Friedrich-Schiller-Universität Jena
Biologisch-Pharmazeutische Fakultät
Institut für Allgemeine Zoologie und Tierphysiologie
Max Planck Institut für chemische Ökologie
Abteilung für Evolutionäre Neuroethologie
Direktor: Prof. Dr. Bill S. Hansson

„Orientation of gravid *Manduca sexta* females to host plant odours“
(Orientierung von eiablagebereiten *Manduca sexta* Weibchen zu Pflanzendüften – Verhalten und chemische Analytik)

Bachelorarbeit
zur Erlangung des Grades eines
Bachelor of Science - Biologen
vorgelegt von

Aileen Gluschak
aus Erfurt

Jena, den 27.09.2011

Gutachter:

Prof. Dr. Bill S. Hansson

Dr. Andreas Reinecke

INHALTSANGABE

Abb.1: ***Manduca sexta***

http://www.marylandmoths.com/Moths/Springidae/Sphinginae/Sphingini/Manduca_sexta_M.jpg,

(17.09.2011, 19:30) Copyright © 2003 Larry Line

ZUSAMMENFASSUNG

In der vorliegenden Arbeit wurde das Präferenzverhalten eiablagebereiter *Manduca sexta* Weibchen (Lepidoptera: Sphingidae) für Düfte potentieller Wirtspflanzen im Windtunnel analysiert. Während der Stechapfel *Datura wrightii* und der Tabak *Nicotiana attenuata* (Solanaceae) zu den bevorzugten Eiablagepflanzen des Tabakschwärmers *M. sexta* gehören, wird der Rosenkohl *Brassica oleracea* (Brassicaceae) in natürlichen Gegebenheiten nicht als Wirtspflanze genutzt. Es wurde untersucht, ob allein die Pflanzendüfte, in Kombination mit Blattattrappen als visuelle Stimuli ausreichen, um die bekannte Wirtspflanzenpräferenz von *M. sexta* Weibchen auszulösen. Den Versuchstieren wurden entweder Pflanzendüfte gegen gereinigte Luft oder die Düfte zweier Arten gegeneinander angeboten. Dabei wurde beobachtet und ausgewertet, welche Düfte zuerst angeflogen wurden und wie häufig es zur wiederholten Kontaktaufnahme mit der Blattattrappe kann. Darüber hinaus wurde geprüft, ob die angebotenen Duftstimuli einen Einfluss auf den zeitlichen Aspekt der Verhaltensabläufe haben.

In der Reihenfolge *D.wrightii*, *N. attenuata* und *B. oleracea* war sowohl hinsichtlich der Häufigkeit der Erst-, als auch auch der wiederholten Kontakte eine deutliche Präferenz der *M. sexta* Weibchen zu erkennen. Vollständige, der Eiablage entsprechende Verhaltenssequenzen wurden aber nur selten beobachtet. Insofern scheint der Duft die Präferenz der Weibchen zu bestimmen, doch zur tatsächlichen Eiablage werden vermutlich weitere Kontakt- oder visuelle Stimuli benötigt.

Zeitliche Aspekte des Verhaltens, etwa die Zeit bis zum Beginn des Flugs oder die Dauer bis zur Kontaktaufnahme mit einer Blattattrappe wurden durch die angebotenen Duftstimuli nicht beeinflusst.

Zusätzlich wurden die Düfte, der im Windtunnelexperiment verwendeten Pflanzen gesammelt und analysiert. Die chemische Komponente (Z)-3-Hexenyl-acetate kommt in allen drei Pflanzendüften, am meisten jedoch im Duft der Pflanze *D. wrightii* vor. Dieses Molekül ist elektrophysiologisch aktiv und ruft starke Signale in den Antennen weiblicher Tabakschwärmer hervor. Diese Verbindung könnte maßgeblich für die Wirtssuche sein, denn *M. sexta* Weibchen wählen ihre Wirtspflanze vor allem nach dem Geruch aus. Der Pflanzenduft enthält charakteristische Moleküle, die in ihrer Gesamtheit über die Antennen der Weibchen wertvolle Informationen über Spezies und Qualität vermitteln. So kann *M. sexta* zwischen den angebotenen Pflanzen unterscheiden und Präferenz zeigen.

INTRODUCTION

The tobacco hornworm *Manduca sexta* (Lepidoptera: Sphingidae) (literature: Hill DS, 1987, p. 421-422) is a nocturnal insect (Cutler D et al., 1995) and lives in the tropical and temperate zones of America (Grant V, 1983). A lot of plant species with hawkmoth flowers occur also there (Grant V, 1983). *Manduca s.* is a specialist on Solanaceae (Del Campo M & Renwick JA, 2000) and the primary pollinator of the night – blooming plant *Datura wrightii* (Solanales: Solanaceae) (Goyret J et al**.,** 2008). But *Manduca s.* larvae are herbivorous and eat the leaves of their main host plants *Datura w.*, *Nicotiana spec*. (Solanales: Solanaceae), *Solanum lycopersicum* (tomato) or *Solanum tuberosum* (potato) (literature: Hill DS, 1987, p. 421).

Current studies in Max Planck Institute for Chemical Ecology, Department for Evolutionary Neuroethology (not published yet), have found out that *Manduca s.* larvae become larger and bigger on *Datura w.* leaves than on *Nicotiana attenuata* under laboratory conditions. Moreover Mira A & Bernays EA have published a study about the mortality rate of *Manduca s.* larvae on *Datura w.* and *Proboscidea parviflora* (Martyniaceae) in 2002. Their results show that more larvae survived and developed on *Datura w.*, which means that they will prefer this host species for feeding and oviposition in the future as adults (see Hopkins host selection principle, 1917). Other studies have already proven that the experience plays an important role for the host plant selection in moths (Olsson POC et al., 2006) and in polyphagous insect herbivore (Coyle DR et al., 2010).

To locate and choose a host plant, olfactory (Willis MA & Arbas EA, 1991) and visual stimuli are crucial (Goyret J et al., 2007). In a recent study researchers detected that the compounds of plant odours and their concentration to each other are the key for ovipositional host plant selection in the grape berry moth *Paralobesia viteana* (Cha DH et al., 2011). Thereby not only one compound is determining the host selection behaviour of *Manduca s.* (Fraser AM et al., 2003). Especially aromatic esters, such as (Z)-3-hexenyl benzoate, (Z)-3-hexenyl-acetate and (Z)-3-hexenyl propionate, evoke stronger electroantennographic (EAG) responses in female moths than in males (Fraser AM et al., 2003). According to the researchers, these compounds can be very important for the ovipositional behaviour.

In the following experiment, the preference of gravid *Manduca sexta* females for potential host plants (*Datura w.* & *Nicotiana a.*) and for a not normal host plant *Brassica oleracea* (Brassicaceae) is tested in a wind tunnel. It is examined, whether host plant odours evoke the

oviposition behaviour. The expected behavioural sequence happened: anemotaxix in zigzagging flight (Willis MA & Arbas EA, 1991), approaching, touching the source (artificial plant leaf) and curling the abdomen to lay eggs (compare with pheromone stimulation of males: Tumlinson JH et al., 1989).

The following hypotheses are concluded, which must be verified in this project.

Null hypothesis for A/B/C: The plant odour wasn't preferred over the odour of an empty cylinder – so there is no interest of gravid *M. sexta* for (A) *Datura wrightii* / (B) *Nicotiana attenuata /* (C) *Brassica oleracea*.

Hypothesis 1: The dummy with the odour source of *D. wrightii* was preferred over *N. attenuata* and *B. oleracea;*

Hypothesis 2: The dummy with the odour source of *N. attenuata* was preferred over *D. wrightii*

Hypothesis 3: The dummy with the odour source of *N. attenuata* was preferred over *B. oleracea*.

Additional, major plant odour molecules are identified, which determine the reaction of affinity or aversion in gravid *M. sexta* females. Plant volatiles of *D. wrightii*, *N. attenuata* and *B. oleracea* are analyzed and compared with each other.

MATERIALS AND METHODS

Insects

Manduca sexta (Lepidoptera: Sphingidae) larvae were raised on an artificial diet food (Große-Wilde E et al., 2010). The pupae were sexed and subsequently kept in cages (45 x 75 x 45 cm) in separate climate chambers for males and females under same conditions (70 % r.h. 27°C). The day-night rhythm was reversed (16 h light). Every day emerged adults were separated from the pupae and supplied with 25% sugar- water solution as nectar surrogate in artificial flowers. The insects were fed ad lib. until the wind tunnel experiments.

Two-day old females have been mated in a 150 x 200 x 150 cm cage with males and food. The gravid and fed female moths were tested in the wind tunnel in their fourth night.

Plants

Datura wrightii (Solanales: Solanaceae), *Nicotiana attennuata* (Solanales: Solanaceae) and *Brassica oleracea* (Brassicales: Brassicaceae) were grown in a greenhouse (23 to 25°C, 50 - 70 % r.h., 16 h light, Philips Sun-T Agro 400 W Na-vapour bulbs, 350 - 500 µmol m^{-2} s^{-1} photosynthetic photon flux at plant level). The plants were about 40 days old when they were used for wind tunnel experiments.

D. wrightii seeds came from B & T World Seeds, Paguignan, France and were subsequently harvested from plants bred in the greenhouse. They grew in 2 L pots and were used in experiments 35 - 45 days after sowing. All *N. attenuata* seeds were obtained after 30 - 31 generations of inbreeding an isogenic line derived from a genotype collected from a burn in south- western of the American state Utah. After germination seedlings were planted in seed flats at day 10, transferred to 1 L pots at day 20 and grown under greenhouse conditions until use. Plants were tested between 30 - 40 days after sowing. *B. oleracea* var. *Rosella* seeds were purchased from Erfurter Samen und Pflanzenzucht GmbH, Germany. These Plants grew in 1 L pots and used in experiments 35 - 45 days after sowing.

Wind tunnel experiments

Test set-up

The 250 x 90 x 90 cm wind tunnel consisted of a transparent Plexiglas chamber. Air was pushed into the wind tunnel with a flow rate of 0,4 m/s. An activated charcoal filter between the chamber and the fan cleaned the contaminated air (see: Lacey ES & Cardé RT, 2010). In

the wind tunnel were 63 - 72 % r.h. and a temperature of 19 to 24°C. The parameters checked always after three animals were tested. Experiments were conducted under 2% white light intensity. The wind tunnel was further illuminated by infrared LEDs to enable behavioural observations through night vision goggles. In between experiments additional red light was used to handle the tested animals without exposing them to daylight.

Two surrogate plant leaves were hung 40 cm apart from each other at the upwind end of the wind tunnel at a height of about 50 cm. Plant volatiles or clean air was added via Teflon tubing at a flow rate of 800 ml/min. Plant volatiles were collected from whole plants in 50 l glass cylinders (radius: 18 cm) besides the wind tunnel. 1,1 l/min clean air were pressed into the cylinders via Teflon tubing through an activated carbon filter . Before the test was allowed to be started, both cylinders were cleaned with acetone and purged with filtered air (push: 1,1 l/min, pull: 1,1 l/min, during a half hour). The start position of *Manduca sexta* was located on the downwind end. The distance from start position to the both dummies with odour source measured 1,4 m. There were also little green scattered patches at the bottom of the wind tunnel, which made an orientation of the insect's residence possible in this chamber. But near the odour sources (about 30 cm) this patches were distributed evenly. So, no target was given. Before the bioassay started, the wind tunnel was tested for neutrality.

Behavioural data aquisition

The test started with clean air, which was recorded as control, against *D. wrightii*, against *N. attennuata* and against *B. oleracea*. After this, the experiments *D. wrightii* against *N. attennuata* and *N. attennuata* against the odour of *B. oleracea* followed.

Each animal was allowed to fly in the wind tunnel for ten minutes. During this time the behaviour of gravid *Manduca sexta* females was recorded following the Time - Event - Sampling method. Long lasting and mutually exclusive behavioural pattern, e.g. sitting (s), flying (f), wing fanning (w), were recorded with their full duration, while brief behavioural pattern, e.g. hovering (h), contact to the odour source while still in flight (c) or abdomen bending (= ovipositions attempt) (o) were recorded as single events at the time of their occurrence. If two or more events occurred in the same sequence, then they were recorded together (For example: hc or hco). If two or more events occurred in the same time, but not in the same sequence, then they were separated by a semicolon (For example: s; w; or w; f). The tendency of flight was represented by arrows. When the moth was still sitting on start position

after a full duration of three minutes, the animal which were still sitting on start position after a full duration of three minutes, the females were stimulated by gentle prodding.
The symbol “d” stands for *Datura wrightii*, “n” stands for *Nicotiana attennuata*, “b” stands for *Brassica oleracea* and “k” stands for an empty cylinder, without plant odour (= clean air). Always after three moths were tested, the positions of plant odour sources were changed. Moths, which couldn’t flight or extended their proboscis, were not counted.

Plant volatile analysis

Headspace sampling

The volatile collections were performed on whole plants. Overall five plants of each species (*D. wrightii*, *N. attennuata* and *B. oleracea*) were used. The plants have been transferred in six 25 l glass cylinders, where 1,2 l/min charcoal filtered air was pressed into permanently. The odour - laden air was pulled 1 l/min through outlets in the sidewall. Custom-made sorptive filters with 25 mg each of Carbotrap C, B and X (Sigma Aldrich) were connected via short Teflon tubing. The Odour collections began 30 min after onset of the scotophase and were running 7 h. From 10:00 to 17:00 empty cylinders (Blanks) and from 22:30 to 5:30 plant odours were collected on the same day.
Between every volatile collection the cylinders were thoroughly cleaned with acetone and purged with clean air (push: 3 l/min, pull: 1,1 l/min, during 10 min).
The adsorbents were eluted about 2h after the end of headspace collecting.

First the internal standard with 25 ml Dichloromethane (DCM) and 20 µl 1-Bromodecane (Stock solution: 1 ng/µl) had to be produced. Each trap was rinsed in 400 µl DCM with internal standard. The solution with odour molecules of each trap came in a separate vial. Then the eluates were concentrated under a gentle stream of nitrogen to 50 µl. This rest was transferred into an insert (volume: 100 µl). After that, the eluates were concentrated under a gentle stream of nitrogen again to 25 µl. Finally the trials were stored at -80°C until an analysis.

Gas chromatography - mass spectrometry

The chemical compounds of the plant odours were analyzed on 7890A gas chromatographs (GC) (Agilent Technologies, CA, USA) (Department: Evolutionary Neuroethology) operated in split-less mode. The injection port was kept at 230°C, and 1 µl of sample injected. A non-

polar column (HP-5 MS ui & Innowax; 30 m, 0.25 mm ID, 0.25 µm film thickness; J&W Scientific, Folsom CA, USA) operated under constant He flow (1.1 ml/min) were used.
There was a condensation in the -60°C cold trap at the beginning for ten minutes. After that a 12° per second heating followed. So, there was a temperature of 210° after ten minutes. The temperature was nearly 230°, when 1µl of sample was injected. Then the GC oven heated up the system to 240°, which was hold on for 5 minutes. The transfer line to the MS was maintained at 280°C and the MS operated in electron impact mode (70 eV) with the ion source at 230°C, the quadrupole at 150°C, and a mass scan range from 33 to 350 amu.
The total ion chromatograms were recorded by an Agilent 5975C mass spectrometer connected to the GC. The nuclear compounds were shot with electrons, which speeded up in an electric field. Unlike this the electrons were deflected depending on their mass in a magnetic field. A detector measured the intensity of deflecting.

Data analysis

First the Kovats retention time index (KI) had to be determined by 10ng alkane mixture saturated. Then a safe analysis was possible.
For each plant mass spectra (MS) report the blank MS report of the same trap and the blank MS report of the trap that was used on the same day were subtracted. The remained chemical compounds, which were accepted as plant odours, were analyzed with the help of MSD Chem Station, the NIST05 Library (National Institute of Standards and Technologies; Gaithersburg MD, USA) and the calculated KI - values by MPI.

Statistics

The Wilcoxon Two Sample Test was used by an online – program (http://www.online-datenanalyse.de/Vorzeichenrangtest/2vS-Test.html) to examine the differences in contact with dummy or abdomen bending between the two odour sources in one run.
Furthermore the unpaired T - Test was applied by an online - program (http://www.graphpad.com/quickcalcs/ttest1.cfm) to compare the times (stationary phase, activation time, event time and approaching time) of the five various experiments to each other.

RESULTS

Wind tunnel experiments

When offered *D. wrightii* headspace versus clean air, 52,5 % of the tested females contacted the dummy leaf with *D. wrightii* odour first in flight (Fig. 2A; SD = 17,24). In addition 14 % of these moths bent their abdomen, thus showing oviposition intent (Table 1). In contrary, 10% had their first contact with the clean air dummy and 6% of these moths bent their abdomen subsequently.

31 % of the tested females contacted the dummy with *N. attenuata* odour source (Fig. 2B; SD = 17,62) and 19 % touched the artificial plant leaf with clean air first. In this experiment only one insect responded with abdomen bending (Table 1). Only few insects (26 %) had their first contact with the dummy in conjunction with the *B. oleracea* odour source (Fig. 2C; SD = 11,93) compared to 15 % having first contact with the clean air dummy.

When the odour of *D. wrightii* and *N. attenuata* was presented simultaneously, a lot more moths responded by contacting the surrogate plant leaf with *D. wrightii* odour source primarily (*D. wrightii*: 49 %, *N. attenuata*: 17 %, see Fig. 3A; SD = 12,01). 8 % of the moths selecting the *D. wrightii* odour bent their abdomen (Table 1).

When odour from *N. attenuata* and *B. oleracea* was presented synchronously, the females did not show a preference (Fig. 3B; SD = 20,81). 22 % had their first contact with the dummy in conjunction with *N. attenuata* odour source and 24 % contacted the dummy with *B. oleracea* odour first. In this experiment only one insect responded with abdomen bending (Table 1).

Additionally all repeated contacts with both surrogate leaves per female were counted and a preference index was calculated on that basis (Fig. 4, see Annex II for the raw data).

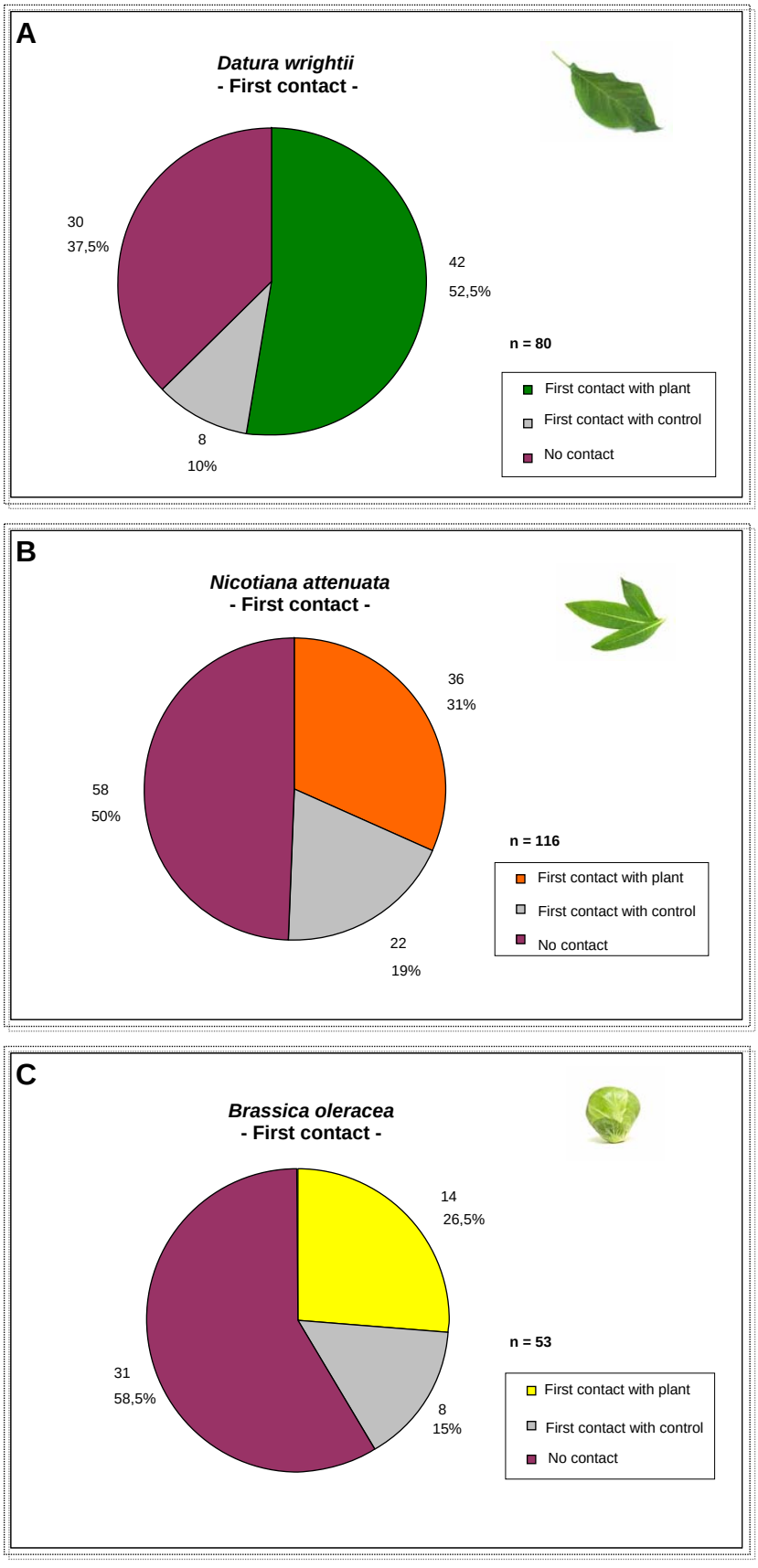

Fig. 2: Preference of gravid *M.* sexta females to plant odours in a flight tunnel. First contact with dummy leaves scented with plant odour (A: *Datura wrightii*, green; B: *Nicotiana attenuata*, orange; C: *Brassica*

***oleracea*, yellow) or clean air as control (grey). Non-responding females are coded in purple. (Plant photos: http://www.okaypix.com)**

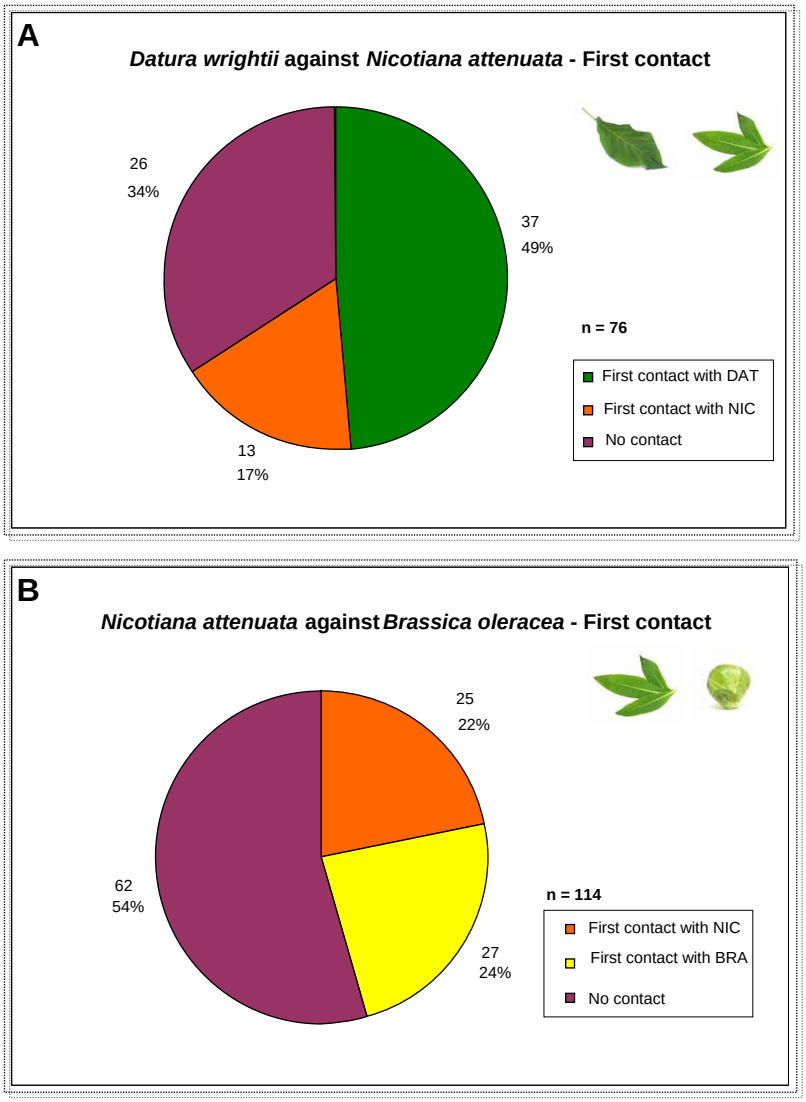

Fig. 3: Preference of gravid *M.* sexta females to plant odours in a flight tunnel. First contact with dummy leaves scented with plant odour (A: *Datura wrightii*, green; B: *Nicotiana attenuata*, orange; C: *Brassica oleracea*, yellow) or clean air as control (grey). Non-responding females are coded in purple. (Plant photos: http://www.okaypix.com)

Table 1: Number of first abdomen bending. See Annex I for the raw data.

First abdomen bending	DAT/Control	NIC/Control	BRA/Control	DAT/NIC	NIC/BRA
Plant 1	7	1	0	4	0
Plant 2/ Control	3	0	0	0	1
No	40	58	0	46	51

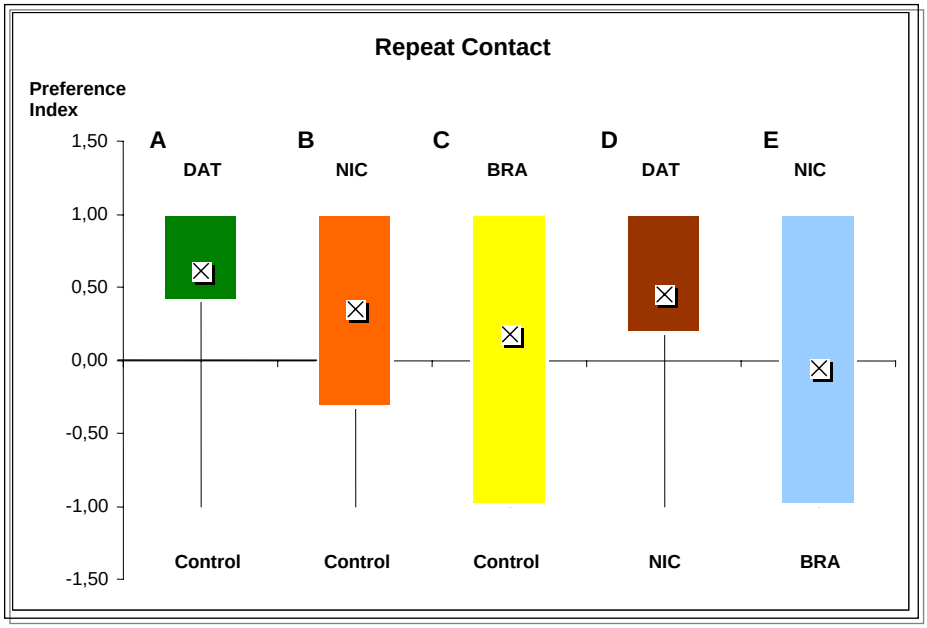

Fig. 4 (Table 3): Preference of gravid *M.* sexta females to plant odours in a flight tunnel. Repeat contact with dummy leaves scented with plant odour. DAT = D*atura wrightii*, NIC = *Nicotiana attenuata*, BRA = *Brassica oleracea*, Control = clean air as odour source. A: n = 50; B: n = 58; C: n = 22; D: = 50; E: n = 52. Wilcoxon signed - rank test (paired samples: plant one and plant two/ control): A: significantly different (0 < α = 0,05); B: significantly different (0,0006 < α = 0,05); C: not significantly different (0,1766 > α = 0,05); D: significantly different (0,0009 < α = 0,05); E: not significantly different (0,6977 > α = 0,05).

There was a clear preference for *D. wrightii* odour, when *D. wrightii* odour and clean air odour were presented in the wind tunnel (Fig. 4A; average = 0,61; significantly different: 0 < α = 0,05; SD = 0,56). Also the surrogate plant leaf with *N. attenuata* odour source was contacted by gravid *M. sexta* females more often than the dummy with clean air odour source (Fig. 4B; average = 0,35; significantly different: 0,0006 < α = 0,05; SD = 0,83). But there is no evidence for *B. oleracea* odour preference in contacting (Fig. 4C; average = 0,18; not significantly different: 0,1766 > α = 0,05; SD = 0,92).

Contrary to expectations the dummies with *N. attenuata* odour source wasn't preferred to *B. oleracea* odour (Fig. 4E; average = 0,45; significantly different: 0,0009 < α = 0,05; SD = 0,8). However and as expected, the dummies with *D. wrightii* odour were more often repeatedly contacted compared to *N. attenuata* odour scented dummies (Fig. 4D; average = -0.05; not significantly different: 0,6977 > α = 0,05; SD = 0,83).

Next, all repeated abdomens bendings to the surrogate plant leaves were counted per tested female and the corresponding preference index was calculated (Fig. 5, see Annex II for raw data).

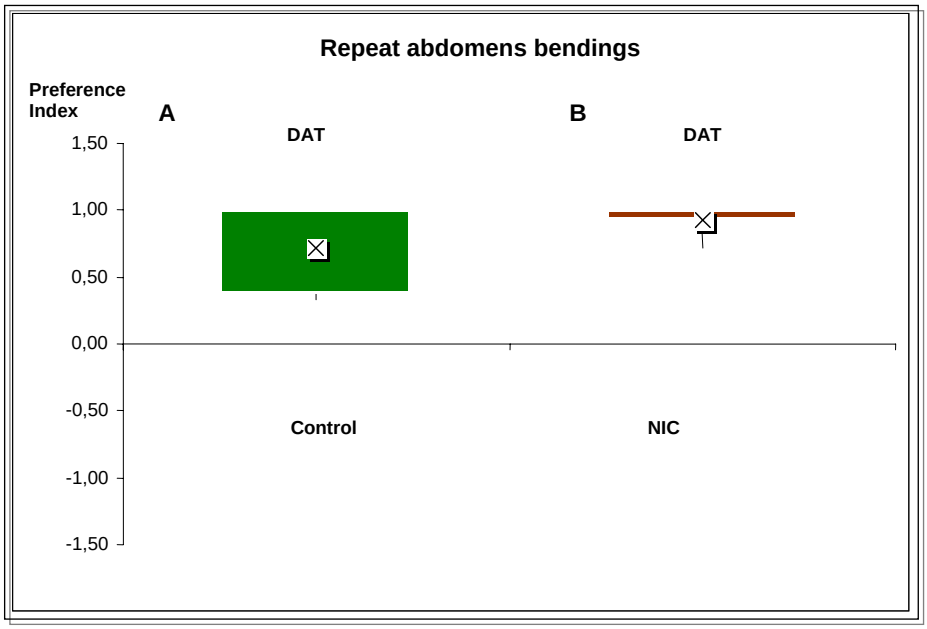

Fig. 5 (Table 3): Preference of gravid *M.* sexta females to plant odours in a flight tunnel. Repeat abdomens bendings to the surrogate plant leaves scented with plant odour. DAT = D*atura wrightii*, NIC = *Nicotiana attenuata*, Control = clean air as odour source. A: n = 8; B: n = 4.

Figure 5 shows the preference of *M. sexta* for *D. wrightii* odour in abdomen bending, when the odour of *D. wrightii* and clean air was presented simultaneously (Fig. 5A; average = 0,72; SD = 0,32) or when the odour of *D. wrightii* against *N. attenuata* was tested in the wind tunnel (Fig. 5B; average = 0,93; SD = 0,15).
No statistical test was performed, because only a few moths bent their abdomens to the surrogate plant leaves.

The times of single events were recorded. The stationary phase, activation time, take off and event times of all moths in contacting and abdomen bending to the surrogate plant leaves were compared to find differences between the different experiments.

Table 2: The activation time (sit till start with wing fanning) of each individual (Annex I) is combined of all animals, which were tested.

Stationary Phase	DAT/ Control	NIC/ Control	BRA/ Control	DAT/ NIC	NIC/ BRA
Average	88,31	96,92	83,3	95,05	102,3
Q1	155	200	200	200	200
Max	280	270	260	240	460
Min	1	1	1	1	1
Q2	1	1	1	1	1
n	67	96	44	63	107

No significant difference between treatments was detected, when analysing the time until females started wing fanning (Table 2; T - Test, unpaired samples: $\alpha = 0,05$, Annex III.I). Because these females, which were still sitting on start position after a full duration of three minutes, were stimulated by gentle prodding. The moths started wing fanning as warm up between one and two minutes.

Table 3: The take off time (sit till start with flight) of each individual is combined of all animals, which were tested.

Activation Time	DAT/ Control	NIC/ Control	BRA/ Control	DAT/ NIC	NIC/ BRA
Average	186,49	183,7	174,55	191,03	212,38
Q1	300	320	320	300	315
Max	440	400	380	390	600
Min	5	5	10	5	5
Q2	85	70	60	80	90
n	67	96	44	63	107

The take off time of all gravid *M. sexta* females, which were tested, is not significantly different in the different experiments (Table 3; T - Test, unpaired samples: $\alpha = 0,05$). The moths needed around three minutes to start flying. Consequently the duration of wing fanning as warm up was between one and two minutes.

Table 4: Time until first contact.

First contact	DAT/ Control	NIC/ Control	BRA/ Control	DAT/ NIC	NIC/ BRA
Average	110,7	102,16	100,91	88,4	106,54
Q1	162,5	97,5	165	90	162,5
Max	520	510	250	530	500
Min	10	10	10	10	10
Q2	30	30	30	30	30
n	50	58	22	50	52

The duration till the first contact of a moth with a dummy, which was associated with an odour source, is not significantly different between the treatments (Table 4; T - Test, unpaired samples: $\alpha = 0,05$) and varied between 88 and 110 seconds.

Table 5: Time until first abdomen bending.

First abdomen bending	DAT/ Control	DAT/ NIC
Average	135	60
Q1	145	82,5
Max	340	90
Min	40	30
Q2	72,5	37,5
n	8	4

No statistical test was performed, because only a few moths bent their abdomens to the surrogate plant leaves. The second event time is about two minutes, when the odour of *D. wrightii* and clean air was presented simultaneously and about one minute, when odour from *N. attenuata* and *D. wrightii* was presented synchronously (Table 5).

Plant volatile analysis

The chemical headspace compounds of *D. wrightii*, *N. attenuata* and *B. oleracea* were collected. Five samples per species were analyzed (Table 6).

Only 12 compounds were collected from the odour of *D. wrightii*. The highest number of chemical compounds was identified in *N. attenuata* headspace (71, see table 6). 25 compounds were collected from the odour of *B. oleracea*. 4-methyl-1-(1-methylethyl)-bicyclo[3.1.0]hexane has the greatest volume in that plant (10 %, see table 6).

(Z)-3-Hexenyl-acetate occurs in all three plant odours together (*D. w.*: 7,7 %, *N. a.*: 2 %, *B. o.*: 3,3 %), whereas methyl benzoate (*D. w.*: 3,8 %, *N. a.*: 1 %) appears not in the odour of *B. oleracea*, but of *D. wrightii* and *N. attenuata*. *B. oleracea* and *N. attenuata* odours have a number of compounds in common. Alpha-pinene (*N. a.*: 2 %, *B. o.*: 8,3 %), (+)-sabinene (*N. a.*: 2 %, *B. o.*: 8,3 %), beta-pinene (*N. a.*: 2 %, *B. o.*: 6,7 %), beta-myrcene (*N. a.*: 1 %, *B. o.*: 5 %), R-(+)-limonene (*N. a.*: 2 %, *B. o.*: 8,3 %) and another compound, which could not be identified (*N. a.*: 1,5 %, *B. o.*: 1,7 %), were collected from the headspace of both plants.

Table 6: Volatiles present in the headspace of *D. wrightii*, *N. attenuata* and *B. oleracea*. Compounds were identified based on the comparison of mass spectra and retention time indices (Kovats index, KI) to those of authentic reference compounds (bold letters) or spectra and KI for the same type of column available in the National Institute of Standard (NIST) mass spectra data base (standard letters). Compounds with low similarity values (< 800) and KI differences > 5 are printed in grey.

Compound	**CAS Number**	**RT (min)**	**KI calculated**	**KI (R)/(L)**	***Datura wrightii*** n = 5	**SD (%)**	***Nicotiana attenuata*** n = 5	**SD (%)**	***Brassica oleracea*** n = 5	**SD (%)**
total number of compounds in five plants total number of different compounds					26 12 **relative amount**		203 71 **relative amount**		60 25 **relative amount**	
(3-Buten-2-ol, 2,3-dimethyl-)	(10473-13-9)	4,3	651	677 (L)	3,8%	±0,95				
(Butanoic acid, methyl ester)	(623-42-7)	4,4±	658	686 (L)			6,4%	±0,6		
(1-Butanol, 3-methyl-)	(123-51-3)	4,5	661	697 (L)			0,5%	±0,1		
Pyridine	110-86-1	4,7	670	674 (L)			1,0%	±0,1		
(Propanoic acid, 2-methyl-, ethyl ester)	(97-62-1)	5,1	687	721 (L)			1,5%	±0,2		
(Cyclobutene, 2-propenylidene-)	(52097-85-5)	5,2	690	735 (L)			0,5%	±0,1		
1,4-Hexadiene, 4-methyl-	(1116-90-1)	5,2	691	692 (L)	11,5%	±1,15				
(2-Buten-1-ol, 3-methyl-/ 2-Buten-1-ol, 2-methyl-)	(556-82-1/ 4675-87-0)	5,5±	704	746 (L) 746 (L)			3,9%	±1,7		
(Butanoic acid, 2-methyl-, methyl ester)	(868-57-5)	5,5	705	747 (L)			1,5%	±0,2		
(trans-2-Pentenal)	(1576-87-0)	5,7	715	715 (L)			0,5%	±0,1		
Ethyl butyrate	105-54-4	6,1	804	805 **(R)**			2,0%	±0,2		
4-Methyl-1-pentanol	626-89-1	7,1	836	838 **(R)**			2,5%	±0,7		
3-Methyl-1-pentanol	589-35-5	7,3	845	845 **(R)**			2,0%	±0,1		
(Ethyl 2-methylbutyrate)	(7452-79-1)	7,6	851	852 **(R)**			0,5%	±0,1		
cis-3-Hexen-1-ol	928-96-1	7,7±	855	855 **(R)**			3,4%	±1,6		
(2-Methylbutyric acid)	(000075-09-2)	8	867	856 **(R)**			0,5%	±0		
trans-2-Hexen-1-ol	928-95-0	8	867	866 **(R)**			0,5%	±0,1		
Hexanol	111-27-3	8,1	868	868 **(R)**			2,5%	±0,7		
Pentanoic acid, 3-methyl-, methyl ester	2177-78-8	8,6	886	887 **(R)**			1,0%	±0,1		
(Heptanal)	(111-71-7)	9,1	904	902 **(R)**			0,5%	±0,1		
3-Pentenoic acid, 3-methyl-, methyl ester	2258-58-4	9,3	910	907 **(R)**			1,0%	±0,1		

Origanene	2867-05-2	9,9	926	925 (L)			1,5%	±0,1		
Bicyclo[3.1.0]hexane, 4-methyl-1-(1-methylethyl)-, didehydro deriv	58037-87-9	9,9±	927	926 **(R)**					10,0%	±0,1
alpha-Pinene	80-56-8	10,1	932	932 **(R)**			2,0%	±0,1	8,3%	±0
alpha-Phellandrene	99-83-2	11,2	968	969 **(R)**					1,7%	±0,3
(+)-Sabinene	3387-41-5	11,4	973	973 **(R)**			2,0%	±0,1	8,3%	0
beta-Pinene	127-91-3	11,4	976	975 **(R)**			2,0%	±0,1	6,7%	±0,1
(Hexanoic acid)	(142-62-1)	11,6	981	988 **(R)**	3,8%	±0,7				
(6-Methyl-5-hepten-2-one)	(110-93-0)	11,9	988	988 **(R)**			0,5%	±0,1		
6-Methyl-5-hepten-2-one	110-93-0	11,9	988	988 **(R)**					5,0%	0
beta-Myrcene	123-35-3	12	991	992 **(R)**			1,0%	±0,1	5,0%	±0,2
Decane	124-18-5	12,2	999	1000 **(R)**			0,5%	±0		
Octanal	124-13-0	12,4	1004	1004 **(R)**			0,5%	±0		
3-Carene	13466-78-9	12,5	1009	1009 **(R)**			0,5%	±0		
cis-3-Hexenyl acetate	3681-71-8	12,5	1008	1008 **(R)**	7,7%	±1,7	2,0%	±0,1	3,3%	±0,3
alpha-Terpinene	99-86-5	12,8	1016	1016 **(R)**			1,0%	±0,1		
p-Cymene	99-87-6	13	1025	1024 **(R)**			0,5%	±0,1		
Eucalyptol	470-82-6	13,2	1032	1031 **(R)**					8,3%	±0
R-(+)-Limonene	5989-27-5	13,2	1029	1028 **(R)**			2,0%	±0,1	8,3%	±0
Benzyl Alcohol	100-51-6	13,3	1034	1034 **(R)**			2,0%	±0,1		
(4-Acetylocta-1,2-diene)	(250445)	13,6	1042	1497 (L)					1,7%	±0,1
(Ocimene)	(502-99-8)	13,8	1049	1043 **(R)**	3,8%	±1,0				
(Butanoic acid, 3-methylbut-2-enyl ester)	(299118)	14	1057	1068 (L)			2,0%	±0,1		
gamma-Terpinene	99-85-4	14,1	1060	1060 **(R)**			2,0%	±0,1		
(gamma-Terpinene)	(99-85-4)	14,1	1060	1060 **(R)**					1,7%	±0,3
(Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1a,2a,5a)-)/ (Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1a,2ß,5a)-)	(17699-16-0)/ (15537-55-0)	14,4	1068	1041 (L) 1041 (L)					8,3%	±0
(1-Octanol)	(143-08-8)	14,5	1072	1070 **(R)**			1,5%	±0,1		
(1,1-Di(isobutyl)acetone)	(40264-43-5)	14,6	1074	1058 (L)					1,7%	±0,3
(cis-5,6-Dimethyl-4,7,9-trioxabicyclo[4.2.1]nonane)	(62759-64-2)	14,6	1074	1083 (L)			0,5%	±0,1		
(N-Methylhomopiperazine)	(4318-37-0)	14,6	1074	1098 (L)	15,4%	±3,4				
Terpinolen	586-62-9	15,1	1089	1088 **(R)**			2,5%	±0,5		
Methyl benzoate	93-58-3	15,3	1096	1095 **(R)**	3,8%	±1,0	1,0%	±0,1		

(Butanoic acid, 2-pentenyl ester, cis-)	(42125-13-3)	15,5	1102	1091 (L)			0,5%	±0		
(2-Hexanone, 6-(acetyloxy)-)	(4305-26-4)	15,8	1112	1120 (L)					1,7%	±0,1
(2-Phenylethanol)	(60-12-8)	15,8±	1114	1105 **(R)**			2,5%	±0,3		
(O-Trifluoroacetyl-isomenthol)	(28587-51-1)	15,9	1116	1138 (L)			1,5%	±0,9	1,7%	±0,1
Nonanal	124-19-6	15,9	1116	1114 **(R)**					3,3%	±0,3
(3-Acetyl-2,5-dimethyl furan)	(10599-70-9)	15,9	1116	1057 (L)					3,3%	±0,2
(Octane, 1-bromo-)	(111-83-1)	16,7	1143	1113 (L)	3,8%	±1,0	0,5%	±0,1		
cis-3-Hexenyl butyrate	16491-36-4	16,8	1187	1187 **(R)**			2,5%	±0,1		
(Propanoic acid, 2-methyl-, hexyl ester)	(2349-07-7)	17,1	1158	1151 (L)			1,0%	±0,5		
(2-Butenoic acid, 3-hexenyl ester, (E,Z)-)	(65405-80-3)	17,6	1173	1199 (L)			1,5%	±0,1		
(1-Cyclohexyl-2-methyl-prop-2-en-1-one)	(25183-82-8)	17,8	1178	1182 (L)			0,5%	±0,1		
(3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-)	(562-74-3)	17,8	1179	1137 **(R)**					1,7%	±0,3
((-)-Lavandulol)	(498-16-8)	17,8	1179	1165 (L)			0,5%	±0		
alpha-Terpineol	98-55-5	18,2	1192	1190 **(R)**					1,7%	±0,3
(alpha-Terpineol)	(98-55-5)	18,2	1192	1190 **(R)**			2,0%	±0,1		
Methyl salicylate	119-36-8	18,3	1196	1195 **(R)**			0,5%	±0		
(Octanoic acid, 7-oxo-)	(14112-98-2)	18,8	1214	1309 (L)					1,7%	±0,1
cis-3-Hexenyl isovalerate	35154-45-1	19,4±	1236	1241 **(R)**			3,9%	±0,2		
Undecane	1120-21-4	21,2	1300	1300 **(R)**			1,0%	±0,1		
(1,1'-Bicyclohexyl)	(92-51-3)	21,9	1326	1341 (L)			1,0%	±0,1		
(alpha-Cubebene)	(17699-14-8)	23,4	1381	1344 (L)	3,8%	±2,0				
((-)-beta-Elemene)	(515-13-9)	23,8	1397	1391 (L)			1,0%	±0,1		
Cedrene	11028-42-5	24,5	1426	1424 **(R)**			2,0%	±0,9		
Thujopsene	470-40-6	24,5	1426	1431 (L)			0,5%	±0,1		
Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	13877-93-5	24,5	1426	1494 (L)	3,8%	±0,7				
trans-alpha-Bergamotene	17699-05-7	24,9	1440	1442 **(R)**			2,0%	±0,1		
(Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-)	(16728-99-7)	24,9	1442	1440 (L)			2,5%	±0,7		
(Clovene)	(469-92-1)	25,2	1452	1446 (L)			2,0%	±0,1		
(Geranylacetone)	(3879-26-3)	25,3	1457	1420 **(R)**					1,7%	±0,3
(Seychellene)	(20085-93-2)	25,4	1459	1448 (L)			0,5%	±0,1		
(Tetradecane, 2-methyl-)	(1560-95-8)	25,5	1464	1448 (L)			2,0%	±0,1		
Acoradiene	24048-44-0	25,7	1473	1474 (L)			1,0%	±0,1		

(Humulen-(v1))	(159394)	26,1	1490	1494 (L)			0,5%	±0,1		
Pentadecane	629-62-9	26,4	1500	1500 **(R)**			1,0%	±0,1		
(beta-Chamigrene)	(18431-82-8)	26,5	1506	1507 (L)			2,0%	±0,1		
(alpha-Bulnesene)	(000075-09-2)	26,7	1512	1526 (L)			0,5%	±0,1		
(2R,6s-2,6,8,8-Tetramethyltricyclo[5.2.2.0(4,6)]undecan-3-one/ 9-Cedranone)	(140213)/ (156232)	28	1569	1564 (L) 1564 (L)			2,0%	±0,7		
Pentadecane, 3-methyl-	629-62-9	28,1	1500	1500 **(R)**			0,5%	±0		
(Hexadecane, 2-methyl-/ Hexadecane, 4-methyl-)	(1560-92-5/ 25117-26-4)	30,2	1664	1647 (L) 1647 (L)			1,0%	±0,1		
((E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene)	(77898-97-6)	34,4	1739	1777 (L)	3,8%	±0,7				
7-Octadecyne, 2-methyl-	35354-38-2	34,5	1866	1863 (L)			1,5%	±0,1		
(3,7,11,15-Tetramethyl-2-hexadecen-1-ol)	(102608-53-7)	34,8	1884	2045 (L)			1,5%	±0,1		
((E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene)	(70901-63-2)	35,4	1911	1922 (L)	19,2%	±2,5				
(ç-Gurjunenepoxide-(2))	(184705-51-9)	37,4	2016	1558 **(R)**					1,7%	±0,3
(Hedycaryol)	(21657-90-9)	37,4	2017	1694 (L)					1,7%	±0,1
(Squalene)	(7683-64-9)	50,6	2834	2914 (L)					1,7%	±0

DISCUSSION

The results show clear differences of gravid *M. sexta* females in contacting the dummy leaves with plant odour in the different treatments. Furthermore there were a different number of non-responder moths depending on offered odour.

It could be proven, that gravid *M. sexta* females have been attracted from the host plant odours in varying degrees, depending on the plant species (Fig. 2 and 3). This indicates that the insects can choose between the plant species, based on the different odour alone.
Thus, the null hypothesis for (A) *Datura wrightii* and (B) *Nicotiana attenuata* could be rejected, but not for the non-host plant (C) *Brassica oleracea* (Fig.4C).

The *Datura wrightii* and *Nicotiana attenuata* odour was preferred from gravid *M. sexta* females over clean air when comparing both the first choice (Fig. 2A and 2B) and repeated contacts to the surrogate plant leaves (Fig. 4A and 4B). This result confirms that an odour can guide a moth to its source and it can increase a moth's responsiveness to a visual target (Goyret J et al., 2007).

As expected, more than a half of individuals had their first contact with their main host plant odour source *D. wrightii* (Hill DS, 1987, p. 421) in the test against clean odour (Fig. 2A).
The attractivity of *N. attenuata* odour seems to be lower than *D. wrightii*, because the half of tested *M. sexta* females had no contact with an artificial plant leaf (Fig. 2B) and wanted to search a better host plant maybe. When offered *B. oleracea* headspace versus clean air (Fig. 2C), a greater number of non-responder moths occurred. This result points out that the moths don't have been attracted from the non-host plant odour of *B. oleracea*.

It was shown that *D. wrightii* odour was preferred over *N. attenuata* odour in first and repeat contacting the surrogate plant leaf (Fig. 3A and 4D), because *D. wrightii* is the main host plant of *M. sexta* (Hill DS, 1987, p. 421). This result has refuted the hypothesis 2 "The dummy with the odour source of *N. attenuata* was preferred over *D. wrightii*" with regard to contacting the odour source.
The Hypothesis 3 couldn't be confirmed. Neither the odour of *N. attenuata* nor the odour of *B. oleracea* was preferred of gravid *M. sexta* females. The differences in repeat contacting the dummy weren't significantly different (Fig. 4E).

Moreover, the repeat contact with *N. attenuata* odour source was reduced, when *B. oleracea* odour was presented instead of clean odour in the wind tunnel (Compare Fig. 4B and 4E). Therefore, *B. oleracea* odour seems to affect adversely the attractivity of *N. attenuata* odour.

The hypotheses can't confirm or refute easily, because there were not enough moths, which bent their abdomens to the odour source as ovipositions attempt in the experiments (Table 1). So the full behavioural sequence for host plant searching wasn't completed. Host plant odours are not enough to evoke an oviposition preference in *M. sexta*. Visual or contact stimuli comprising both surface texture and surface chemicals may be required to induce full oviposition behaviour.
In conclusion, all of this makes it impossible to confirm or refute the hypothesis 1. It's clear that *D. wrightii* odour was preferred over *N. attenuata* odour in contacting the odour source, but there wasn't carried out a test between *D. wrightii* and *B. oleracea* odour. This should be caught up in following experiments in the future.

No significant difference was detected when analysing activation time, take off time contact and abdomen bending time to the odour source. Consequently the odours of *D. wrightii*, *N. attenuata* or *B. oleracea* don't trigger a faster starting flight or a faster host plant selection of gravid *M. sexta* females. This indicates that in the performed experiments these times did not dependent on the plant odour offered but did rather depend in intrinsic states of the tested animals.

The activation time (sitting time) was uniform between the different tests, because these animals, which were still sitting on start position after a full duration of three minutes, were stimulated by tickling to start wing fanning.
Also the period of wing fanning of all moths was similar, across treatments. This is most probably due to the insects having a determined period of maximum two minutes for warming up (Fig. 6).
Even the event times weren't significantly different between the moths, which mean that they need the same period of time to notice the odour source.

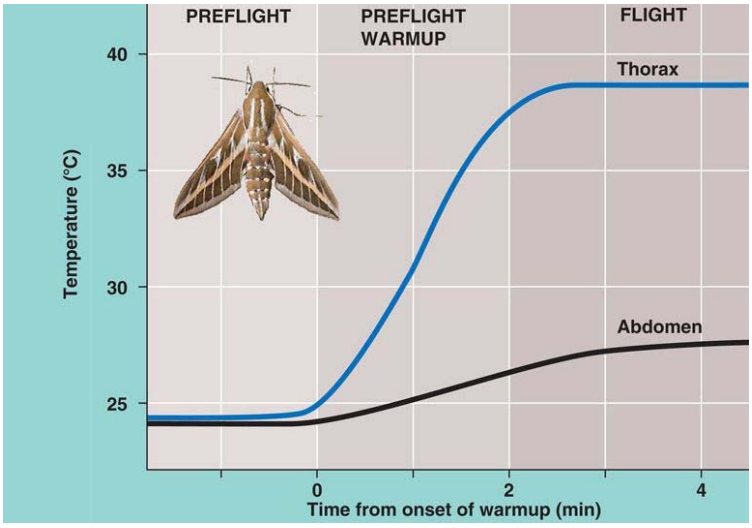

Fig. 6: Preflight warm up in the hawkmoth. The hawkmoth (*Manduca sexta*) uses shivering mechanism for preflight warm up of thoracic flight muscles. Once airborne, flight muscle activity maintains a high thoracic temperature.
(http://bio1152.nicerweb.com/Locked/media/ch40/hawkmoth-warmup.html. Copyright © 2005 Pearson Education, Inc. publishing as Benjamin Cummings: Campbell NA & Reece JB, Biology 7th edition, Chapter 40.5: Figure 40.20. All rights reserved.)

The relative amounts of compounds, which are electrophysiologically active in the odours of plants, represent the attractivity of a host plant and navigate gravid *M. sexta* females to the odour source.

Only one compound occurs in all three plants (See table 13). It’s (Z)-3-hexenyl-acetate, which is a specific odour cue and is detected by a number of olfactory sensillum types on the female antenna. This compound is actually electrophysiologically active and evokes stronger electroantennographic responses in female moths than in males (Fraser AM et al., 2003). According to the researchers, this is a very important compound maybe to activate the ovipositional behaviour. Most of this compound was collected by *D. wrightii* (Relative amount: 7,7 %) and much less in *B. oleracea* and *N. attenuata* (Table 13: *B. o.*: 3,3 %, *N. a.*: 2 %). This correlates with the wind tunnel result, in which more gravid *M. sexta* females had their first contact with *D. wrightii* than with *N. attenuata* (Fig. 3A).
N. attenuata and *B. oleracea* share many compounds together, from which beta-myrcene (*N. a.*: 1 %, *B. o.*: 5 %), R-(+)-Limonene (*N. a.*: 2 %, *B. o.*: 8,3 %) and 6-Methyl-5-hepten-2-one (*N. a.*: 0,5 %, *B. o.*: 5 %) evokes weak electroantennographic responses only (Fraser AM et al., 2003). Withal, there are other analyzed compounds, which evoke strong electroantennographic responses (Fraser AM et al., 2003 and an unpublished article yet):

nonanal in *B. oleracea*, methyl salicylate, (Z)-3-hexen-1-ol, (Z)-3-hexenyl butyrate and benzyl alcohol in *N. attenuata*, plus ocimene in *D. wrightii* (table 13). So the odour of *N. attenuata* shows the most chemical compounds, which are electrophysiologically active. But in relative amount they are low available, because there are many chemical compounds in the odour of that plant.

The species – specific compounds in an odour, give the moth information about the plant. Thus, *M. sexta* females can distinguish between different plant species and choose the very best host plant.

REFERENCES

Literature:

Cha DH, Linn Jr. CE, Teal PEA, Zhang A, Roelofs WL and Loeb GM 2011. Eavesdropping on Plant Volatiles by a Specialist Moth: Significanca of Ratio and Concentration. *PLoS ONE* **6**(2): 1-8.

Coyle DR, Clark KE, Raffa KF and Johnson SN 2010. Prior host feeding experience influences ovipositional but not feeding preference in a polyphagous insect herbivore. *Entomologia Experimentalis et Applicata* **138**: 137-145**.**

Cutler D, Benett R, Stevenson R and White R 1995. Feeding behavior in the nocturnal moth *Manduca sexta* is mediated mainly by blue receptors, but where are they located in the retina? *The Journal of Experimental Biology* **198**: 1909-1917.

Del Campo M and Renwick JA 2000. Induction of host specificity in larvae of *Manduca sexta*: chemical dependence controlling host recognition and developmental rate. *Chemoecology* **10:** 115-121.

Fraser AM, Mechaber WL and Hildebrand JG 2003. Electroantennographic and behavioral responses of the sphinx moth *Manduca sexta* to host plant headspace volatiles. *Journal of Chemical Ecology* **29**(8): 1813-33.

Grant V 1983. The Systematic and Geographical Distribution of Hawkmoth Flowers in the Temperate North American Flora. *Botanical Gazette* **144**: 439-449.

Goyret J, Markwell PM and Raguso RA 2007. The effect of decoupling olfactory and visual stimuli on the foraging behaviour of *Manduca sexta*. *The Journal of Experimental Biology* **210**: 1398-1405.

Goyret J, Markwell PM and Raguso RA 2008. Context- and scale-dependent effects of floral CO_2 on nectar foraging by *Manduca s*. *Pnas* **105**(12): 4565-4570.

Große-Wilde E, Stieber R, Forstner M, Krieger J, Wicher D and Hansson BS 2010. Sex-specific odorant receptors of the tobacco hornworm *Manduca sexta*. *Frontiers in Cellular Neuroscience* **4**: 22.

Hill DS 1987. Agricultural insect pests of temperate regions and their control. *Cambridge: Cambridge University Press* pp. 421-422.

Lacey ES and Cardé RT 2010. Activation, orientation and landing of female Culex quinquefasciatus in response to carbon dioxide and odour from human feet: 3-D flight analysis in a wind tunnel. *Medical and Veterinary Entomologie* **25**(1): 94-103.

Mira A and Bernays EA 2002. Trade-offs in host use by *Manduca sexta*: plant characters vs natural enemies. *Oikos* **97**: 387-397.

Olsson POC , Anderbrant O and Löfstedt C 2006. Experience influences oviposition behaviour in two pyralid moths, *Ephestia cautella* and *Plodia interpunctella*. *Animal Behaviour* **72**: 545-551.

Tumlinson JH, Brennan MM, Doolittle RE, Mitchell ER, Brabham A, Mazomenos BE, Baumhover AH and Jackson DM 1989. Identification of a Pheromone Blend Attractive to *Manduca sexta* (L.) Males in a Wind Tunnel. *Archives of Insect Biochemistry and Physiology* **10**: 255-271

Willis MA and Arbas EA 1991. Odor – modulated upwind flight of the sphinx moth, *Manduca sexta* L. *Journal of Comparative Physiology A* **169**: 427-440.

Photos:

http://www.marylandmoths.com/Moths/Springidae/Sphinginae/Sphingini/Manduca_sexta_M.jpg 25.08.2011, 14:30.

http://www.okaypix.com, 07.09.2011, 20:00, *D. wrightii* leaf © Unclesam, *N. attenuata* leaf © Uros Petrovic, *B. oleracea* single sprout © Chris leachman.

Campbell NA and Reece JB 2005**.** Biology 7th edition. *Pearson Education, Inc. publishing as Benjamin Cummings* Chapter **40**(5): Figure 40.20.

(alternative: **http://bio1152.nicerweb.com/Locked/media/ch40/hawkmoth-warmup.html,** 17.09.2011, 19:30).

DANKSAGUNG

Ein besonderer Dank gilt meinem Betreuer Dr. Andreas Reinecke, meiner praktischen Einweiserin Anna Henning und Prof. Dr. Bill S. Hansson, die die Durchführung meiner Bachelorarbeit am Max – Planck – Institut für chemische Ökologie ermöglichten.
Ich bedanke mich auch bei allen studentischen Hilfskräften und Praktikanten, die mich bei der praktischen Arbeit unterstützt haben.

EIDESSTATTLICHE ERKLÄRUNG

Hiermit erkläre ich, Aileen Gluschak, geboren am 12.12.1989 in Erfurt, dass ich die vorgelegte Arbeit selbstständig verfasst und keine anderen als die im Literaturverzeichnis und Abbildungsnachweis angegebenen Hilfsmittel verwendet habe.

Erfurt, den

Unterschrift:

ANHANG