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Can alteration in host odor blends change the olfactory preferences in *Spodoptera littoralis*?

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Can alteration in host odor blends change the olfactory preferences in *Spodoptera littoralis*?

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Abstract

Gustatory and olfactory stimuli have been shown to induce feeding preferences in generalist phytophagous insect species. Generalists have to process a lot of information while performing host selection and it has been suggested that this may be a limitation, while it makes the host detection slower than for specialist species that process a lower amount of information. Experiences from earlier stages may therefore work as a way for generalists to make the processing of information faster (Anderson et al., 2013). Webster et al. (2010) showed, in an experiment where the insect had been exposed to volatiles alone and in combination, that the insect responded stronger to a blend than to single compounds. By changing the blend it may therefore be possible to disturb the host recognition.

In this study the Egyptian cotton leaf worm *Spodoptera littoralis* was used as model species. The aim of the study was to study interactions between olfactory preferences and host odor blends by manipulating the preferences of S. *littoralis* through prior experience, and by artificial manipulation of odor blends. The study was targeted towards a specific set of questions: 1) Will larvae fed on cowpea develop a preference for cowpea when presented two choices? 2) Will larvae exposed to the odor of cotton plants develop a preference for cotton over cowpea? 3) Will larvae exposed to altered forms of cotton plant odor behave like larvae exposed to cotton odor or will the detection mechanism of host detection be disturbed? 4) Will adult female *Spodoptera littoralis* prefer the same plant species as they preferred as larvae? The larvae were tested in a y-tube olfactometer for dual choices and the adults were tested for oviposition preference. This study shows that larvae of *S. littoralis* are able to make a choice when presented two alternatives. A tendency for an induced feeding behavior prior to earlier experiences could be seen. Larvae exposed to odors did not show a preference prior to earlier experiences and the adults did not show any preference in their oviposition choices.

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1 Introduction

Insects and plants experience many close relationships to each other. Some relations are mutualistic, were both parts benefit from the relationship, but there are also many antagonistic relationships where only one part benefits from the other (Schoonhoven et al., 2005). A mutualistic relationship can for example be between a honeybee and a nectar rewarding flower, where the insect gets nectar and the plant gets help with spreading pollen between flowers (Bronstein et al., 2006). Antagonistic relationships can be found for example when an insect feeds on the green parts of a plant (an herbivorous insect species). It is beneficial for the insect to feed on the plant but this will also cause damage to the plant. Insects may use plants for different reasons than feeding. Mating and oviposition sites are two other common behaviors, for which insects use plants.

1.1 Searching for a host

Herbivorous insect species feed on plants and it is therefore of major importance that the insect can make a good choice when selecting a host. But the choice is important not only for the feeding but also the oviposition site. The next generation will be dependent on the plant on which the mother chose to lay her eggs (Carrasco et al., 2015).

Schoonhoven et al. (2005) describe that a typical host-plant selection sequence starts with the insect perceiving plant-derived cues from a distance. The cues can be either visual, like color or shape, or olfactory, or a combination of both. The next step for the insect is to decrease the distance between itself and the source of these cues, by walking or flying. Eventually the plant is found and the insect can make contact with the plant by touching it. The insect can now examine the surface by, for example, palpation of the leaf structure. The plant may be damaged by test biting, probing or puncturing (depending on the insect’s mouthparts). The plant will now be either rejected or accepted as a host by the insect. The insect may also examine several potential hosts before a decision is made (Schoonhoven et al., 2005). This behavior has been described as a direct search. Directed search requires good detection mechanisms and the insect has to be able to process information (cues) from plants to locate the food source (Bruce et al., 2005; Schoonhoven et al., 2005).

Insects can also perform undirected searches (Schoonhoven et al., 2005). An undirected search can be triggered when an insect becomes motivated to search for food. Only internal stored information (like memory) and proprioceptive information (the ability to sense the position, location and orientation) are used. That means no cues from the plants are involved; the insect searches for a food source according to an internal program. An undirected search can change into a directed search if the insect receives information from a plant during a random walk (Schoonhoven et al., 2005).

1.2 Plant volatiles

Plants produce complex odor blends that can interact with other organisms (Bruce and Pickett, 2011). The number of volatiles in the air around plants may run up to several hundred, but the blend is often dominated by a few major compounds (Schoonhoven et al., 2005). The chemical make-up of a plant is not constant and fixed. It may exhibit great temporal variability. Mature plants differ from young ones; it can also vary between seasons and the time of day (Gomez et al., 2003; Gouinguene and Turlings, 2002). There are also other factors that affect the chemical composition of a plant, like soil and light intensity (Bruce and Pickett, 2011; Schoonhoven et al., 2005).

Other organisms can also cause a change in the chemical composition of a plant. Herbivory can induce biosynthesis of several compounds in a plant

(Schoonhoven et al., 2005). The induced changes can affect the herbivore but also the natural enemies of the herbivore. The enemy gets attracted to the plant and attacks the herbivore (Van Poecke, 2001). The changes that occur due to herbivory vary among genotypes as well as plant species. Mechanical damage can also induce biosynthesis in a plant but it often differs from the changes induced by insects (Schoonhoven et al., 2005). The compounds being induced can also differ depending on which insect species that attack the plant (Van Poecke, 2001). The area of the attack is not always the only part of a plant being affected by induced biosynthesis. The plant can respond with systemic induced biosynthesis, which means that damage to one leaf can lead to biosynthesis in other parts of the plant (Ryan, 2000).

As mentioned before, the volatiles released by plants often comprise hundreds of components. The insect will only detect or respond to some of them. The number of compounds that an insect uses for host recognition is normally between 3-10 compounds (Wadhams, 1990). Host-produced volatile compounds that insects can perceive are called kairomones. The insect can perceive kairomones through olfactory receptor neurons (ORNs). Two hypotheses have been proposed to explain the recognition of olfactory cues. One suggests species-specific compound recognition and the other ratio specific recognition (Bruce et al., 2005). Webster et al. (2010) showed in an experiment where the insect had been exposed to volatiles alone and in combination that the insect responded stronger to a blend than to single compounds.

1.3 Generalists and specialists

The degree of feeding specialization of herbivorous insects can differ between species. They can be on a continuum between specialists or generalists. A specialist insect only accepts a few plant species as hosts. Generalist herbivores on the other hand accept a wider range of plant species as hosts and can feed from several plant families (Schoonhoven et al., 2005). Most insects exhibit a high degree of specialization; less than 10% of the insect species known feed on plants from more than three families (Bernays and Graham, 1988).

Specialists need to only process a few plant cues selecting hosts. Generalists on the other hand must process several plant cues since they have a wider range of possible hosts (Bernays, 2001). Bernays, (2001) showed that specialists were able to make better and faster choices than generalists. Anderson et al. (2013) suggest that neural limitations may be more noticeable in generalists and probably represent a problem for fast and accurate choice of hosts, for feeding as well as for oviposition.

1.4 Induction of feeding preference

In 1968 Jermy et al. demonstrated that larvae from the order *Lepidoptera* exhibited a food preference based on earlier food experiences. This phenomenon was called induction of feeding preference. Induction of feeding preference is common in several insect larvae and is not restricted to certain taxonomic groups (Jermy et al., 1968). Other studies have also found induction of feeding; Saxena and Schoonhoven (1978, 1982) found that larvae preferred the diet they had been cultured on. It has been suggested that experiences from earlier life stages could work as a way for generalists to make the processing of information faster while making a host decision (Anderson et al., 2013). Induction of feeding begins early in the larval life and increases in strength with each instar feeding on the inducing plant (Ting et al., 2002).

It seems like it is not only the feeding itself that induce the preference; volatiles may also play an important role. Carlsson et al. (1999) examined if there was an orientation response in larvae of *Spodoptera littoralis* to odors they had experienced before. The odors were either the odor from a previously eaten host plant or a synthetic plant odor present in conjunction with feeding but not incorporated in the food. It was revealed that an increase of orientation response towards an odor is not restricted to the food source itself. A synthetic plant compound present in conjunction with feeding, but not added to the food source, also elicited an increase in orientation response in experienced larvae. Inexperienced larvae of *S. littoralis,* on the other hand, showed low attraction to volatiles from host plants, whereas larvae that had experienced a host plant showed strong attraction to volatiles for this plant (Carlsson et al., 1999).

It has also been shown that adult insects can be affected by previous experiences. Anderson et al. (2013) and Thöming et al. (2013) showed that reproductive behavior of adult *S. littoralis* was influenced by experiences from their larval stages. Females oviposited more often on plant species they had been feeding on as larvae. The males also showed a change in the reproductive behavior due to earlier experiences. They chose to fly towards sex pheromones with a background of plant odor from the plant they had been reared on as larvae. By selecting a female on the same plant species as the one where he grew up, the male can control the quality of the food for his offspring (Anderson et al., 2013; Thöming et al. 2013).

1.5 Spodoptera littoralis

Spodoptera littoralis is a moth belonging to the family *Noctuidae*. The moth is a great generalist and has been found to feed on at least 84 plant species within 40 different families (Holloway, 1989). It is considered as a major pest species and can destroy several hectares of important agricultural crops since it can have several generations per season and reach very high population densities. *Spodoptera littoralis* can be found in Africa, the Mediterranean region and the Middle East (Brown and Dewhurst, 1975).

1.6 Objectives

Earlier studies have shown that feeding preferences can be induced by previous feeding experiences (Carlsson et al., 1999; Saxena and Schoonhoven, 1978, 1982). Both gustatory and olfactory stimuli seem to be of importance but Carlsson et al. (1999) showed that odor exposure itself can also trigger a preference. This study will investigate further the importance of gustatory and olfactory stimuli for preference induction by aiming to answer the following questions:

1. Will larvae fed on cowpea develop a preference for cowpea when presented with two choices?

2. Will larvae exposed to the odor of cotton plants develop a preference for cotton over cowpea?

3. Will larvae exposed to altered forms of cotton plant odor behave like larvae exposed to cotton odor or will the detection mechanism of host detection be disturbed? Three alterations will be used; cotton plants together with maize plants, cotton together with phenylacetaldehyde and a synthetic cotton blend.

4. Will adult female *S. littoralis* prefer the same plant species that they preferred as larvae?

2 Materials and methods

2.1 Plants

Plant species used in the study were cowpea (*Vigna unguiculata*), cotton (*Gossypium hirsutum*) and maize (*Zea mays*). They were grown from seeds at SLU, Alnarp in 1,5L round plastic pots (diameter 15cm). The plants were grown in the biotron at SLU, Alnarp. The temperature was 22 °C with a day length of 16h and the relative humidity was 75%. The plants were treated with a half spoon of nematodes mixed with 1 liter water right after seeding to prevent sciarid flies. The plants were grown for approximately four weeks before exposure to insects began.

2.2 Insect rearing

The insect species used in the study was *S. littoralis* (Egyptian cotton leaf worm). The insects were reared at SLU, Alnarp in a controlled climate chamber at 25°C ± 2°C with 65 % ± 2 % relative humidity and with a 17h day length until they reached 2nd larval instar. The larvae were fed with artificial diet during all larval instars (table 1). Potatoes were sliced and mashed in a mixer. Oil, vitamin-E and ethanol were added and after that the yeast mixture (wheat germ, dried yeast flakes, methyl-4-hydroxybenzoate, sorbic acid, ascorbic acid and cholesterol). Agar powder was put in boiling water. Agar and potato-yeast-mixture were combined and stirred. When the temperature was approximately 50-60 degrees the vitamin mixture and the sodium benzoate were added. The mixture was put in candy boxes that were left to cool down for 2 hours and later put in the freezer for longer storage.

Table 1. *Content of the artificial diet for Spodoptera littoralis*

Wheat germ	400g
Dried yeast flakes	240g
Methyl-4-hydroxybenzoate	20g
Sorbic acid	22g
Ascorbic acid	22g
Cholesterol	8g
Vitamin mixture	24.4g
Sodium benzoate	6.4g
Distilled water	3.6L
Plant agar (powder)	65g
DL-alpha-Tocopherol acetate (vit. E)	4ml
Oil	10ml
96% ethanol	100ml
Peeled potatoes	1700g

2.3 Experiment setup

2.3.1 Olfactometer setup

Dual choice test in a Y-tube olfactometer was used to test odor preferences of the larvae (fig. 1). Air was pushed by means of a pump through wash bottles (activated charcoal for filtering followed by water for moistening of the air). The air through the wash bottles was flowing into cooking bags, where odor sources were placed. From the cooking bags the air was lead into the Y-tube through an air flow meter that controlled the air flow that went out in the Y-tube. The air flow used was 0.5L/min through each of the arms of the olfactometer. One larva at a time was placed in the Y-tube; if the larva didn't start moving after a while it was gently touched with a brush. The larvae had ten minutes to make a choice. If the decision time took longer it was counted as a non-choice. The odor delivery tubes were regularly switched from one arm to the other to ensure that no position effects influenced the results.

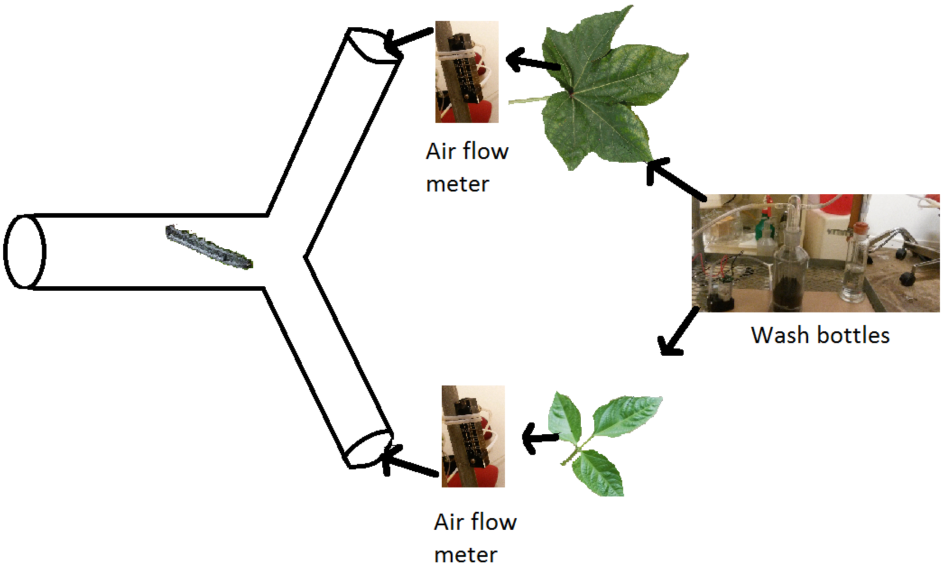

Figure 1. *Schematic illustration over the setup of the dual choice tests in y-tube olfactometer; here an example with cotton and cowpea odors.*

2.3.2 Oviposition experiment setup

When the females were between 2-4 days old they were mated in single pairs. When female and male were *in copula* they were transferred to plastic cages (BugDorms) 30x30x30cm. One cotton leaf and one cowpea leaf were placed in each cage, in plastic tubes filled with water. The tubes were placed diagonally in the corners (fig. 2). The leaves were selected to be similar in size. The couple was provided honey mixed with water as a food source. They were kept in the BugDorm for two days.

After two days the leaves were checked for egg batches that were counted, scratched off the leaves with a cut filter paper and weighed. Egg batches on walls and floor closer than one cm to a leaf were counted as a choice for this leaf and were scratched off the wall and weighed along with the rest.

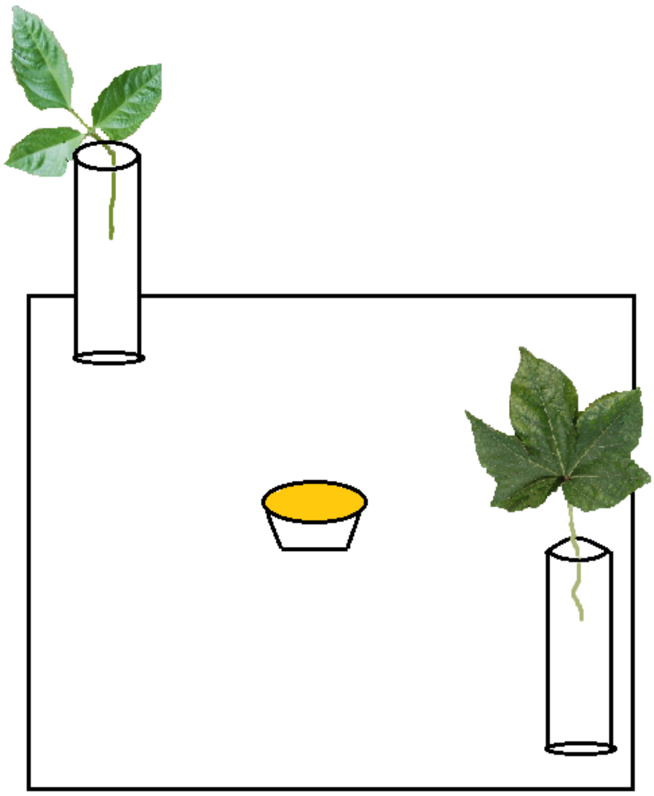

Figure 2. Schematic illustration over the oviposition experiment setup. The plants were placed diagonally from each other and a honey water mixture was provided as food source. The mated coupled stayed in the cage for two days before egg batches were collected.

2.4 Experimental conditioning

2.4.1 Will larvae fed on cowpea develop a preference for cowpea when presented two choices?

2.4.1.1 Treatment group artificial diet

Approximately 60 2^{nd} instar larvae were put in plastic candy boxes with a lid that had been modified with mesh to allow air to go into the box. The larvae were kept in a controlled climate chamber at 25°C ± 2°C with 65 % ± 2 % relative humidity and with a 17h day length. The larvae were fed with artificial diet (recipe in table 1).

When the larvae had reached 3rd to 4th larval instar they were tested for preference toward artificial diet over fresh air to see if the larvae where able to make a choice in the y-tube olfactometer.

2.4.1.2 Treatment group cowpea

The larvae had since 2nd instar been fed with cowpea. The larvae were kept in a controlled climate chamber (25°C ± 2°C with 65 % ± 2 % relative humidity and with a 17h day length) during their whole life development.

When the larvae had reached 3rd to 4th larval instar they were tested in the y-tube olfactometer for preference toward cowpea and fresh air in a y-tube olfactometer. A control group fed with artificial diet was tested simultaneously as the larvae fed on cowpea. Due to limited rearing possibilities only a small number of individuals could be tested.

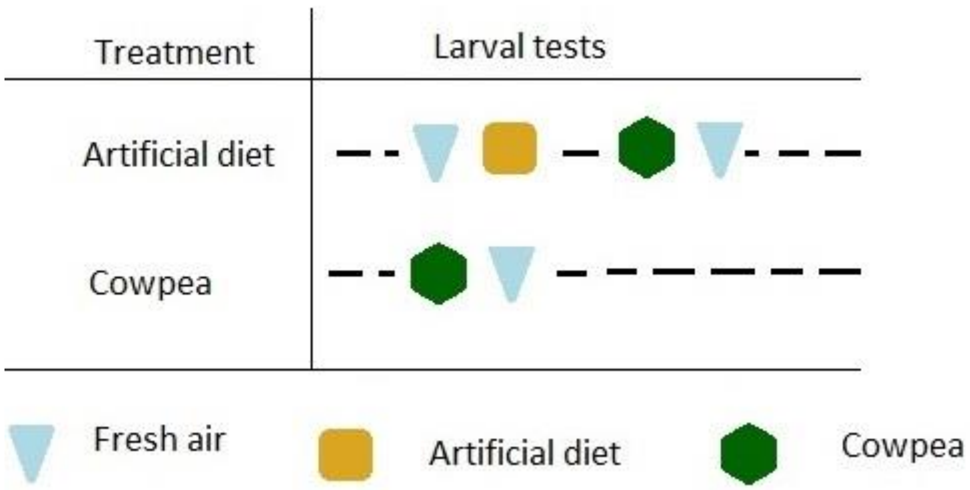

Figure 1. Schematic illustration over the treatment groups and tests performed for previously feeding experienced larvae. Larvae previously fed with artificial diet were tested for preference between fresh air and artificial diet and also functioned as a control group for previously cowpea fed larvae (test cowpea vs. fresh air). Larvae previously fed with cowpea were tested for preference between cowpea and fresh air.

2.4.2 Will larvae exposed to the odor of cotton plants develop a preference for cotton over cowpea?

2nd instars larvae were put into plastic candy boxes where the sides had been cut off and mesh metal was glued on the sides to secure odor exposure into the cage. This was repeated four times with 1 to 1.5 weeks between each new batch of larvae. 60 larvae were put into each box, except from the fourth time when 80 larvae were put in each box due to mortality and individuals escaping in previous rearing.

Three cotton plants grown for approximately four weeks were put close to the larval cage. The plants and the larval cage were put in a bigger cage to enclose them from the surrounding. Before 2nd instar the larvae had no previous experience of the odors tested.

In the olfactometer the larva was exposed to cowpea in one arm of the y-tube and cotton in the other to see if preference had develop for cotton which had been exposed to the larvae. The plants were enclosed in cooking bags that were tightly closed to minimize air escape and ensure airflow to go through the y-tube. The larvae were tested simultaneous with a control group fed on artificial diet. The control group were kept in a different chamber, (25°C ± 2°C with 65 % ± 2 % relative humidity and with a 17h day length), to secure that they were not exposed to any plant odors.

2.4.3 Will larvae exposed to altered forms of cotton plant odor behave like larvae exposed to cotton odor or will the detection mechanism of host detection bee disturbed?

Usually, 60 larvae were put into each box, but the fourth time 80 larvae were put in each box due to mortality and individuals escaping in previous rearing, with 1 to 1.5 weeks between each new batch of larvae. Before 2nd

instar the larvae had no previous experience of the odors tested treatment groups.

After 2nd instar the larvae were moved from their previous climate chamber (25°C ± 2°C with 65 % ± 2 % relative humidity and with a 17h day length) to a new one with 25°C, 70% relative humidity and with a 12h day length. The control group, which was not exposed to additional odors and was fed on artificial diet, was left in the first chamber (25°C ± 2°C with 65 % ± 2 % relative humidity and with a 17h day length).

2.4.3.1 Treatment group cotton maize

2nd instars larvae were put into plastic candy boxes were the sides had been cut off and glued with mesh metal to secure odor exposure into the cage. Three cotton plants and three maize plants functioned as odor exposure. The cotton odor had here been altered with the presence of maize. After 2nd instar the larvae were moved from their previous climate chamber (25°C ± 2°C with 65 % ± 2 % relative humidity and with a 17h day length) to a new one with 25°C, 70% relative humidity and with a 12h day length.

In the olfactometer the larvae were tested for preference toward cowpea or cotton but also for preference toward cotton or cotton and maize together (fig. 5). The plants were enclosed in cooking bags. Larvae fed on artificial diet functioned as a control group. The control group was kept in a different chamber (25°C ± 2°C with 65 % ± 2 % relative humidity and with a 17h day length). The groups were tested simultaneous.

2.4.3.2 Treatment group cotton phenylacetaldehyde

2nd instars larvae were put into plastic candy boxes were the sides had been cut off and glued with mesh metal to secure odor exposure into the cage. Three cotton plants together with phenylacetaldehyde functioned as the odor treatment. *Spodoptera littoralis* expresses the olfactory receptor slitOR14, which has shown a high response to phenylacetaldehyde, on the antenna (W. Walker, M. Larsson, F. Schlyter, P Anderson unpublished data), which is why it was interesting to test if the phenylacetaldehyde would disrupt the perception of cotton plant. After 2nd instar the larvae were moved from their previous climate chamber (25°C ± 2°C with 65 % ± 2 % relative humidity and with a 17h day length) to a new one with 25°C, 70% relative humidity and with a 12h day length.

Dispensers with phenylacetaldehyde were put in the pots of the cotton plants (fig. 4). 10µl phenylacetaldehyde (1 µg/ µl) was added to 4 ml octane. The release rate for the phenylacetaldehyde was 336ng/h.

The dispensers were made out of 4 ml vials. The lid was drilled so that a cotton wick in a plastic tube could be fitted in. The wick soaked up the solution and released it to the surroundings. Octane was used as a solvent. Before solvent and the compounds were added to the dispensers the dispensers were washed out with 2ml of pentane. The cotton plants with phenylacetaldehyde and the box with larvae were enclosed in a bigger cage.

The larvae were tested in the olfactometer for preference towards cotton over cowpea and in additional tests preference for cotton over cotton and phenylacetaldehyde (fig. 5). Larvae fed on artificial diet functioned as a control group and was tested simultaneous with the cotton phenylacetaldehyde group. The control group was kept in a different chamber (25°C ± 2°C with 65 % ± 2 % relative humidity and with a 17h day length). Fig. 4 shows the set up with the larvae and the plants together with phenylacetaldehyde in the bigger cage.

Figure 4. Larvae were put in candy boxes with metal mesh on the sides and on the lid. Three cotton plants with dispensers with phenylacetaldehyde dissolved in octane were in closed in a bigger cage. One dispenser with phenylacetaldehyde was placed in the pot of each plant.

2.4.3.3 Treatment group cotton synthetic blend

2nd instars larvae were put into plastic candy boxes which solid sides but with a lid with a whole covered with plastic mesh. The larvae were after 2nd instar moved to a new climate chamber to a new one with 25°C, 70% relative humidity and with a 12h day length from their previous climate chamber (25°C ± 2°C with 65 % ± 2 % relative humidity and with a 17h day length).

A synthetic cotton blend was mixed based on blend 1 reported from (Borrero-Echeverry et al., 2015). The solvent used was octane. The dispensers used for release were 4 ml vials designed the same way as the dispensers for phenylacetaldehyde release. Table 2 shows the content of the synthetic cotton blend. The dispenser was put in the candy box with the larvae. A “fence” in the form of a plastic mesh protected the dispenser from larvae touching it. The box with larvae and the synthetic cotton blend was not placed in a bigger cage.

The larvae were tested in the olfactometer for preference for cotton over cowpea and also for preference toward cotton over the synthetic cotton blend (fig. 5). A control group reared on artificial diet in a climate chamber with 25°C ± 2°C with 65 % ± 2 % relative humidity and with a 17h day length was tested simultaneous.

Table 2. *Content of the synthetic cotton blend used as odor exposure treatment*

	release rate ng/h	µl in 4 ml octane	C, store solution
DMNT	30	9.6	1µg/µl
Benzaldehyde	20	6.4	1µg/µl
β-caryophyllene	15	4.8	1µg/µl
β-myrcene	14	4.5	1µg/µl
Nonanal	11	3.5	1µg/µl
Z-3-hexenylacetate	11	3.5	1µg/µl
E-ocimene	8	2.6	1µg/µl
R(+)-Limonene	4	12.8	100ng/µl
α-humulene	4	1.3	1µg/µl
Decanal	1.5	0.5	1µg/µl
R(-)-Linalool	0.5	16	10ng/µl

2.4.4 Will adult female *Spodoptera littoralis* prefer the same plant species as they preferred as larvae?

Larvae in treatment groups described in section 2.4.2-2.4.3 were raised until adults. They were exposed to the odors until they reached pupal stage. The pupae were sexed and females and males were kept in different climate chambers with a temperature of +25 degrees. The day length was 16h and the relative humidity was 60% which differed from their larval conditions (25°C with 70% relative humidity and with a 12h day length). Adults reared on artificial diet functioned as a control group.

When the females were between 2-4 days old they were mated in single pairs. When female and male were *in copula* they were transferred to plastic cages (BugDorms) 30x30x30cm. One cotton leaf and one cowpea leaf were placed in each cage, in plastic tubes filled with water.

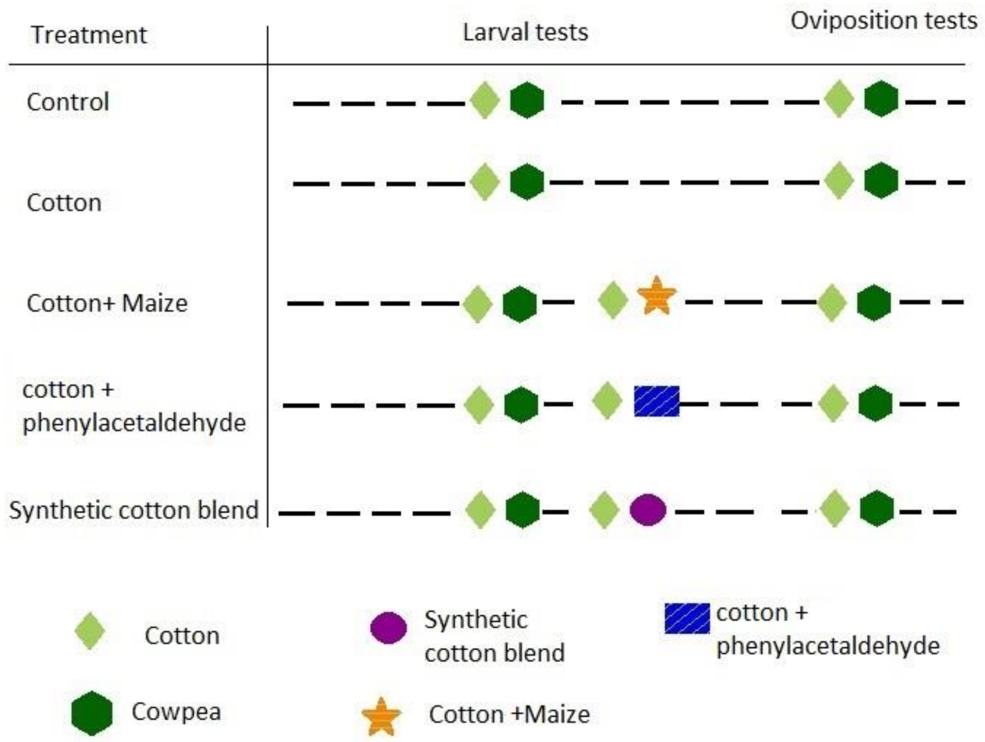

Figure 5. Schematic illustration over the tests made for the treatment groups with odor exposure. Both larval tests in Y-tube olfactometer and oviposition tests were performed.

2.5 Statistics

The statistical software R was used for all the statistical analyses.
Larval preference was analyzed with chi-square test to see if the proportion of larval choice differed from 50%, which was the expected value for no preference. A GLM model was used to determine if the treatments differed from each other.

Oviposition preference was calculated as an index (egg weight plant A - egg weight plant B)/total weight of eggs on plants (A+B). A GLM model was used to determine if the treatments differed from each other.

3 Results

3.1 *Will larvae fed on cowpea develop a preference for cowpea when presented two choices?*

Larvae fed on artificial diet showed a significant preference for artificial diet in the choice between artificial diet and fresh air, with 87 percent of the larvae tested choosing the artificial diet over the fresh air ($p<0.002$, Fig. 6). The ability of the larvae to make a directed choice shows that the y-tube olfactometer correctly functioned in the way it allowed the larvae to make choices based on the information perceived.

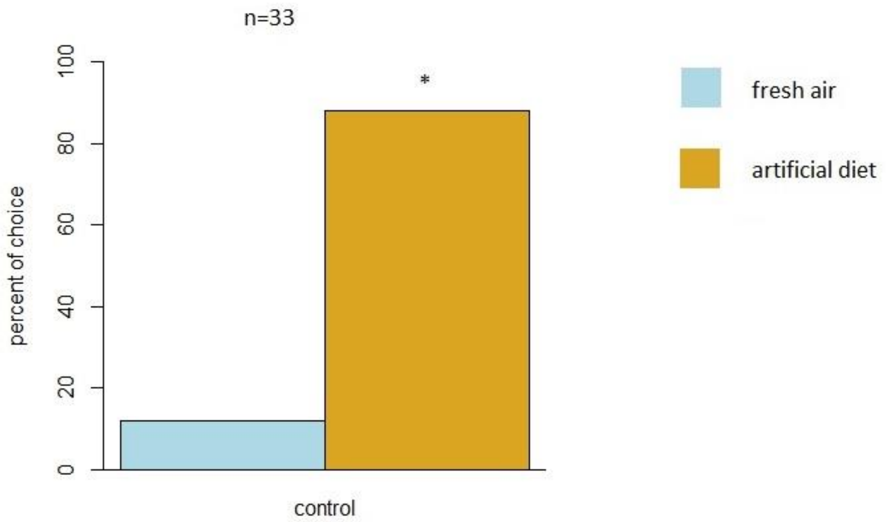

*Figure 2. Percent choice of artificial diet from larvae previously fed with artificial diet, in dual-choice test choosing between artificial diet and fresh air. *=p< 0.002, chi-square test.*

Larvae fed on cowpea did not show a statistically significant preference towards cowpea. Due to limitations in rearing possibilities for both plants and insects only a small number of insects were tested (10 larvae in each group), which likely contributed to the lack of statistically significant results. 80% of the larvae fed on cowpea chose cowpea over fresh air, indicating a strong tendency for preference towards cowpea over fresh air even though no significance can be shown. The larvae with no previous experience of cowpea (control group in fig. 7) also showed a tendency toward cowpea. This tendency is, however, not as strong as for the larvae with previous experience of cowpea.

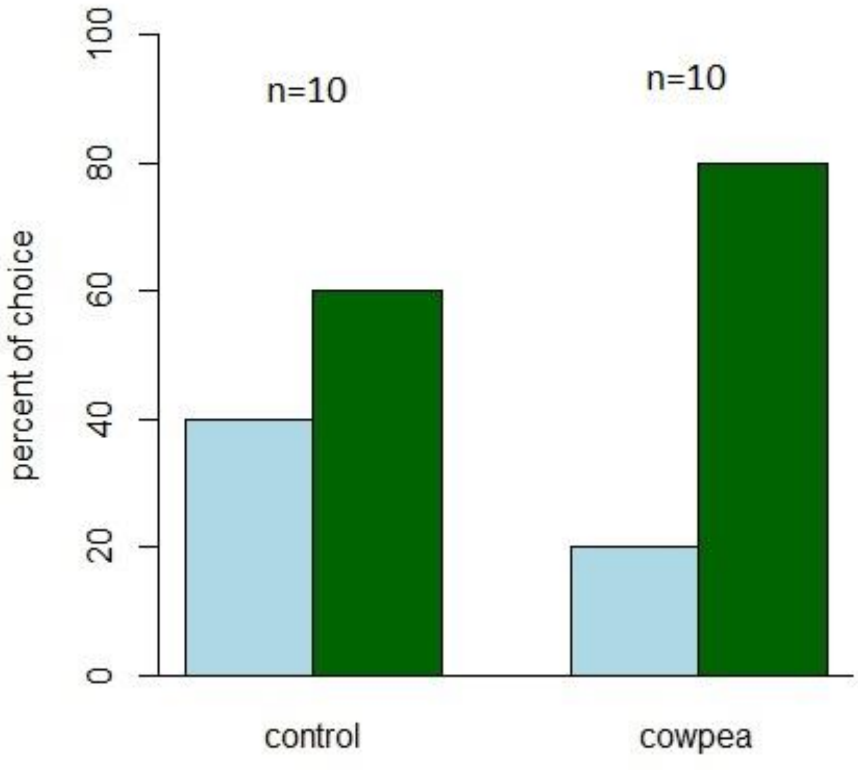

Figure 3. Percent of choice for larvae fed on cowpea and larvae from control group fed on artificial diet, in dual-choice test choosing between cowpea and fresh air. None of the groups differ in their choice from 50 % but larvae with previous experience of cowpea showed a strong trend toward choice for cowpea (chi-square tests, control; p=0.527, cowpea; p= 0.0578).

3.2 Induction by odor stimuli

3.2.1 Will larvae exposed to the odor of cotton plants develop a preference for cotton over cowpea?

Larvae previously experienced with cotton odor did not show a significant preference for either cowpea or cotton. A higher number of larvae chose cowpea over cotton but more larvae would need to be tested to conclude a trend. The control group with no previous plant odor experienced showed an almost identical trend indicating that the trend is not affected by the presence of cotton in earlier experiences (fig. 8).

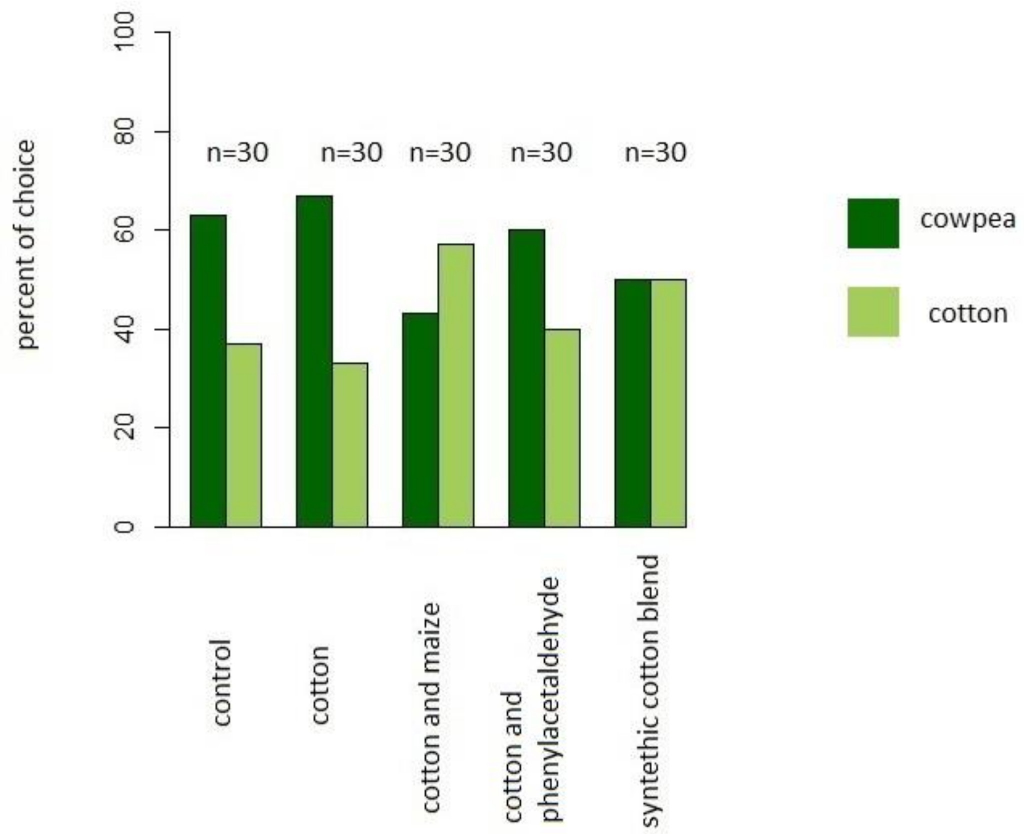

Figure 4. Percent of larvae in dual-choice test choosing between cotton and cowpea. The larvae had previous experience of odor exposure (cotton, cotton and maize, cotton and phenylacetaldehyde or synthetic cotton blend). Control larvae had no previous experience of odor exposure. No significant differences could be seen between the groups (GLM, Tukey's test, p>0.374) and none of the groups differed in their choice from 50% (chi-square tests, p>0.0679)

3.2.2 Will larvae exposed to altered forms of cotton plant odor behave like larvae exposed to cotton odor or will the detection mechanism of host detection be disturbed?

3.2.2.1 Treatment group Cotton and maize

Larvae tested for preference toward cotton or cowpea did not show any significant preference toward either of the plants. More individuals chose cotton over cowpea but more individuals would be needed to show a trend.

Larvae exposed to cotton and maize were tested in y-tube olfactometer for preference toward cotton or cotton and maize. Control larvae used were larvae with no previous odor exposure. The two groups did not show any trend towards differing from each other in preference (fig. 9).

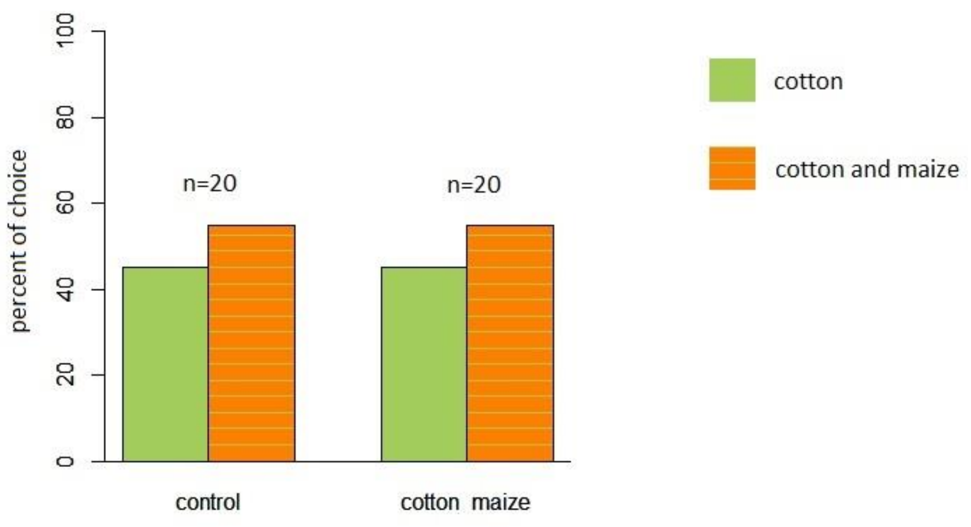

Figure 5. Percent of larval choice in dual choice tests with larvae previously exposed to cotton and maize and a control group with no previous odor exposure. Larvae chose between cotton and cotton and maize. None of the groups differed in their choice from 50% (chi-square tests, p=0.655 for both groups).

3.2.2.2 Treatment group Cotton and phenylacetaldehyde

Larvae treated with cotton and phenylacetaldehyde did not show a significant preference for cotton or for cotton and phenylacetaldehyde. A tendency can however be seen toward choices for cotton over cotton and phenylacetaldehyde in both the control group and in the treatment group (fig. 10). The tendency is slightly stronger in the control group.

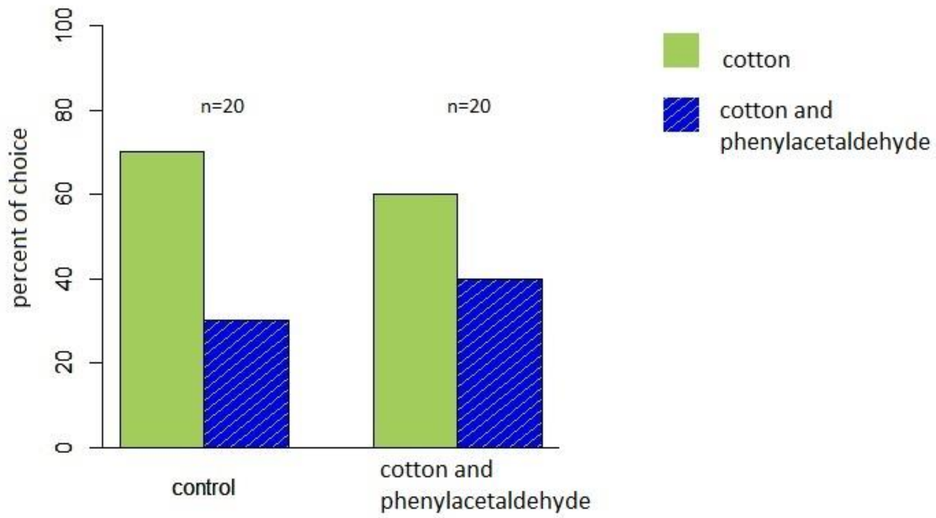

Figure 6. Percent of choice of larvae previously exposed to cotton and phenylacetaldehyde and control group with no previous odor exposure, in dual choice test choosing between cotton and cotton+ phenylacetaldehyde. No significant preference for a choice could be seen (chi-square tests, p>0.0736).

3.2.2.3 Synthetic cotton blend

Larvae previously exposed to the synthetic cotton blend did not show any preference for the previous odor exposure. The control group also did not significantly favor one odor over the other. Some tendencies can be seen in the result however. The control group tended to choose cotton more often than the larvae previously experienced to the synthetic blend but more individuals would be needed to see if the trend is stronger (see fig. 11).

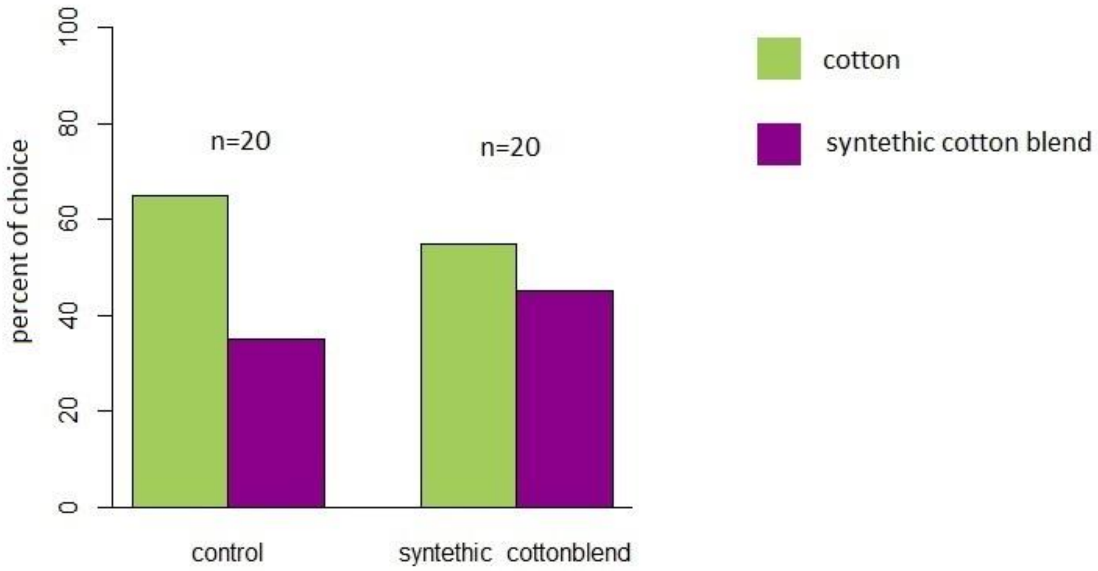

Figure 7. Percent of choice of larvae previously exposed to synthetic cotton blend and control larvae with no previous odor exposure, in dual choice test choosing between cotton and cotton synthetic blend. The groups did not differ in their choice from 50% (chi-square tests, p>0.180).

3.3 Will adult female Spodoptera littoralis prefer the same plant species as they did as larvae?

Females were tested for their oviposition preferences for cowpea and cotton plants. Due to mortality and no-choice replicates, with egg masses placed only on the walls of the oviposition cages, the replicate numbers were unfortunately low. The overall tendency can be seen for preference toward cowpea over cotton but no significant preference could be measured for any individual treatment group (fig. 12). The control larvae show the greatest tendency toward preference for cowpea and the females from treatment group with cotton and phenylacetaldehyde showed the least preference toward cowpea in the groups.

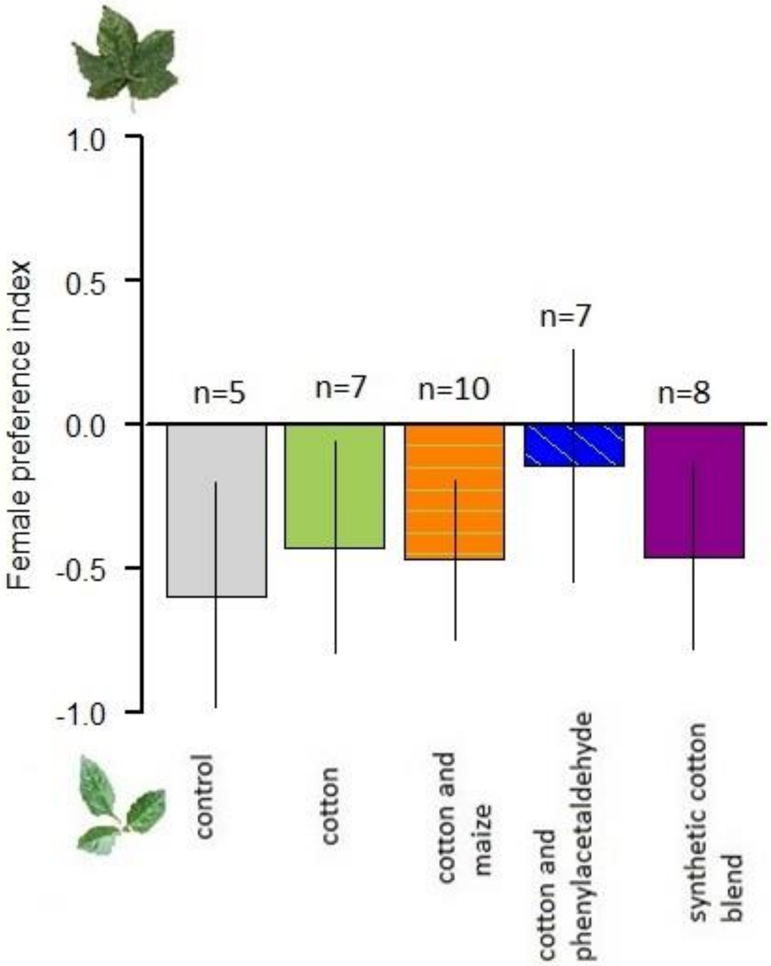

Figure 8. Choices of females with larval experience of odor exposure (cotton, cotton and maize, cotton and phenylacetaldehyde and synthetic cotton blend). Control group had no previous experience of odor exposure. Index scale varying between 1.0 (full preference for cotton) and -1 (full preference for cowpea). The statistical test did not show any significant differences between the groups (GLM, Tukey's test, p>0.906) neither did the individual groups differ in their choices between cotton and cowpea (Wilcoxon tests, p>0.258).

4 Discussion

Induction feeding can begin early in the larval life. With each instar while the larvae are feeding on the inducing plant the induction gets stronger (Ting et al., 2002). Experiences from earlier stages may work as a way for generalists to make a faster processing of information while making a host decision. It has been shown that generalists have a host preference hierarchy among their hostplants. This hierarchy can be changed by earlier experiences in both larvae and adult insects (Thominget al 2013). Several studies have shown that experienced larvae show preference toward their host plant (Carlsson et al., 1999); (Saxena and Schoonhoven, 1978, 1982).

4.1 Will larvae fed on cowpea develop a preference for cowpea when presented two choices?

This study did not significantly show that larvae fed on cowpea developed a preference for cowpea. However, only a small number of individuals could be tested in this study due to rearing opportunities and mortality rates but 80% of the larvae tested chose cowpea over fresh air. This indicates that larvae fed on cowpea do prefer cowpea over fresh air. In the test used, the larvae were allowed to choose between their previous host and fresh air and it would be interesting to also give a choice of two plants where one of them is the previous host. In the control group fewer larvae chose cowpea over fresh air, indicating that they did not connect the plant odor with a food source, but also here the result was not significant. This is in contrast with Khalifa et al. (1973) that found that inexperienced larvae were attracted to plant odors. However other studies support that inexperienced larvae show no or only a weak attraction to plant odors (Carlsson 1999). This study supports that larvae show a low preference toward plant odors when they have not previously had experience with them. Larvae fed on artificial diet showed a strong preference for artificial diet, indicating that the larvae were able to make a choice when presented with two options.

4.2 Will larvae exposed to the odor of cotton plants develop a preference for cotton over cowpea?

The larvae exposed to cotton did not show any significant preference for cotton over cowpea. More larvae chose cowpea over cotton but the number of larvae is too small to conclude that they really did prefer cowpea over cotton. This shows that the larvae did not develop a preference for cotton by being exposed to the odors. Other studies have shown that such an induction is possible (Carlsson et al. 1999). The plants used in the experiment may have suffered from shading as the lighting in the controlled climate chamber was not optimal. Light quality can have strong effect on the release of plant volatile compounds and their role in biotic interaction and shading can negatively affect the amount of volatile compounds that are emitted (Kegge et al., 2013). This may have been one contributing factor for the lack of induced host preference in this experiment.

4.3 Will larvae exposed to altered forms of cotton plant odor behave like larvae exposed to cotton odor or will the detection mechanism of host detection be disturbed?

Both olfaction and gustation have been seen to mediate important information for the larvae to discriminate food plants (Hanson and Dethier, 1973; de Boer and Hanson, 1987). Odor alone has also been seen to be enough to induce a preference (Carlsson et al., 1999).

The larvae that were exposed to altered forms of cotton did not discriminate between cotton and cowpea. Neither did they discriminate between their previously experienced altered odor and cotton plants. If the larvae exposed to cotton plants actually would have preferred cotton it could be argued that the larvae exposed to altered forms of cotton odor were not able to detect cotton from cowpea because of lack of previous experience. The larvae would then probably also have been able to discriminate between cotton and altered forms of cotton.

The larvae with previous experience with phenylacetaldehyde and the larvae previously experienced with the synthetic cotton blend chose more often their previous odors compared with their control groups. The octane as solvent may have contributed to an avoidance behavior of the larvae. The larvae that had previous experience with octane may have been better adapted to it.

4.4 Will adult female Spodoptera littoralis prefer the same plant species as they preferred as larvae?

The mechanism behind the transferring of preference during metamorphis has been debated. Two mechanisms have been proposed, the first one is that the female is exposed to chemicals from the larval environment (postimaginal conditioning) and the other one is retention of memory through metamorphosis into adulthood (preimaginal conditioning) (Barron, 2001). In both the fly *Drosophila melanogaster* and in the moth *Manduca sexta* learning through metamorphosis have been demonstrated (Tully et al., 1994; Blackiston et al., 2008).

Jaenike (1978) proposed the preference-performance hypothesis (also referred to as the “mother-knows-best” hypothesis). The hypothesis proposes that oviposition preference should correspond with host suitability for offspring development. The female thus maximize the fitness of their offspring by ovipositing at the most appropriate host sites. Recent studies have, however, shown that the mother doesn’t always know the best (Rizvi et al., 2016). The decision of the female in the choice of a plant could be based on reasons favoring her performance rather than that of her offspring (Valladares and Lawton, 1991).

In that case the female fails to make the seemingly optimal choice and oviposits on a plant that will not be optimal for the growth and development of her offspring. Reproduction in several plant-feeding taxa has been demonstrated to be closely linked to feeding preferences during larval stages (Awmack and Leather, 2002; Anderson et al., 2013; Akhtar and Isman, 2003).

In this study no significant preference could be seen for the female´s choice of oviposition site. If it would have been a clear tendency toward preference for cowpea it could be argued that there had been no induction of feeding preference and that the adult females have an innate preference for cowpea which would have been in consistence with (Thöming et al., 2013) study that showed that inexperienced adults prefer cowpea over cotton. It may be possible that with more individuals a stronger trend toward cowpea could be seen but further investigations would be needed to assure that.

5 Conclusions

This study shows that larvae from Spodoptera are able to make a host choice when presented two alternative hosts. Previous host experience has been found to impact the choice the larvae will make when the larvae were allowed to feed on the source.

Larvae fed on artificial diet showed a clear significance when choosing between artificial diet and fresh air, showing that the larvae in fact were able to make a choice in the olfactometer. Larvae fed on plant did show a strong trend toward their previous host and with more individuals that could be tested it is possible that a significance host choice could be seen.

The larvae exposed to only odors did not show a preference toward their previous host and did not discriminate between cotton and altered forms of cotton odor. The larvae were able to make a choice in the olfactometer (shown by the significant choice made by larvae tested for preference toward artificial diet and fresh air and larvae fed on cowpea showing a strong trend) which leads to the conclusion that larvae treated with only odor exposure were not affected by the odors exposed to them. It cannot be concluded that the larvae perceived the added plant odors or connected them with something positive. Neither did they connect them with something negative as no avoidance choice could be seen. The odor from the artificial food given may have had too strong impact on their feeding preferences to be changed by additional odor stimulation.

The larvae that were only exposed to odors did not develop a host preference and neither did they show any preference as adult females. It would however, have been interesting to see if the larvae fed on plant (cowpea) would show the same trend toward preference for cowpea as adult females but due to low number of individuals that could not be tested.

In conclusion the larvae with only odor exposure were uninfluenced by their stimuli and showed no significant preference for their previous experienced odor. Larvae that were not only exposed but also allowed to feed on a plant showed however, a strong trend toward their previous host. The adult females did not prefer one plant over the other. The overall trend is pointing toward preference for cowpea but more individuals would be needed to confirm such a trend.

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