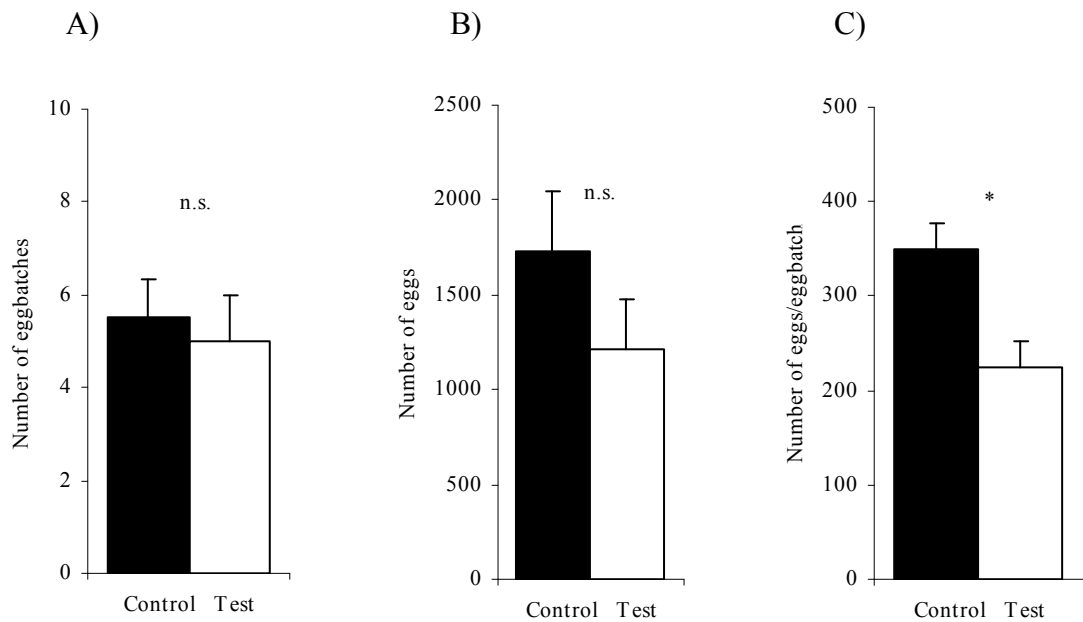
Pooling the two groups of results for Pactol gave a significant difference in the number of egg batches [ $p=0.046$ ], the number of eggs deposited on the plants [ $p=0.008$ ] and also the number of eggs per egg batch [ $p=0.047$ ] (figure 5.11).





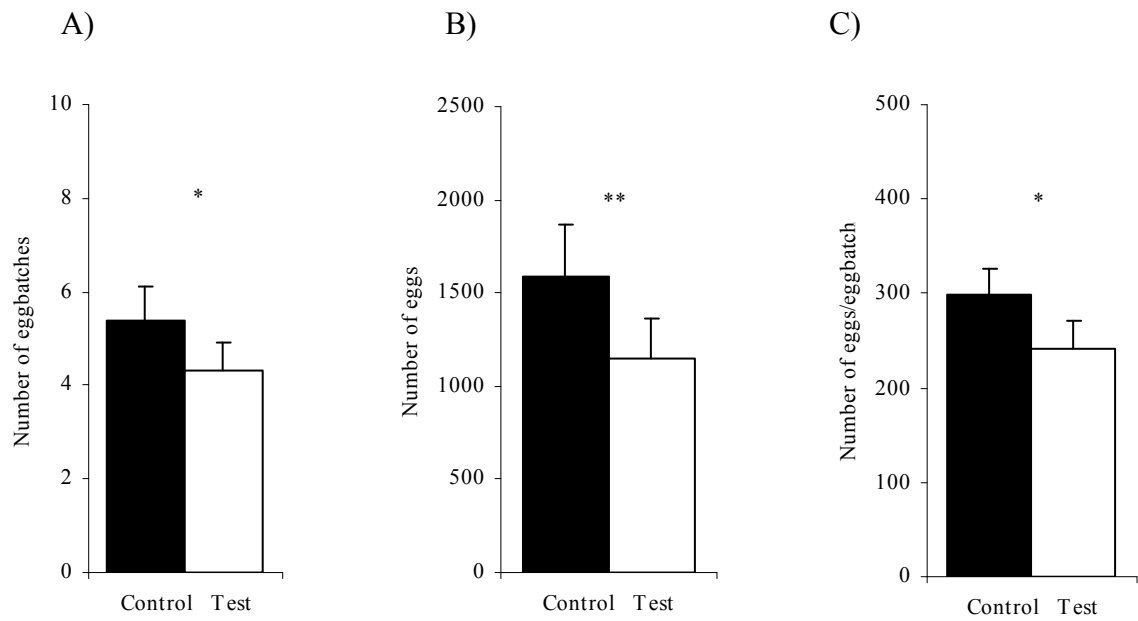
**Figure 5.9 Two-choice experiment comparing damaged (test) and undamaged (control) Pactol plants inside the cage I: No removal of damaged leaves**

Mean numbers of A) egg batches and B) eggs laid on undamaged control plants and damaged plants respectively and C) eggs per egg batch. Error bars indicate SEM, n=12, Wilcoxon signed rank test.



**Figure 5.10 Two-choice experiment comparing damaged (test) and undamaged (control) Pactol plants inside the cage II: Removal of the damaged leaves**

Mean numbers of A) egg batches and B) eggs laid on undamaged control plants and damaged plants respectively and C) eggs per egg batch. Error bars indicate SEM, n=12, Wilcoxon signed rank test.



**Figure 5.11 Two-choice experiment comparing damaged (test) and undamaged (control) *Pactol* plants inside the cage: pooled results of removed and not removed leaves**

Mean numbers of A) egg batches and B) eggs laid on undamaged control plants and damaged plants respectively and C) eggs per egg batch. Error bars indicate SEM, n=24, Wilcoxon signed rank test.

## 6. Discussion

My data show that *S. littoralis* females avoided ovipositing on plants that were affected by induced volatiles emitted from plants both in and on the outside of the cage. Females of other lepidopterans also avoid plants using odours from damaged plants when they oviposit (De Moraes et al., 2001). Landolt (1993) showed that females of *T. ni* were primarily attracted to undamaged cotton plants when they were about to oviposit. The results of the total amount of eggs in the bioassay with the ongoing damage on the cotton plants fit with this study, and it also fit with the results of Jönsson (2005), and shows that the insects were behaving normally.

Faeces from the larvae may only have influenced the choices of the *S. littoralis* females in the bioassay with the ongoing damage on cotton. In the bioassays with the ceased herbivory, with the damaged leaves still left on the plants, the faeces probably had stopped to emit volatiles and hence had no impact on the choice of the females. This fits with the results from Hilker & Klein (1989) which pointed out that the faeces stop to emit odours after three days.

The damaged area seems not to be important since the results were the similar in the presence or absence of the damaged leaves in both cotton and maize. This indicates that systemically occurring plant chemicals are important to the insects during oviposition and not only compounds associated with the wound. However, it cannot be excluded that other factors during the removal of the leaf can influence the female choice.

The results with the dummy plants inside the cage and the damaged/undamaged plants outside the cage surprised me to some extent. Abul-Nasr et al. (1972) stated that *S. littoralis* oviposit on almost any vertical object, but in the present work no eggs at all were placed on the dummy plants. In total only a few egg batches were laid by the females and no clear choice between the sides was seen. However a small overweight towards the undamaged plant could be seen, from the eggs placed on the mesh of the cages. Many cues stimulate *S. littoralis* females to oviposit on the plant, such as the odour, contact chemical and the texture of the leaves, which were obviously lacking in the dummy plant bioassay. Regarding the amount of eggs, the number of egg batches and the eggs per egg batch, it was obvious that the dummies did not fool *S. littoralis* in any way being a host plant good enough to oviposit on. The odours from the surrounding plants were alone not enough to stimulate the females to oviposit. The dummy plants were not watered, which may have removed another stimulus close to the dummy plants to the females; the females could be stimulated by humidity (Moussa et al., 1963) when they are about to oviposit.

The decision by the female on how many eggs to oviposit can be controlled at two levels. It seems like *S. littoralis* can control both the number of eggs per egg batch and the number of egg batches in their choice between different oviposition places. The numbers of eggs per egg batch were shown to be controlled in the case of Pactol and Delprim and the number of egg batches in the case of cotton and Pactol.

The females choose to oviposit on the undamaged control Pactol maize plant compared to herbivore damaged plants, making similar choice as in the same kind of bioassay with the cotton. A difference in the response of the females to the induced

response was found between Pactol and Delprim. Different cultivars of maize vary considerably in the release of volatiles (Turlings et al., 1998). Gouinguené et al. (2001) pointed out that the induced plant volatiles vary between the two varieties, both qualitatively and quantitatively, which may be the reason for the behavioural differences between the two varieties. There are many volatiles that Pactol and Delprim differ from each other in the amount of volatiles emitted but also in what compounds that is released. For example,  $\beta$ -caryophyllene is not released by Delprim but from Pactol (Gouinguené et al., 2001).

When herbivory by *S. littoralis* on maize is stopped, the emissions of the induced plant volatiles drop rapidly already after 10 hours (Turlings et al., 1998). Turlings et al., (1995) showed how the emissions of plant volatiles from damaged maize plants are reduced to almost zero until day 3 after the damage ceased. However, the relaxation of release of plant odours that can be detected by insects is not well studied, (Metlen et al. 2009). In our experiments the damage ceased just the day before the experiments, and it could be that *S. littoralis* can sense some volatile release longer after the damage has ceased in the Pactol variety compared to Delprim. The induced plant volatile emissions from cotton last longer than from maize (Turlings et al., 1995). I believe that *S. littoralis* most probably would be able to do a choice regarding both Delprim and Pactol if there was an ongoing damage on the maize plants. The question is if there is a difference at all between the damaged and undamaged maize plants to the adults in the case of the Delprim at the time for our experiments.

Bishara (1934) wrote that that *S. littoralis* only attack maize sporadically and when there are no other green plants to attack. However, even if *S. littoralis* may have problem to sustain a living on maize, they readily oviposit on maize in our experiments. The reason could be that I performed no-choice experiments and the insects had no alternative. Furthermore, attack of natural enemies can also be important. *S. littoralis* have also been shown to be more often parasitized by *M. rufiventris* in a mixed planting of cotton and maize than in a planting of pure cotton (Shalaby et al., 1988). A high survival rate of *S. littoralis* even after being attacked by *M. rufiventris* (above 60% survive to adults) (Ibrahim, 1987) maybe is not enough to affect *S. littoralis* to change their behaviour to avoid the maize plants.

If I was to repeat the bioassays again I would try to be more careful with using plants that are not affected in some way. Timing the induced defence in the two maize varieties would also be changed, to test the females of *S. littoralis* during the time when the maize is known to release their volatiles. I would choose to use only 1 female per cage to rule out that the females interact with each other or their egg batches.

How can oviposition of *S. littoralis* females be controlled in the field? One way could be to spray plants with larval frass as have been tested in a bioassay (Klein et al., 1990). Another way of trying to disturb the herbivore when they are about to oviposit could be to use genetically modified plants that are more attractive to predators and parasitoids (Degenhardt et al., 2003) or include non-host plants that disturb host attraction (Zhang & Schlyter, 2003, Cook et al., 2007). Furthermore could varieties of different crops that are stronger in their induced defence, such as Pactol, be recommended to farmers.

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