Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

– kunskap för en hållbar utveckling

Feeding Preferences and Foraging in Larvae of *Manduca sexta* and *Spodoptera littoralis*

- a laboratory study based on olfaction

Elin Nyström

Självständigt arbete vid LTJ- fakulteten, SLU

Hortonomprogrammet, Alnarp 2013

Faculty of Landscape Planning, Horticulture and Agricultural Sciences

Feeding Preferences and Foraging in Larvae of *Manduca sexta* and *Spodoptera littoralis*- a laboratory study based on olfaction

Födopreferenser och sökande av föda hos larver av *Manduca sexta* och *Spodoptera littoralis*- en laboratoriestudie baserad på olfaktion

Elin Nyström

Handledare (Supervisor): Docent Anna Balkenius and Professor Peter Anderson, Chemical Ecology, Department of Plant Protection Biology SLU

Examinator (Examiner): Docent Mattias Larsson, Chemical Ecology, Department of Plant Protection Biology SLU

Credits: 15hp (ECTS)

Level: G2E

Course title: Kandidatarbete i Biologi/Horticultural Science Programme

Institute: Institute of Biosystems and Technology

Course code: EX0493

Programme/education: Hortonomprogrammet/Horticultural Science Programme

Place of publication: Alnarp

Year of publication: 2013

Title of series, no: Självständigt arbete vid LTJ- fakulteten, SLU/Independenet Project at Faculty of Landscape Planning, Horticulture and Agricultural Sciences, SLU

Online publication: http://stud.epsilon.slu.se

Keywords: *Manduca sexta, Spodoptera littoralis,* larvae, foraging, olfaction, larval dual choice test, induced feeding preference

1. Abstract

Larvae of Lepidopteran *Manduca sexta* and *Spodoptera littoralis* are pests of economical importance in several horticultural and agricultural crops. Knowledge of insect feeding preferences and behavior is crucial in order to find economically and ecologically sustainable solutions to the problems caused by these herbivores.

The aim of this thesis was to study the behavior and the induced feeding preferences in larvae of *M. sexta* and *S. littoralis* when reared on certain host plants. The hypothesis was that larvae of *M. sexta* would have an innate preference for plants of the Solanaceae family and therefore discriminate the previously experienced non-host when faced with the choice between a true host and the previous food source. *S. littoralis* where hypothesized to prefer the plants on which they previously have been feeding of.

Larval dual choice tests were made in an olfactometer, testing the olfactory-based preference of larvae of *M. sexta,* reared on foliage of oilseed rape, tomato, potato or synthetic diet, and the olfactory- based preference of *S. littoralis,* reared on foliage of cowpea, cotton, cabbage or synthetic diet. Among the larvae of *M. sexta*, reared on foliage of potato, there was significant attraction to the prior experienced host while the larvae of *S. littoralis* preferred cabbage in all experiments, regardless of prior host. *M. sexta* reared on oilseed rape did not survive long enough to be tested.

2. Sammanfattning

Larver av *Manduca sexta* och *Spodoptera littoralis*, tillhörande ordningen Lepidoptera, är skadegörare av ekonomisk betydelse i flera trädgårds- och jordbruksgrödor. Kunskap om insekters födopreferenser och beteende är avgörande i sökandet efter ekonomiskt och ekologiskt hållbara lösningar på de problem som orsakas av dessa växtätare.

Syftet med detta arbete var att studera beteende och inducerad födopreferens hos larver av *M. sexta* och *S. littoralis,* uppfödda på vissa värdväxter. Hypotesen var att larver av *M. sexta* skulle ha en medfödd preferens för växter av familjen Solanaceae och därför diskriminera värden den blivit uppfödd på när den ställs inför valet mellan en ”sann värd” och tidigare näringskälla, om den tidigare näringskällan är en icke-optimal värd. Hypotesen för *S. littoralis* var att de skulle föredra de växter som de blivit uppfödda på när de ställs inför ett val av värdväxt.

Två- valsförsök med larver utfördes i en olfactometer. Larver av *M. sexta*, uppfödda på blad av raps, tomat, potatis eller syntetisk diet, fick i testet göra ett val mellan värdväxter baserat på dofter. Detsamma gällde larverna av *S. littoralis*, uppfödda på blad av vignaböna, bomull, kål eller syntetisk diet. Bland de larver av *M. sexta*, som var uppfödda på blad av potatis, föredrog ett signifikant antal den tidigare förtärda värden medan larver av *S. littoralis* i alla experiment valde kål, oavsett tidigare värd. *M. sexta* uppfödda på raps överlevde inte länge nog för att testas i olfaktometern.

3. Table of Contents

4. Introduction

4.1. Insect-host plant relationship

Depending on their host ranges, phytophagous insects can be separated into different categories: monophagous, oligophagous or polyphagous. Monophagous insects feed on one or a few plant species within a single genus while oligophagous insects feed on a number of plants within one family, or a small number of species from different families. Insects feeding on a large number of plants, from different families, are considered as polyphagous herbivores (Bernays & Chapman, 1994).

Most phytophagous insects are specialists with a strong feeding preference and a narrow host specificity. As some of these insects specialize on crops of agricultural importance, they cause economic consequences; both in decreased crop yield and increased costs in order to control the insects (Ting et al., 2002).

The impact of phytophagous insects, of both economical and environmental importance, indicates the need of understanding the underlying mechanisms and behavioral patterns in order to find economically and ecologically sustainable solutions to problems caused by herbivorous insects.

4.1.1. Host-plant finding

To locate a host, the insect uses a variety of senses including smell (olfaction) and taste (gustation) (Bernays & Chapman, 1994). Olfaction is the ability of detecting compounds in the gaseous state. Taste, in insects, differs from the sense of taste in humans. Insects have gustation receptors, similar to those in humans, located on many parts of their bodies, but they are not exclusively used for feeding purposes. The sense of taste in insects is therefore usually referred to as "contact chemoreception". The olfactory receptors are present on insect antennae, including larval forms (Chapman, 1998).

Many species of insects have behavior that enables them to follow odor plumes to the odor source, and in this way find distant resources such as host plants or mates (Murlis et al., 1992). The specific recognition of odors depends on the occurrence of sensory neurons, only responding to specific compounds. Furthermore, the response to an odor depends on the physiological state of the insect. During feeding periods the insect might get attracted by a certain odor, but if the insect is ready to pupate the response to the very same odor is limited; the age of the insect matters. Furthermore a hungry insect responds different to an odor compared with an satiated insect, as well as a mated insect reacts different than a non mated (Chapman, 1998).

4.1.2. Host-plant selection

Holometabolous adult insects, such as butterflies and moths, mostly oviposit on a host plant suitable for larval development. However, sometimes the female does not choose the most appropriate host for oviposition, with respect to the needs of the larvae. Therefore, in order to survive, the newly hatched larvae may need to locate and recognize a more suitable host plant (Bernays & Chapman, 1994).

Also when food supply is diminished, larvae move to new plants, proving that the offspring is fully capable to distinguish host plants from nonhost plants. The larvae do not only possess the ability to select the right plant species, but also to choose an individual plant within the species, appropriate for its needs (Bernays & Chapman, 1994).

4.2. Induced feeding preference

The term “induction of preference” is particularly used for phytophagous insects that tend to prefer a plants they have fed on before, with no regard of the plants advantageous features for the larva’s development (Bernays & Chapman, 1994). Induced preference for host plants occur in many phytophagous insects (Ting et al., 2002), but the understanding of the underlying mechanisms of induced preference is limited (Bernays & Weiss, 1996). Inductions of preference begins early in the larval stage, at first- and second instar level, and also increase in strength with each instar feeding on the inducing plant (Ting et al., 2002).

4.3 Model species

As model species of my study I chose the oligophagous tobacco hornworm, *Manduca sexta* (Lepidoptera: Sphingidae) and the highly polyphagous Egyptian cotton leaf worm *Spodoptera littoralis* (Lepidoptera: Noctuidae).

Studies of the tobacco hornworm have resulted in an increased understanding of induced host preference. It has been reported that dietary experience with host plants "tunes" the peripheral taste system, resulting in an intensified acceptability of host foliage (del Campo et al., 2001).

The reproductive behavior of *S. littoralis*, and other behavioral patterns, has been broadly studied, providing a useful array of knowledge for continued studies of the moth's plant preferences (Sadek et al. 2010).

4.3.1. The herbivorous larvae of *Manduca sexta*

Newly hatched larvae of *Manduca sexta* are polyphagous and become oligophagous when feeding on Solanaceous plants. They remain polyphagous when reared on artificial diet or nonhost plants (Yamamoto, 1974). As facultative specialists on Solanaceous plants, the larvae develop a strong preference for plants of this family and the induced preference could be so strong that the larvae would rather die of starvation than eat an alternative food (del Campo & Renwick, 1999). Larvae that are reared on nonhost plants or an artificial diet have shown neutrality to host foliage (de Boer, 1993).

4.3.2. The herbivorous larvae of *Spodoptera littoralis*

The Egyptian cotton leaf worm is very polyphagous, with a host range that covers over 40 families, including at least 87 plants species of economical importance (Holloway, 1989). *Spodoptera littoralis* is a major agricultural pest, distributed in Africa, the Mediterranean region, and the Middle East (Brown and Dewhurst 1975), causing considerable damage on crops such as cotton, cowpea and cabbage.

The feeding of Spodoptera larvae is influenced by previous experience (Anderson et al., 1995) and when adapted to a host plant, larvae tend to specialize to this specific plant, discriminating other hosts of choice (Yamamoto, 1974).

4.4. Aims and hypothesis

The main aim of this thesis was to study the behavior and the induced feeding preferences in larvae of *M. sexta* and *S. littoralis* when reared on certain host plants.

The hypothesis was that larvae of *M. sexta* would have an innate preference for plants of the Solanaceae family and therefore avoid the previously experienced non- host when faced with the choice between a true host and the previous food source. An induced feeding preference of a non-host plant will not be strong enough to keep the larvae from choosing their natural host.

S. littoralis were hypothesized to prefer the plants on which they previously have been feeding of. Due to their polyphagous nature one might assume that the larvae wouldn´t discriminate against any food of choice (Yamamoto, 1974, Anderson et al., 1995).

5. Materials and Methods

5.1. Insects

In the experiment I used larvae of:

- The tobacco hornworm, *M. sexta,* provided by Max Planck Institute Jena (see Appendix 1, Manduca Colony History), and further reared at Department of Chemical Ecology, Swedish Agricultural University Alnarp.
- African cotton leaf worm, *S. littoralis*, reared for several generations at Department of Chemical Ecology, Swedish Agricultural University Alnarp.

5.2. Rearing of *Manduca sexta*

Adults were kept in a rearing chamber under 16:8 L: D, 25 ± 2 °C and 70 ± 2 % relative humidity (RH). Larvae are kept in another climate chamber under 17:7h L: D, 25 ± 2 °C, 70 ± 2 % RH.

Two batches of *M. sexta* were used. The first batch was hatched on, and fed, synthetic food (see Table 1.) for approximately a week and then separated into three groups. One group continued on the synthetic diet, while the two remaining groups was fed either foliage of tomato (*Solanum lycopersicum)* or foliage of oilseed rape (*Brassica rapa*).

The second batch was put as newly hatched larvae on either potato foliage, tomato foliage and synthetic diet. Leaf of tomato and potato (*Solanum tuberosum*) were placed in the rearing chamber, allowing the adult moths to choose a plant to oviposit on in order to ease the collection of the eggs. Oviposition also occurred on plastic containers of sugar water (food for the adults) and on the net of the rearing cage. These eggs were put on synthetic diet and used as a control group in further studies.

5.3. Rearing of *Spodoptera littoralis*

Fourth to fifth instar larvae were taken from a laboratory culture maintained in Alnarp, Sweden, originated from wild-collected *S. littoralis* from Alexandria, Egypt, in 2008. The culture has been refreshed with new wild-collected moths at least once a year. Adult insects were kept in a rearing chamber under 25 ± 2 ºC, 70 ± 2% RH and 16:8h L: D. Larvae were kept in another climate chamber under 17:7h L: D, 25 ± 2 °C, 70 ± 2 % RH.

Several batches of *S. littoralis* were used in the experiment. Eggs of *S. littoralis* were separated into four groups and the hatching larvae were fed either foliage of cowpea (*Vigna* spp), cabbage (*Brassica* spp), cotton (*Gossypium* spp) or synthetic diet (see Table 1.).

5.4. Synthetic diets

The content of the synthetic food differs for the two different insect species according to their nutritious need.

Table 1. Content of synthetic diet for larva of *Manduca sexta* and *Spodoptera littoralis*

Insect	Content	Amount (g)
Manduca sexta	agar	46
	wheat germ	144
	corn meal	140
	soy flour	76
	casein	75
	salt mix	24
	sugar	36
	cholesterol	5
	ascorbic acid	12
	sorbic acid	6
	methyl paraben	3
	vitamin mix (nicotinic acid, riboflavin, thiamine, pyridoxin, folic acid, biotin, water)	30 (ml)
	formalin (37%)	60(ml)
	linseed oil	9 (ml)
Spodoptera littoralis	Potato	1700
	yeast mixture (wheat germ, dried yeast flakes, methyl-4-hydroxybensoate, sorbic	710

	acid, asorbic acid, cholesterol)	
	vitamin mix (nicotinamidae, d- panthothenic acid calcium salt, riboflavin (Vit. B2), thiamine (Vit. B1), pyridoxolhydrochlorid (Vit. B 6- hydrochlorid), folic acid, D- biotin (Vit. H), cyanocobalanin (Vit. B12)	24.4
	sodium benzoate	6.4
	distilled water	3.6 (l)
	plant agar	65
	DL- alpha- tocopherol acetate (Vit. E)	4 (ml)
	oil	10 (ml)
	96% ethanol	100 (ml)

5.5. Plants

Oilseed rape, potato, tomato, cowpea, cotton and cabbage were used for rearing and in testing.

All plants, except tomato, were cultivated in 1.5 l pots in a commercial substrate (Kronmull, Weibull Trädgård AB, Hammerhög, Sweden) for five to six weeks at 25 ± 2 ºC, 70 ± 5% RH in a greenhouse until they were used for experiment. The tomato plants were purchased at local garden center Plantagen. All plants were in a non-flowering stage when they were tested.

5.6. Larval dual choice test

5.6.1. Set up

The olfactometer used for the experiments was originally a test chamber for conditioning fruit flies, modified into a version suitable for the experiments. The olfactometer consists of a Plexiglas box separated into five chambers by porous metal walls between the distal chambers and nylon meshes between the second air baffle and the central chamber. The central chamber is 200 × 200 mm and serves as the area of the experiments (Carlsson et al., 1999).

An aquarium pump pumped the initial air through a tube at a flow of 0.4 l/min, containing activated charcoal in order to eliminate unwanted odors, into polyethylene cooking bags that were either empty or contained one of the odor sources (synthetic food (control), tomato plant, potato plant, rape plant, cowpea plant, cotton plant or cabbage plant). From the two sources of odor, plastic tubes were connected to either of the short sides of the olfactometer box. The odor was drawn into the olfactometer, passing the two distal chambers, continuing through half of the central chamber and sucked out through a hole in the lid of the olfactometer by a second pump with a flow of 3.5 l/min, connected to the top of the box by a tube.

The airflow was adjusted and controlled by flow meters and in a smoke test the flow of air was proven satisfactory for the experiments. Before every test session the olfactometer was sterilized with 70% ethanol and aired for approximately 5 minutes.

5.6.2. Experimental procedure

The larvae were individually placed in the middle of the central chamber, the lid was closed and the pumps were started. A timer, set on 5 minutes, was started and the larva was considered to have made a choice when it had entered an area, marked 1cm from the nylon mesh walls. If the larva did not make a choice within 5 minutes it was recorded as a non- choice.

5.7. Statistical analysis

In the statistical analysis, through a Binomial test, the probability of the outcome not being 50% ($p = 0.5$ vs p not $= 0.5$) is tested, using the program Minitab 16.1.1 Statistical Software, in order to determine the significance of the gained results.

6. Results

Manduca sexta dual choice test

Of the larvae reared on potato foliage 53.4 % made an active choice and a significant difference was found in the choice of potato and tomato (Fig. 1, P < 0.001) as the majority of the individuals chose potato.

Out of the larvae reared on tomato, 66.7 % made an active choice in the olfactory dual choice test. Of all tested larvae 48.9 % choose tomato and 17.8 % potato, which was found marginally non-significant (P = 0.052). The remaining 33.3% of the larvae did not make a choice.

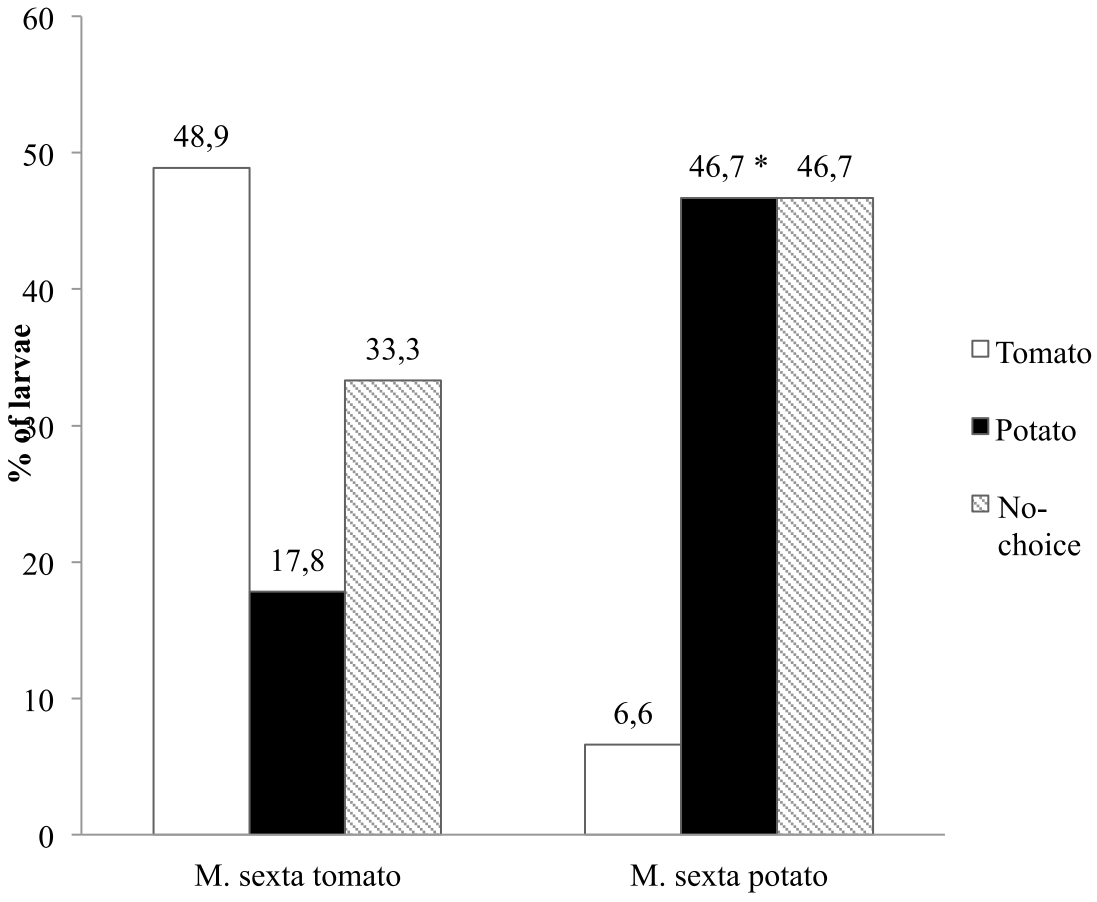

Fig. 1. Percental distribution of *M. sexta*, reared on foliage of tomato or potato, in larval dual- choice test, choosing between odors from tomato and potato leaves. N = 45 (* = p < 0.05, ** = p < 0.01, *** = p < 0.001).

The larvae reared on foliage of oilseed rape all died after a couple of weeks on the diet and larvae reared on synthetic diet never grew big enough for the testing

Spodoptera littoralis dual choice test

Cabbage vs cowpea

In the choice between cabbage and cowpea, 65 % of the larvae reared on cabbage chose cabbage (fig. 2, P = 0.021). Of the remaining larvae 15 % chose cowpea and the remaining 20 % made no choice. Of the larvae reared on cowpea 85 % chose cabbage (fig. 2, P < 0.001) and 5 % cowpea. The remaining 10 % made no choice.

Cotton fed larvae preferred cabbage as 80 % of them chose it (fig. 2, P = 0.001). 10 % chose cowpea and the remaining 10 % made no choice. Larvae fed with synthetic food preferred cabbage, 75 % chose cabbage (fig.2, P = 0.001) and 5 % cowpea. 20 % made no choice.

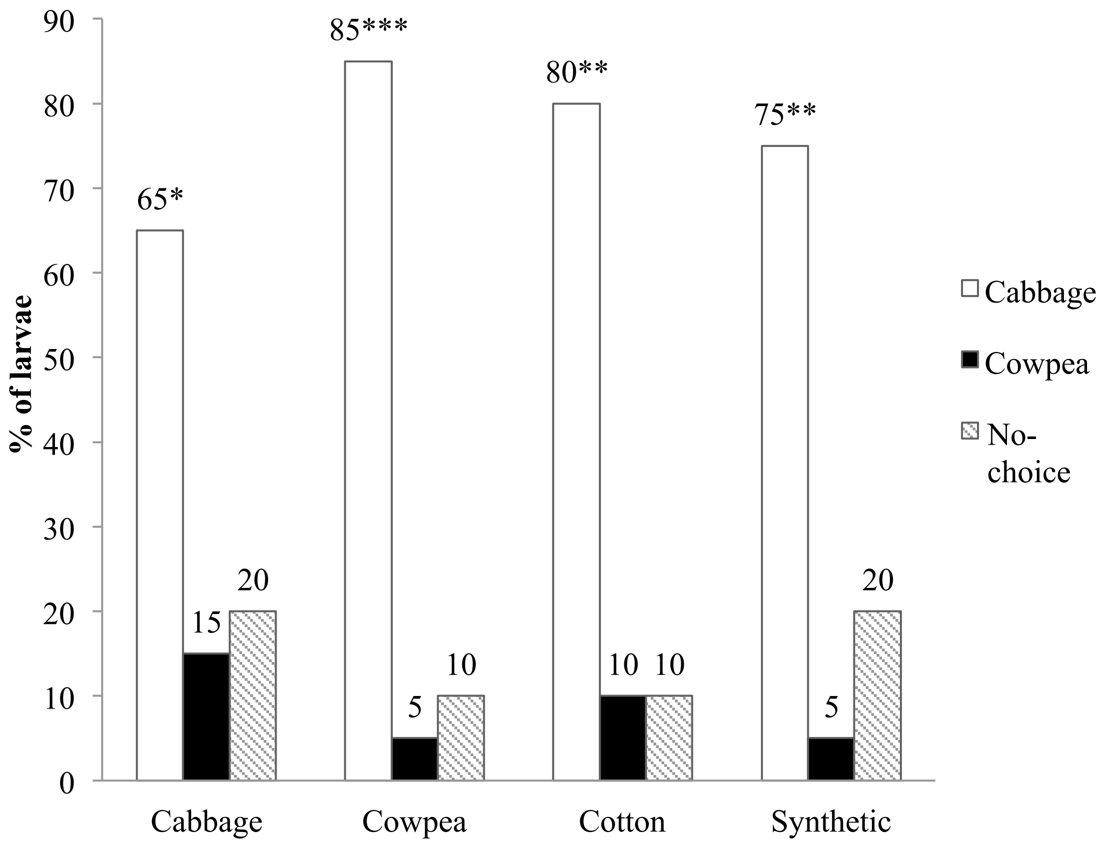

Fig. 2. Percental distribution of *S. littoralis*, reared on foliage of cabbage, cowpea, cotton or synthetic diet, in larval dual- choice test, N = 20 (* = p < 0.05, ** = p < 0.01, ***= p < 0.001).

Cabbage vs cotton

Larvae reared on cabbage, preferred cabbage when choosing between cabbage and cotton. 55% chose cabbage (fig. 3, P = 0.001), 0 % went for cotton and 45 % made no choice at all.

Of the larvae fed with cowpea, 60% chose cabbage, 30 % went for cotton and 10% made no choice.

Larvae reared on cotton did not show any preference when choosing between cabbage and cotton, 38.5 % chose cabbage and 38.5 % chose cotton. The remaining 23 % did not make a choice.

The larvae with prior feeding experience of synthetic food did not show any significant preference. 45 % chose cabbage, 25 % selected cotton and the rest, 30 %, made no choice.

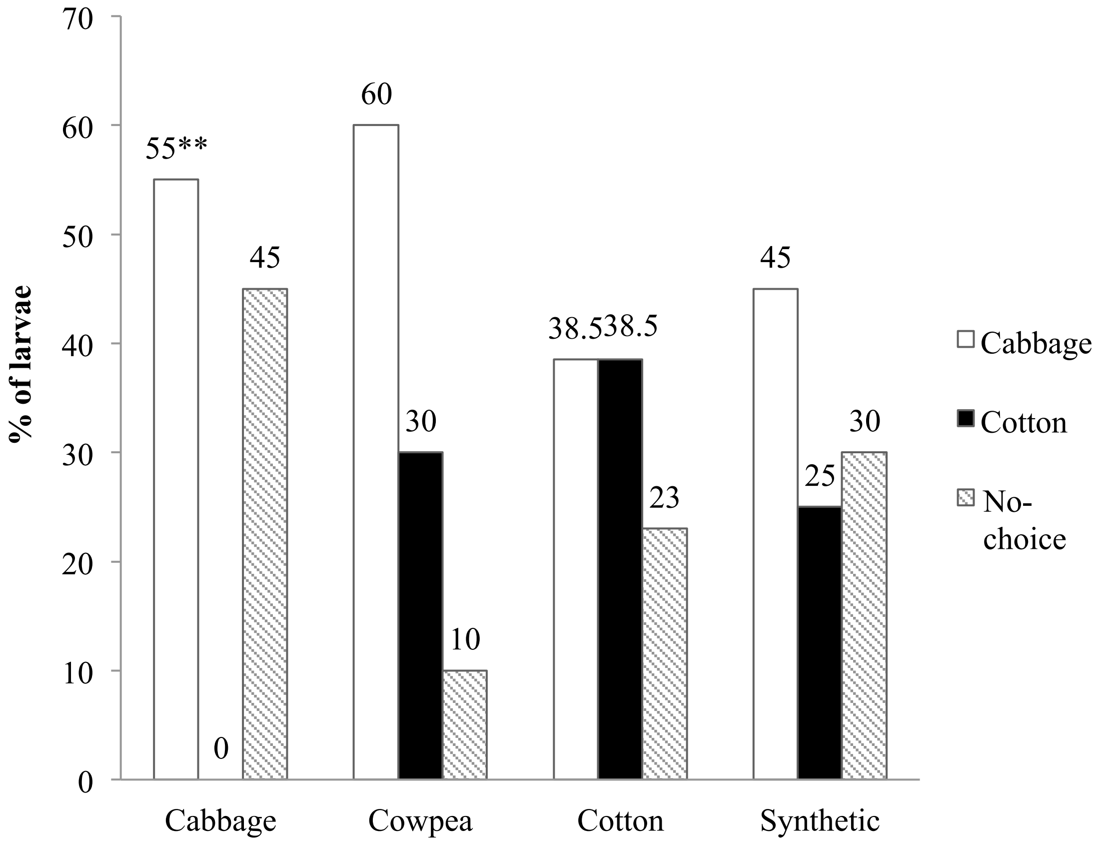

Fig. 3. Percental distribution of *S. littoralis*, reared on foliage of cabbage, cowpea, cotton or synthetic diet, in larval dual- choice test, N = 20 (* = $p < 0.05$, ** * = $p < 0.01$, *** *= $p < 0.001$).

Cowpea vs cotton

Larvae fed with cabbage did not show any significant preference in the choice between cowpea and cotton. 25% chose cowpea, 70 % cotton (P = 0.064) and 5 % made no choice (fig. 4).

The larvae reared on cowpea did not show any preference for their prior host, 30 % (fig. 4) chose cowpea and 55 % selected cotton. The remaining 15 % made no choice.

Of the larvae reared on a synthetic diet, 45 % chose cowpea (fig. 4), 30 % went for cotton and the rest, 25 %, made no choice.

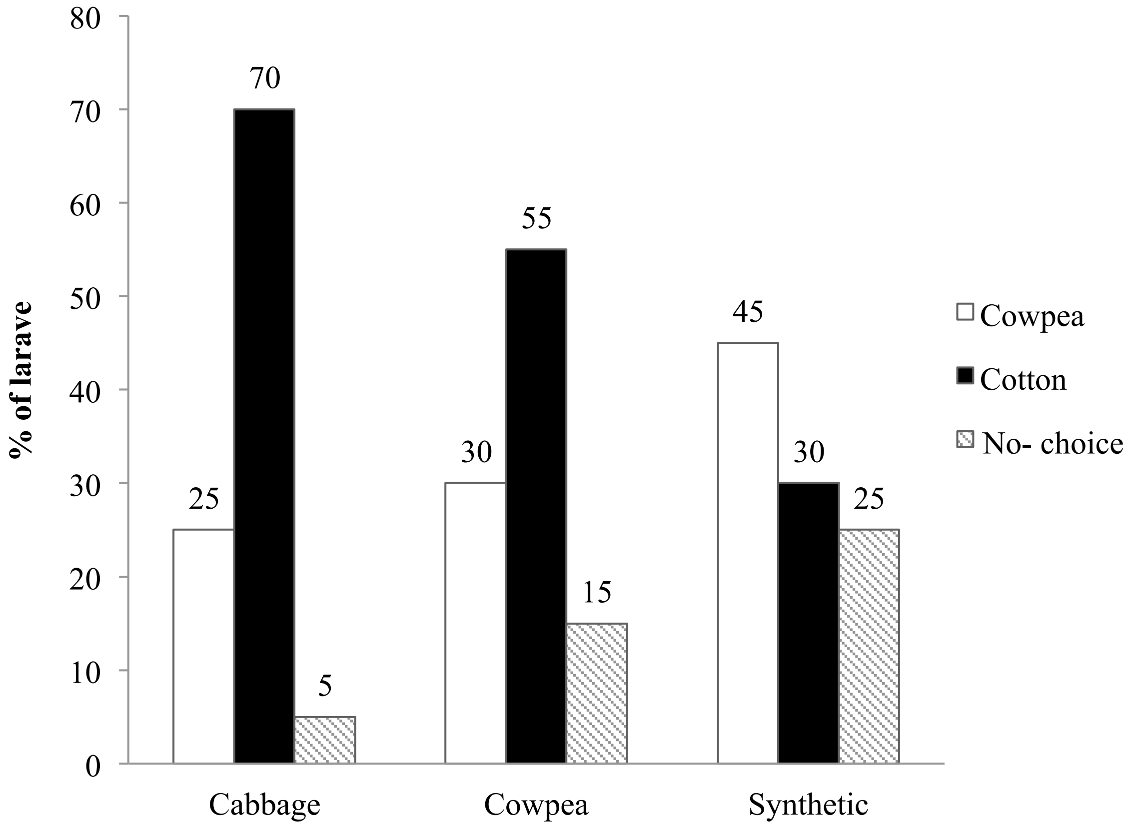

Fig. 4. Percental distribution of *S. littoralis*, reared on foliage of cabbage, cowpea or synthetic diet, in larval dual- choice test, N = 20.

7. Discussion

Through the derived results, that significantly displays that the tobacco hornworm larvae preferred the odour of which they had prior experience, one can assume that the feeding preference of the larvae very much depend on their prior feeding experience and when choosing between two true hosts, the known one is the first choice. Results gained through an experiment conducted by del Campo and Renwick (2000) clearly demonstrates that feeding on Solanaceous plants causes induction of host specificity in tobacco hornworm larvae. That the newly hatched larvae feed on foliage of oilseed rape indicates the lack of an innate preference for Solanaceous plants.

The preference for cabbage is a recurring result in the study, regardless of what plant they had prior experience of. *S. littoralis* where hypothesized to prefer the plants on which they previously had fed, but the gained results shows another tendency. The fact that larvae with prior feeding experience of a specific plant discriminate its prior host when faced with another host indicates that they do not specialize towards the experienced plant but might search for other qualities when foraging through odour. When choosing between the two hosts cabbage and cowpea, Spodoptera larvae reared on synthetic diet, preferred cabbage.

Larvae reared on cabbage mostly chose cabbage in the test, not always with significant results but the percental figures and the studied behavioral patterns of the larvae clearly displays the tendency of cabbage as a preferred host. For example, larvae with prior feeding experience of cowpea chose cabbage over cowpea and larvae fed with cotton chose cabbage over cowpea. For a more satisfying result, in future studies larvae reared on cotton need to be faced with the choice between cotton and cabbage in order to enhance the hypothesised "cabbage- preference".

As mentioned earlier, Chapman (1998) stated that the insect's response to a certain odour depends on the physiological state of the insect. All larvae used in the experiment were starved for the same amount of time and were all of the same age. The odour of cabbage might be a dominant odour compared with the other odours in the olfaction-based test, but if the larvae were allowed to rely on other senses, such as sight and taste, cabbage might not have been dominant. Further studies, where the insect rely on other senses than smell, would provide further understanding to the insects choice of feeding host.

No significant results were derived for the tobacco hornworm larvae reared on tomato, choosing between tomato and potato, but one could tell, studying the behavioral patterns during the testing and with the percental figure, that they had a tendency to choose tomato over potato. The potato fed larvae, faced with the choice between potato and tomato mostly chose potato or did not make any choice at all (fig. 1).

The tobacco hornworm larvae only showed attraction to the odour in experiments conducted before 11 AM, indicating that they follow a diurnal rhythm. If placed in the olfactometer and encouraged to make a choice after 11 AM, the larvae simply stayed put or aimlessly wandered around inside the central chamber of the olfactometer, not responding to the odours. As discussed in a study by J. W. Truman (1972) the moulting of the tobacco hornworm is associated with a circadian rhythm and is dependent of the photoperiod and temperature of the surrounding environment. A theory is that the larvae´s attraction to odours is influenced by this circadian rhythm, simply changing their foraging behaviour in benefit of the moulting behaviour. Bernays and Woods (2000) concluded, in an in vivo study, that the base of the rhythm is an endogenous neural oscillation, influencing the pattern of foraging. A study, where the larvae´s diurnal rhythm is disturbed or turned would give further understanding in this matter.

Compared to the larvae of *M. sexta, S. littoralis* did not seem to follow any diurnal rhythm that would affect their odour based foraging. Regardless of the time of the day they were active when tested in the olfactometer.

The reason for the death of the tobacco hornworm larvae reared on oilseed rape can be discussed; one theory could be that they cannot handle the secondary metabolites, glucosinolates, of the oilseed rape, which are released as a plant defence. Lack of essential nutrients is another possible reason for the death of the larvae. The development of a strong preference for Solanaceous plants (del Campo & Renwick, 1999) could be a mechanism used to prevent neonates from feeding on the "wrong kind" of plants.

The slow growth and low survival rate of the tobacco hornworm larvae fed with synthetic food could be an indication of that the synthetic food is not optimal for the rearing of *M. sexta.* Though it might as well have been the case that these specific batches of food had gone bad and could not fully sustain the larvae´s needs.

Difference in size could also be noted between the Egyptian cotton leaf worm larvae reared on different plants. Those feeding on cabbage grew big fast while the larvae on cowpea and cotton grew slower, indicating lack of nutrition or appetite.

8. Acknowledgements

I would like to thank my supervisor docent Anna Balkenius who has been cheerful and positive throughout this project, making me feel secure and certain of my thesis. In the same way, thank you professor Peter Anderson, for being my supervisor, providing me with helpful information, comments and knowledge.

Amanda Karlström, classmate and friend, thank you for your help and support.

Several persons in the Chemical Ecology group have been very helpful and friendly to me and I would like to thank every one of you.

9. References

Anderson P., Hilker M., Löfqvist J. (1995) Larval diet influence on oviposition behavior in *Spodoptera littoralis. Entomologia Experimentalis et Applicata* 74:1, 71- 82.

Bernays, E.A., Weiss, M.R. (1996) Induced food preferences in caterpillars: The need to identify mechanisms. *Entomologia Experimentalis et Applicata* 78, 1-8.

Bernays, E.A., Chapman E.F. (1994) Host- plant selection by phytophagous insects. *Chapman & Hall,* Great Britain, London.

Bernays, E.A., Woods H.A. (2000) Foraging in nature by larvae of *Manduca sexta*- influenced by an endogenous oscillation. *Journal of Insect Physiology* 46:825- 836.

de Boer, G. (1993) Plasticity in food preference and diet- induced differential weighting of chemosensory information in larval *Manduca sexta. J. Insect Physiol.* 39:1, 17- 24.

Brown E.S., Dewhurst C.F. (1975) The genus *spodoptera* (Lepidoptera, Noctuide) in Africa and the Near East. *Bulletin of Entomological Research* 65:02, 221- 262

del Campo, M.L., Miles, C.I., Schroeder, F.C., Mueller C., Booker, R., Renwick, J.A. (2001) Host recognition by the tobacco hornworm is mediated by a host plant compound. *Letters to Nature* 411, 186- 189.

del Campo, M., Renwick J.A.A. (2000) Induction of host specificity in larvae of *Manduca sexta*: chemical dependence controlling host recognition and developmental rate. *Chemoecology* 10:115-121.

del Campo, M.L., Renwick, J.A.A. (1999) Dependence on host constituents controlling food acceptance by *Manduca sexta* larvae. *Entomologia Experimentalis et Applicata* 209- 215.

Carlsson, M.A., Anderson, P., Hartlieb, E., Hansson, B.S. (1999) Experience-dependent modification of orientational response to olfactory cues in larvae of *Spodoptera littoralis. Journal of Chemical Ecology,* 25: 11, 2445-2454

Chapman, R.F. (1998) The insects: structure and function. 4 ed. *Cambridge University Press,* United Kingdom, Cambridge.

Holloway, J.D. (1989) The moths of Borneo: Family Noctuidae, trifine subfamilies: Noctuinae, Heliothinae, Hadeninae, Acronictinae, Amphipyrinae, Agaristinae. *Malayan Nature Journal* 42, 57-226.

Murlis J., Elkinton J. S. E., Carde R.T. (1992) Odor plumes and how insects use them. *Annu. Rev. Entomol*. 37:505-32

Nucifora, A. (1985) Successive cultivation and systems of integrated control in protected crops of the Mediterranean area. *Tecnica Agricola* **37**:223-241.

Sadek, M. M., B. S. Hansson, and P. Anderson. 2010. Does risk of egg parasitism affect choice of oviposition sites by a moth? A field and laboratory study. *Basic and Applied Ecology* 11:135–143.

Truman J.W. (1972) Physiology of insect rhythms- Circadian organization of the endocrine events underlying the moulting cycle of larval tobacco hornworms. *The Journal of Experimental Biology* 57:805- 820.

Ting, A., Ma, X., Hanson F.E. (2002) Induction of feeding preference in larvae of the patch butterfly, Chlosyne lacinia. *Acta Zoologica Academiae Scientiarum*

Hungaricae 48 (Suppl.1), 281- 295.

Yamamoto, R.T. (1974) Induction of hostplant specificity in the tobacco hornworm, *Manduca sexta. J. Insect Physiol.* 20, 641- 650.