The effect of domestication on insect resistance in cotton

- Bioassays with the insect herbivores *Spodoptera littoralis* and *Agriotes spp.*

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Effekten av domesticering på insektsresistens i bomull

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Sammanfattning

Växters förmåga att försvara sig varierar beroende på många olika orsaker. En teori säger att domesticeringen av vissa växter har sänkt deras resistens mot herbivorer i en trade-off med andra egenskaper så som ökad skörd som människan selekterat fram.

I det här arbetet har jag undersökt effekten av domesticering på resistensen mot växtätande insekter genom att använda sorter och arter av bomull i olika stadier av domesticering. Detta har gjorts i tre olika experiment. Först genomfördes ett utvecklingsexperiment i vilket jag studerade utvecklingen av larver av bomullsflyet, *Spodopeta littoralis* från ägg till förpuppning, samt puppornas vikt. Den andra delen var ett beteendeförsök där rotlevande larver från skalbaggarna *Agriotes* spp. fick välja mellan rotsystemen av olika bomullsplantor. En tredje del av arbetet var en insamling av växtdofter från bomullsplantor som inducerats antingen av *S. littoralis*, som ätit på bladen, *Agriotes* spp., som ätit på rötterna eller en kombination av skadorna från båda arterna.

Mina data visar att det finns en skillnad mellan olika bomullssorter och att vildare sorter generellt har en högre resistens mot insektsangrepp än domesticerade sorter. De domesticerade sorterna Dpl 90 och glandless cotton gav en signifikant kortare utvecklingstid i utvecklingsexperimentet än de vilda sorterna Tx263, *G. herbaceum* och Tx 2259. Däremot var det inga signifikanta skillnader mellan bomullssorterna i vikten på pupporna mellan dieterna. Tvåvalstestet med Agriotes-larver gav inga signifikanta resultat vilket troligen berodde på ett för lågt antal av replikat. Jag fann dock en tydlig trend där larverna föredrog den domesticerade sorten Dpl 90 över alla andra testade sorter, förutom den vilda arten *G.herbaceum*. Analysen av insamling av växtdofter visar att plantorna som inducerats av *S. littoralis* reagerar starkare och har en högre avgivning av flyktiga ämnen såsom alpha pinene, 1S-beta pinene, myrcene och terpinolene än plantor som inducerats av endast *Agriotes* spp. eller med en kombination av de båda arterna. Möjligtvis kan Agrioteslarverna manipulera bomullsplantan så att den inte startar sitt inducerade försvar eller så kan bomullsplantan inte upptäcka dem. Möjligen har också flyktiga ämnen släppts ut från rötterna av bomullsplantorna vilket inte detekterats eftersom endast ämnen från överjordiska delar insamlades.

Abstract

A plant's defensive ability against herbivore attack can be influenced by many different factors, one of them being domestication. During human selection to improve the yield of plants, the resistance against herbivore attack can have been lowered, due to a trade-off for use of resources between these traits in the plants.

In this thesis I investigated the effect of domestication on resistance against herbivorous insects in cotton plants. I used different varieties and species of wild and domesticated cotton in three different experiments. A feeding assay was conducted, the mortality, development time and pupal weight of larvae of the Egyptian cotton leaf worm *Spodoptera littoralis* was studied. In a second experiment the preference of the root-feeding beetle *Agriotes* spp. was monitored in a two choice assay comparing domesticated cotton to other cotton varieties and species. The third part of the thesis was an odour collection from domesticated cotton plants induced by either *S. littoralis* larvae feeding on the leaves, *Agriotes* spp. feeding on the roots or a combination of damage from both species.

The data show that there is a difference between cotton cultivars and that wild varieties delay development time compared to domesticated cotton. The feeding assay showed that the domesticated varieties Dpl 90 and Glandless cotton had significantly shorter development times than the undomesticated varieties (Tx 263, Tx 2259 and *G. herbaceum*) The two choice assay with *Agriotes* spp. larvae did not give any significant results most likely because of a low number of repetitions. *Agriotes* spp. did not prefer either domesticated nor undomesticated varieties of cotton. However, a trend in favor of the domesticated cotton variety could be observed.

Finally, analysis of the odour collection demonstrates that plants induced by *Spodoptera littoralis* alone release higher amounts of the volatiles alpha pinene, 1S-beta pinene, myrcene and terpinolene than plants induced with either only *Agriotes* spp. or a combination of the two types of larvae. It may be that *Agriotes* spp. can manipulate the defence of the cotton plants. Another explanation is that the cotton plants cannot detect the *Agriotes* larvae. Also, there might have been a release of volatiles below ground that was not detected due to the layout of the experiment.

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Introduction

The importance of resistance and different types of resistance

Plants are faced with a major challenge when they come under attack by herbivores, as they cannot flee from their enemies and therefore need to rely on different defence strategies. One way of defence would be the existence of morphological barriers such as trichomes and waxes, which make it harder for an herbivore to feed or move on the plant, or by having tough leaves which can wear the insects' mandibles down. Plants also possess chemical defences, which affect herbivores in different ways depending on type of chemical and species of insect larvae. The resistance trait can further be either non-preference, also called antixenosis, or antibiotic. Antixenosis means that the host plant has traits that make the plant unattractive as a host plant. Antibiotic resistance means that the plant contains traits that directly affect the pest insects' fecundity, survival or size (Schoonhoven et al., 2005). All these resistance traits help the plants to reduce damage from attacking pest species, but they are also important for farmers as losses in fields vary greatly depending on cultivar.

Direct and indirect defences

The defence in plants can be divided into direct and indirect defence. The direct defences affect pests directly, for example through toxins or hypersensitivity responses around egg batches. Indirect defences are traits that do not directly affect pests but creates a less favourable environment for the pest, for example by attracting natural enemies (Schoonhoven et al., 2005, Price et al., 1980).

Induced and constitutive defences

It is costly for plants to build up and sustain insect resistance mechanisms, especially when the herbivore pressure is low and the resistance is therefore not always needed (Karban, 2011). As a response plants have developed different strategies to cope with pests i.e. constitutive and induced defences (Schoonhoven et al., 2005).

Constitutive defences are structures or compounds that are always present in the plant (Schoonhoven et al., 2005). The benefit of constitutive defences is that they are always present and the plant can directly defend itself to an herbivore attack. Slow growing plants generally rely more on constitutive defences than faster growing plants, as they cannot replace lost tissues as quickly as faster growing plants (Karban, 2011).

Unlike constitutive defences, induced defences are only activated when needed, thereby saving resources for the plant that can be used for growth and reproduction. A typical example for induced resistance is the release of plant volatiles after an herbivore attack. These compounds can recruit parasitoids and predators of herbivores to the infested plant and therefore help to reduce the abundance of herbivores on the plants. The fact that the release of volatiles is induced allows the plant to fine-tune its different volatile blends to specific herbivores and thereby attract specialist beneficial insects. A constant release of volatiles would on the other hand give the plants location away, as well as removing a reliable signal for the beneficials and hence reducing their recruitment. (Zangerl, 2003). The fact that induced defence is turned on or up regulated could be a problem for the plant as it takes a while before they get their optimal defence (Karban, 2011).

Domestication sometimes has been found to have an effect on the induced and constitutive defences in plants. In a comparison between domesticated olive trees and wild ones it was shown that the wild ones had higher levels of phenolics when grazed upon by ungulates (Massei & Hartley, 2000). Domesticated *Brassica* and *Phaseoulus* plants were shown to be less attractive to parasitoids than their wild relatives, which were most likely caused by reduced volatile release, thereby making it more difficult for parasitoids to locate infested plants (Benrey et al., 1998). Another example can be found in cranberries (*Vaccinium macrocarpon*). Cranberries were a good candidate to investigate the effect of domestication in as they were domesticated rather recently and there are both wild and intermediate varieties available to test for resistance against insect herbivores. The experiments showed that the domesticated cultivars had lower levels of volatiles, as well as lower levels of the defence chemical cis-Jasmonic acid (Rodriguez-Saona et al., 2011). Inbred plants have been shown to have a lesser defence against herbivore attack. Inbred horsenettle (*Solanum carolinense*) got significantly lower amounts of spines and trichomes than outbred ones after they had been induced by insect feeding. Inbred plants also had a lower constitutive defence than the outbred ones (Kariyat et al., 2013). Volatile emissions from plants are also lower in inbred plants than in outbred ones. This was shown both for emissions that are part of the constitutive resistance and induced emissions. The low emissions of volatiles lead to lower recruitment of predators and parasitoids when the plants were attacked by herbivores (Kariyat et al., 2012).

Cotton

The genus *Gossypium* is an ideal model organism to study domestication effects on as there are many closely related species that are domesticated that also are present as wild types (Fryxell, 1979). Cotton fibres are a produced by a number of species from the genus *Gossypium* (Malvaceae) (McDougall et al., 1993). The genus is part of the *Gossypieae* tribe, which is distributed in the tropics and subtropics.

Cotton is one of humanity's primary sources of fibres. The cotton fibres are produced by the seeds produce and are elongated epidermal hair cells (McDougall et al., 1993). Selection has been done to get a white fibre (Fryxell, 1979). The harvested cotton fibre consists of a very high level of cellulose (83%), which means that it is a very strong fibre. Apart from the obvious use as fabric, cotton is used for a variety of reasons. Cotton oil is used as a vegetable oil and is often used in snacks. Hulls and meal left over after oil production are used as fodder for farmed fish or other animals and it can also be used as a fertilizer (Caliskan, 2010).

Cotton is produced in 81 countries around the world and in 2014 the production of cotton lint was around 26 million tons, which was harvested from 34 million hectares of land (http://faostat.fao.org/). China, India, USA, Pakistan and Brazil, which are the top five producers, together stood for over 75% of the world's cotton production in 2012 (FAOSTAT, 2014). 95% of the world's cotton production is Upland cotton i.e. *Gossypium hirsutum* (McDougall et al., 1993). Other cultivated species include *G. herbaceum*, *G.arboreum* and *G. barbadense* (Fryxell, 1979).

Direct defences in cotton

Cotton has several direct and indirect resistance mechanisms against insect herbivores (Hagenbucher et al., 2013). Most species have the ability to produce Gossypol or other similar terpenoids (Fryxell, 1979). Gossypol is a toxin that is an important direct defence trait of cotton plants. Although its exact mode of action is still not understood, it might act by binding to proteins in the insects' gut decreasing the amount of proteins that can be absorbed by the larvae. Gossypol might also block enzymes in the larvae's guts, which further decreases the amount of protein the larva can absorb leading to a longer development time for the larva (Schoonhoven et al., 2005). Gossypol is deposited in glands on most surface tissue of the plant, including the seeds (Lusas & Jividen, 1987). Another direct chemical defence in cotton is tannins (Hagenbucher et al., 2013). In recent years genetically engineered Bt cotton

plants were introduced, that express toxins from the insect pathogen *Bacillus thurengiensis* (so called Bt-toxins), which offer protection against important lepidopteran pest species (Hagenbucher et al., 2013, Schoonhoven et al., 2005).

Morphological defences in cotton include leaf structure and trichomes (Schoonhoven et al., 2005, Fryxell, 1979). Trichomes make it harder for insects to move across leaves in turn making them an easier prey for predators and exposing them longer to abiotic stresses (Schoonhoven et al., 2005). Unfortunately the trichomes might also slow down the predators hunting them depending on the predator's species. The effect of trichomes on insect pests also varies vastly; some are warded off while other benefit from hairy leaves. (Hagenbucher et al., 2013).

Indirect defences in cotton

Indirect defence of cotton consist of the release of volatiles by the plant and the presences of extra-floral nectaries. Cotton plants can alter their volatile blend once under attack by an insect pest and the new volatile mixture can be used by predators and parasitoids to locate their prey and hosts on the cotton plants (Hagenbucher et al., 2013). These volatiles can however also influence herbivores. Damaged cotton plants are less attractive for ovipositing female *S. littoralis* moths than undamaged plants, possibly to reduce competition for the larvae or that food quality is reduced. In experiments where volatiles from damaged plants were added to undamaged plants, these were significantly less attractive for the females (Zakir et al., 2013). The same effect has also been shown with root feeders, which made the attacked plants unattractive for ovipositing moth females and larvae (Anderson et al., 2011). In the case of the larvae this might be due to increased levels of terpenoids as root feeding has been shown to increase the levels of terpenoids in the leaves of *G. herbaceum* (Bezemer et al., 2003, Bezemer et al., 2004).

Extra-floral nectaries are another way to attract insect predators to plants (Schoonhoven et al., 2005). Extra-floral nectaries (EFNs) are structures that produce sugar rich nectar outside flowers (Bentley, 1977). In cotton they are found on the underside of the leaves. This nectar attracts predators or parasitoids to the plant, which helps the plant with the defence. The nectar production of cotton EFNs is increased after insect damage, which can increase the recruitment of beneficials such as ants (Schoonhoven et al., 2005, Wackers & Bonifay, 2004).

Domestication of cotton

The domesticated cotton species are geographically separated and therefore they have been domesticated separately at different times and places. There are currently four species of cotton, which were domesticated: *G. barbadense*, *G. hirsutum*, *G. herbaceum* and *G. arboretum*. Two of these were domesticated in the new world (*G.barbadense*, *G. hirsutum*) and are tetraploid while the remaining two were domesticated in the old world and are diploids. Archaeological finds have shown that cotton may have been domesticated to be a feed for animals 4500 years ago (Fryxell, 1979).

Selection of cotton

From the time cotton was domesticated up until today, growers of cotton have selected plants based on different traits. The first trait that was selected was the amount of fibre around the seed. Later bigger fruits became an important trait in the selection. When agriculture became more intense pests became an increasing problem and one solution for this was to select for early varieties. Another trait selected was hairy plants, which provided resistance against certain insect pests. The next step was fibre quality, which became important at the time of the industrial revolution. Quality in this case means uniform, long and strong fibres, which could be used in the industrialized production of fabric (Fryxell, 1979).

In the 1970's there was some breeding done to create cultivars with better resistance against insects and diseases. Cultivars adapted to local conditions were also developed (Lusas & Jividen, 1987). In later years a lot of focus in cotton breeding has been on transgenic cultivars, such as Bt-cotton, which are useful tools for the control of pest insects (Wu et al., 2008). Until recently it was thought that it was the crystals in the toxins that killed cells by making holes in the cell membranes. Recently however, it was shown that a Bt-toxin binds to a Mg^{2+} -dependent signaling system in the insect's cells and thereby kills the cell (Zhang et al., 2005). However, insects have been shown to be able to develop resistance against the Bt-toxins (Akhurst et al., 2003).

All these modifications are probably 'not for free' and there is some evidence that domestication weakened the insect resistance in cotton. Additionally, with the rise of modern pesticides, breeders started to breed varieties for environments with a low herbivore pressure, e.g. discontinuation of early varieties (Bottrell & Adkisson, 1977). Since the 1940`s there have been trials with glandless cotton, varieties without glands and therefore very low levels

of Gossypol (Lusas & Jividen, 1987), which leads to that the plants have a very low defensive capacity against insect herbivores (Sunilkumar et al., 2006). However, they have the benefit of producing seeds and plant material which are not toxic to humans, which means they can be used to produce food or used as feed for livestock (Lusas & Jividen, 1987, Fryxell, 1979).

Some primitive and undomesticated varieties of cotton have been shown to have higher resistance to insect attacks than domesticated cotton due to higher levels of terpenoids (Stipanovic et al., 1978). This effect has also been shown in other species where there were no release of volatiles in domesticated varieties (Tamiru et al., 2011). *Gossypium raimondii* has better defence than *G. hirsutum*, which might be explained by the presence of a special terpenoid called Raimondal (Stipanovic et al., 1980). Furthermore, a naturalized variety of cotton was shown to produce much more volatiles than domesticated cotton when attacked by herbivores (Loughrin et al., 1995), which could make it more attractive to natural enemies

Objectives and hypothesizes

In this project I investigated how different varieties of cotton, with different levels of domestication, could affect herbivores and if domestication had a negative impact on the insect resistance.

Therefore we conducted three different experiments. First I did a feeding assay with larvae of the generalist moth *Spodoptera littoralis* (Noctuidae: Lepidoptera) were reared on different species and varieties of cotton until pupation. Secondly, a two choice preference trial with the generalist root feeders *Agriotes* spp. (Elateridae: Coleoptera) was performed. Lastly I did an odour collection on cotton plants that were either damaged by *S. littoralis*, *Agriotes* spp. or with a combination of both species.

I hypothesize that:

1. Larvae will develop slower on wild varieties of cotton due to their better insect resistance
2. In the two choice assays *Agriotes* larvae will avoid wild cultivars
3. Plants release different blend of volatiles depending on what pests are attacking them

Materials and Methods

Spodoptera littoralis

Spodoptera littoralis from a laboratory colony (Alnarp x wild strain) were used for these experiments. The animals were originally collected in Egypt and the population is regularly refreshed with wild moths. The larvae were reared in a climate chamber at 25°C, 70% relative humidity and 16 L : 8 D long-day conditions and fed with artificial diet based on potato (Hinks & Byers, 1976).

Agriotes spp.

Agriotes spp. larvae were collected in an apple orchard on the Alnarp campus during autumn 2013 and spring 2014. Larvae were kept in a box (30*10cm) containing soil and were fed with potatoes. Climate conditions were 25°C, 70% relative humidity and 16 L : 8 D long-day conditions.

Plants

A selection of wild and domesticated cotton plants were used in these experiments. From the species *G. hirsutum*, which stands for more than 90% of the world's production of cotton (Wendel & Cronn, 2003) the following four plant types were used:

- DPL 90 (Deltapine 90) is a commercial available cotton variety from Delta & Pine Land company (now part of Monsanto)
- TX 8702gl is a glandless cotton variety released by Texas Agricultural Experiment Station (TABS)
- TX 263, which was originally collected in Oaxaca, Mexico.
- TX 2259 is a naturalized cotton variety, which releases high levels of volatiles when attacked by insects (Loughrin et al., 1995). The seeds were collected in the Everglades, FL (USA).

Furthermore two other species of cotton was used.

Gossypium herbaceum is a species of cotton originating in the Indus valley in India. G. herbaceum was domesticated at around the same time as *G. hirsutum* i.e. around 8000 to 4000 years ago (Hancock, 2004). The seeds originate from India.

Gossypium raimondii is a new world species of cotton that contains a chemical called Raimondal which gives resistance against insect attack (Stipanovic et al., 1980). The seeds used in the experiment originate from Peru.

Lastly cabbage (*Brassica oleracea* v. *capitata*, Brassicaceae) was also used as it is known to be an inferior host plant for *S. littoralis* (Thoming et al., 2013). The cultivar was Stenhuvud from Weibulls.

All species and varieties were potted in 1.5 L pots with a commercial substrate (Kronmull, Weibull Trädgård AB, Hammenhög, Sweden) and were grown at 22°C, 75% relative humidity.

Feeding Assay

The effect of different cotton varieties on the development of insect herbivores was tested with *S. littoralis*., the larvae were allowed to emerge overnight and placed in individual 30 ml cups with a plaster layer on the bottom to keep the leaves from wilting. A leaf disc covering the plaster was cut and placed on the wet plaster before the larvae were put in. Once the larvae reached 4th instar they were moved to 250 ml individual cups and fed with complete leaves or large pieces of leaves. Leaves were kept fresh with damp cotton wrapped around the petioles. Larvae were fed with either artificial diet or leaves of various wild and domesticated cotton plants. Additionally, cabbage was used as a control treatment. Plants used during the experiment had at least 4-6 true leaves. Leaves were changed as they dried out or when consumed and artificial diet was changed daily.

To control the effects of plant quality, leaves from a single plant were fed exclusively to four larvae. For one run of the experiment three plants of each of the species and varieties listed above under "Plants" were selected. Twelve insects per diet were used for each run. The larvae were taken from three different egg batches and were distributed accordingly. The experiment was conducted in four different runs making a total of 48 repeats per diet, fed with leaves from 16 different plants. Treatments were randomized. Survival, pupal weight and development time, total and for each larval stage, was recorded. The experiment ended when all larvae had pupated or died.

Agriotes distribution assay

In a box (30*10cm) filled with soil, two different cotton plants were planted in opposite corners. Comparisons were made between DPL 90 and the other cotton varieties listed above under "Plants". In each box six *Agriotes* larvae were placed on the top of the soil between the two plants and were allowed to enter the soil. After a week the larvae's distribution was recorded by examining the position of each larva: within the roots of plant A, within the roots of plant B, if the larvae had died or if they had not made any decision, i.e. the larvae were found in the soil between the cotton plants. The experiment was repeated five times per treatment.

Induced volatile release in DPL 90

DPL 90 cotton plants were infested with either *S. littoralis*, the root feeder *Agriotes* spp. or by both herbivores. After three days S. littorals larvae were removed, while the wireworms were allowed to stay in the soil for the full experimental period. A week after the insect attack started, head space collections of volatiles was performed.

The plants were put in sealed, airtight plastic cooking bag (35x45 cm). Extraction was done using standard aeration columns (length: 60 mm; width: 3mm) using Porapak (60-80 mesh) as adsorbent. Flow rate through each column was established beforehand using a flow-meter (flow-rate: 100– 200 ml/min). Before use the columns were cleaned two times with each methanol, dichloromethane and pentane and dried with nitrogen. The columns were connected to aquarium pumps and inserted into the bags in a top corner of the bag. Incoming replacement air was filtered over charcoal and supplied from a lower corner of the bag diagonally from the column to get an air flow across the plant. The supplied air was ordinary air from the room that the odour collection was done in. Collection of samples were terminated after one 24 hour cycle. Volatiles were extracted from the column using 0.5 ml of pentane. The extracts were stored in a freezer at -20°C until they could be run on the GC-MS.

Analysis of the extracts was done using a 689N Network GC System (Agilent Technologies, Santa Clara CA, USA) GC-MS. A USB351916H 5% phenyl /95% Methyl Siloxane (Length 60meters. diameter 0,25 mm. df 0.25 µm) (Agilent Technologies, Santa Clara CA, USA) column was used as a stationary phase. Helium was used as a carrier gas, with a pressure of 26.69 psi, resulting in a gas flow of 1.9 ml/min or 35 cm/sec. A temperature program with the following specifications was used 0.0–2.00 min: 50°C, 2.01-28 min: temperature was

increased by 8°C/min to 275°C. The temperature was then maintained at 275°C for 10 minutes. A post-run temperature of 300°C was maintained for one minute. Total run time: 40.13 min. The detection of compounds was done using MS with an electron spray ionisator. The MS was set to the following conditions: solvent delay 4:30 min, 70eV ionization energy. Scanning occurred between 29 m/z and 400 m/z. The database used for identification of compounds was Alnarp11. To make verify that the compounds were what the program identified them as Kovats retention indexes were calculated and compared to values for wax columns at pherobase.net.

Statistics

The programmes used were Minitab statistical software 16 and R 3.0.2. The larval mortality and the virus infections was analyzed with a χ2 test in Minitab comparing all other treatments to the domesticated variety Dpl 90. Total larval development time, duration of instars and pupal weight were analyzed in Minitab with ANOVA:s and then followed up by Tukey tests. Lastly the data from the Agriotes two choice trial was analyzed in R using a McNemar's test.

Results

Feeding Assay

During the feeding assay no significant differences in mortality was detected between DPL 90 and the remaining treatments, except for cabbage, which had a significantly lower mortality (p-value 0,038; X2=4,286, df=1) (Table 1).

Tab. 1 Larval mortality. Mortality of *S. littoralis* larvae between 1st instar and pupation, when being fed on different types of *Gossypium*, artificial diet or cabbage. Data was analysed using a χ2 test comparing DPL 90 and the other treatments. (n-values=48 for all treatments).

	Cabbage	DPL 90	Artificial diet	Glandless	Tx 263	Tx 2259	G. herbaceum	G. raimondii
Alive	33	23	23	29	22	24	20	25
Dead	15	25	25	19	26	24	28	23
Mortality %	31,25	52,08	52,08	39,58	54,17	50	58,33	47,92
P-value	0,038	X	1	0,219	0,838	0,838	0,538	0,683
χ2	4,286	X	0	1,151	0,042	0,042	0,379	0,167
DF	1	X	1	1	1	1	1	1

Tab. 2. Total larval development time. Mean development time of *S. littoralis* larvae (days ± standard error) when fed on different types of *Gossypium*, artificial diet or cabbage. Means within one line followed by different letters are significantly different (p ≤ 0.05; Tukey HSD test).

	Total development time (Days)	n-values
G. raimondii	21,6±0,07B	25
G. herbaceum	25,1±0,10A	20
Tx2259	26,3±0,14A	24
Tx263	26,7±0,22A	22
Glandless	18,4±0,05C	29
Artificial diet	18,6±0,06C	23
Dpl 90	20,0±0,07BC	23
Cabbage	19,5±0,04C	33
ANOVA P-value	0,000	

Tab. 3 Duration of instars. Mean duration of larval instars of *S. littoralis* (days ± standard error) when fed on different types of *Gossypium*, artificial diet or cabbage. Means followed by different letters are significantly different (p ≤ 0.05; Tukey HSD test).

Diet	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	n-values
G. raimondii	5,4±0,04A	3,4±0,07BC	4,7±0,08ABC	2,3±0,04B	3,2±0,05AB	2,2±0,06ABC	25
G. herbaceum	6,0±0,03A	4,4±0,11AB	5,4±0,12A	2,9±0,07AB	2,8±0,058C	3,4±0,08A	20
Tx2259	5,7±0,05A	5,9±0,140A	3,9±0,07ABC	3,2±0,05AB	4,3±0,07A	3,3±0,10AB	24
Tx263	5,8±0,08A	4,3±0,09AB	5,1±0,11AB	3,9±0,09A	4,3±0,14AB	3,2±0,09ABC	22
Glandless	5,4±0,04A	2,5±0,030C	3,6±0,04C	2,4±0,03B	2,6±0,04C	1,9±0,04BC	29
Artificial diet	5,23±0,04A	2,4±0,04C	3,7±0,05C	2,4±0,04B	2,1±0,04C	2,8±0,05ABC	23
Dpl 90	5,2±0,04A	2,6±0,07C	4,4±0,07ABC	2,4±0,05B	2,9±0,04BC	2,9±0,07ABC	23
Cabbage	5,53±0,03A	3,4±0,05BC	3,4±0,04C	2,7±0,03B	2,7±0,02C	1,9±0,03C	33
ANOVA P-value	0,172	0,000	0,000	0,000	0,000	0,001	

Development time of larvae was however significantly prolonged in larvae fed with wild cotton plants (F =50,53 ; df =198 ; p <0,001). The total development time from emergence to pupation was longer in *G. herbaceum*, Tx263 and Tx2259, while DPL 90, Glandless cotton and artificial diet had the shortest development time. Cabbage and *G. raimondii* had intermediate development times (Table 2). There was no significant difference between the different diets during the first instar (F =1,49 ; df =201 ; p =0,172), but for later instars there was a difference (Table 3). On average, there was a one week difference between the diets which promoted the fastest pupation, i.e. glandless cotton, and the diet which delayed pupation the most i.e. Tx 263.

Tab. 4. Weight of pupae. Mean weight of *S. littoralis* pupae (mg ± standard error) when being fed with different types of *Gossypium*, artificial diet or cabbage. Means within one line followed by different letters are significantly different (p ≤ 0.05; Tukey HSD test).

Treatment	Weight (mg)	n-values
G. raimondii	198,77±1,179C	25
G. herbaceum	227,65±2,725BC	20
Tx2259	231,37±2,305BC	24
Tx263	206,05±2,966C	22
Glandless cotton	238,15±2,431BC	29
Artificial diet	335,41±3,414A	23
Dpl 90	238,65±2,197BC	23
Cabbage	258,43±2,423B	33

Additionally, there was a significant difference between in the pupal weights depending on larval diet (F =11,47 ; df =176 ; p <0,001) (Table 4). Artificial diet lead to the highest pupal weights followed by cabbage, while *G. raimondii* and Tx 263 had the lowest pupal weights. The only significant difference between treatments was between artificial diet and all the other treatments.

Tab 5. Virus infections. Mortality of *S. littoralis* larvae due to virus between 1st instar and pupation, when being fed on different types of *Gossypium*, artificial diet or cabbage. Data was analysed using a χ^2 test comparing DPL 90 and the other treatments. (n-values=48 for all treatments).

	Cabbage	DPL 90	Artificial diet	Glandless	Tx 263	Tx 2259	*G. herbaceum*	*G. raimondii*
Healthy larvae	43	41	48	44	43	41	42	43
Virus	5	7	0	4	5	7	6	5
P-value	0,537	-	0,006	0,336	0,537	1	0,765	0,537
χ^2	0,381	-	7,551	0,924	0,381	0	0,089	0,381
DF	1	-	1	1	1	1	1	1

In addition, I investigated if there was a connection between virus infection and diet (Table 5), no significant differences between the treatments were found.

Agriotes choice assay

Tab. 6 Agriotes two choice test. Data was analysed using a McNemar's test comparing DPL 90 and the other treatments.

	Dpl vs. Glandless	Dpl vs. *G. raimondii*	Dpl vs. Tx263	Dpl vs. Tx263	Dpl vs. Tx2259	Dpl vs. *G. herbaceum*
Larvae on DPL	13	15	13	13	7	7
Larvae on other plant	4	6	6	7	9	9
$\chi 2$	3.7647	3.0476	1.8947	1.25	0.0625	0.0625
df	1	1	1	1	1	1
p-value	0.05235	0.08086	0.1687	0.2636	0.8026	0.8026

There was no significant difference in the choice behaviour of *Agriotes* spp. when they were presented with different cotton varieties. However, in all comparisons, except between DPL 90 and *G. herbaceum*, significance was close to the significance threshold (Table 6).

Odour blend of damaged cotton plants

Tab. 7 Volatile blends. Compounds released from insect damaged DPL 90 *G. hirsutum* plants during a 24 h time period (µg ± standard error).

Compound	*Spodoptera*	*Agriotes*	Spodoptera+Agriotes
Alpha Pinene	18355±3786	4851±307	10603±1994
1S-beta Pinene	2148±399	410±100	1554±369
Myrcene	1194±153	354±166	182±86
Gamma-Terpinene	254±85	272±12	253±23
Terpinolene	410±116	130±31	0±0
Camphene	60±28	0±0	53±25
3-Carene	0±0	85±22	59±28

Comparing the composition of odour plants of herbivore damaged cotton plants, the following was observed: the amount of alpha-pinene, 1S-beta-Pinene and myrcene was higher in plants infested with *S. littoralis* than in plants infested with *Agriotes* spp. Also, terpinolene follows the pattern of having a higher level in plants with *S. littoralis* and lower in the presence of *Agriotes* spp. The plants with both species of larvae have levels of these compounds intermediate to the other treatments. The other identified compounds, camphene, 3-carene and gamma-terpinene do not vary very much depending on what insects are infesting the plants (Table 7).

Discussion

During the course of evolution plants have developed many different ways to defend themselves against insect herbivores. These can be chemical or morphological, induced or constitutive and direct or indirect as explained above but their purpose is to make the conditions for herbivores trying to use the plant as a host plant as unfavorable as possible. The results showed that domesticated cotton varieties generally mediated shorter developing times than wild varieties for *S. littoralis* larvae, which mean that domestication and selection may have lowered the defensive capacity of the plants. The *Agriotes* two-choice trial showed no significant results but a clear trend towards preference for Dpl 90 in all treatments except *G.herbaceum*. Lastly the odour collection showed that some induced volatiles in the cotton were lower if *Agriotes* were on the plants than when they had not been.

We sought to investigate if domestication has lowered the defensive abilities of cotton in trade-offs with other traits such as yield or whiteness of fibre. The importance of the defence can be seen when looking at the results for glandless cotton. Cotton seeds with very low levels of pigment glands were first observed in the late 1940: s and during the next decennium some crosses were made until a glandless seed was created. During the 60's further crosses between glandless and commercial cultivars where made (Lusas & Jividen, 1987). Since the Gossypol is located in these glands (Fryxell, 1979) plants without glands had very small amounts of Gossypol. A big problem with glandless cotton is that it has very low resistance to insect attack because of the lack of gossypol (Sunilkumar et al., 2006). This was shown in the feeding assay where glandless cotton leads to the shortest development time of the larvae. Short development time means that the larvae have better conditions on the glandless cotton than on other varieties.

The wild cotton varieties' (Tx266, Tx2259, *G.raimondii* and *G. herbaceum*) better resistance could be seen in longer development time and in the case of Tx263 and *G.raimondii* and a lower pupal weight than the other treatments. Pupal weight is often used as an indicator of fitness (Carriere et al., 2004) so even if survival of the larvae was not lowered the adult moth might be affected . Dpl 90 and Glandless cotton had shorter development time than the other cottons, however the pupal weight did not vary much. Cabbage was included as a poor host as it has been reported to delay development of *S.littoralis* larvae (Thöming et al., 2013). The cabbage contains glycosinolates that are toxic to many insect generalist herbivores and also can work as a feeding deterrent due to its bitter taste (Schoonhoven et al., 2005). Larvae also

react differently depending on instar. Older larvae are affected less by growth inhibitors than younger larvae (Wiseman et al., 1996). Even though pupal weight is used as a indicator of fitness it is likely that the larvae on the artificial diet are actually to big as pupae. In nature, individuals this big would have trouble competing with lighter conspecifics and would be out selected. The relatively short development time of *G. raimondii* compared to *G. herbaceum* might be a bigger advantage for the larvae than pupal weight. A shorter development time means that the larvae get a head start and might even be able to have an extra generation during a growing season.

In some cases plants without defensive chemicals might prove to be a disadvantage for larvae. Linalool is a green leaf volatile released from many different plants when they are under herbivore attack (Schoonhoven et al., 2005) and it is also a compound present in some male insects sexpheromones (Landolt & Heath, 1990). This means that male moths raised on plants with low amounts of linalool might be less attractive to females than larvae raised on better defended plants.

In Table 2 it shows that there is a difference in the variance between slow developing diets (*G.herbaceum*; 0,10; Tx2259; 0,14 and Tx263; 0,22) and the diets which promoted the fastest growth (Glandless cotton 0,05; Dpl 90 0,07; Artificial diet 0,06 and cabbage 0,04). This implies that the undomesticated varieties are less genetically uniform meaning that there could be many different genes or traits that offer resistance within the cultivar, but are not necessarily present in each individual plant. Glandless cotton is of special interest as it not only is based on domesticated cottons that have been developed through intensive breeding to not have gossypol (Fryxell, 1979). This can help to explain why glandless cotton lead to one of the shortest development times of all diets. The selection process have lowered the general defensive ability of the cotton plant and the lack of gossypol further makes glandless cotton an ideal host for insect herbivores (Sunilkumar et al., 2006).

The wild cotton varieties could be used in breeding programs to develop more resistant varieties. The strains Tx263 and Tx 2259 could be bred into commercial cultivars to create new insect resistant cultivars that are cheaper for farmers. This could help farmers that do not use Bt-cotton or pesticides, either if they cannot afford it, or if they just do not want to use it. As the variance was relatively high, the plants with the longest development times would have to be identified from these strains.

One important thing to note is that there are other factors that may have effected the outcome of the trial. Domesticated plants have been shown to have fewer stomata than wild varieties (Navea et al., 2002). This could mean that the wild varieties lost water faster than the domesticated ones meaning that their quality deteriorated faster. The slow development might actually just be due to the wild varieties losing nutrients faster.

Domesticated of plants has in some cases lead to leafs that are less though. Tougher leaves are avoided by ovipositioning females (Bellota et al., 2013). The reson for this is unclear, possibly the insects cannot oviposit or perhaps they find the host plant unsuitable for the larvae due to the toughness of the leaves.

In the feeding assay the development time was recorded. For future studies it would be interesting to investigate what made the larvae develop slower on wild varieties. Also a comparison of volatile release between the different *G. hirsutum* varieties would be interesting to investigate if they release the same volatiles and if so, how much does it vary?

The volatile emissions in the odour collections differed depending on which larvae induced the plant (Table 7). In the plants where *Agriotes* spp. were present the levels of alpha pinene, 1S-beta pinene, terpinolene and myrcene were lower than when only *S.littoralis* where present. The lowest release of volatiles occurred when only *Agriotes* larvae induced the plants. Possibly, *Agriotes* spp. can manipulate the plant to not produce as much defensive compounds. It has been shown that some insects have compound in their saliva that can disrupt the induction of the plants induced defences (Weech et al., 2008). Another group of scientists showed that bacteria that live in the gut of Colorado potato beetle (*Leptinotarsa decemlineata*) can be transferred from the beetle and lower the plants defensive response (Chung et al., 2013). However, the fact that *Agriotes* spp. does induce a response in above ground defence is in line with earlier findings (Bezemer et al., 2004). The other compounds did not vary between the treatments; this could be because they are not part of the induced defence.

As the volatiles were only collected from the aboveground parts of the plants, any volatiles released by the roots in the soil would not be detected by this experimental setup. In maize, it was shown that below ground herbivores can induce the roots to start releasing (E)-beta-

caryophyllene, a sesquiterpene which attracts a nematode that attacks the insect herbivores (Hitpold & Turlings, 2008).

The *Agriotes* two choice trial did not give any significant results. There is however a clear tendency in all treatments except *G.herbaceum* that the larvae prefer Dpl 90. The preference for Dpl 90 might be proven with a larger sample size. A resent trial showed that *Agriotes* spp. larvae do not move unless their food source is depleted (Sonnemann et al., 2014). This might explain why there were no significant results in my trial. In a trial with *Agriotes ustlatus* it was shown that the larvae can migrate 60 cm down in the soil if the conditions at soil level is unfavourable, for example to dry or to cold (Furlan, 1998). This shows that *Agriotes* larvae have the ability to move away from bad host plants. If the larvae do prefer Dpl 90 it would be in line with the findings in the feeding assay that domesticated plants are better hosts for insect herbivores.

Today 95% of the cotton produced is of the species *G. hirsutum* (McDougall et al., 1993) but that does not necessarily mean that it is the best species for every farmer in every land. There most likely are local adaptations to pests in for example *G. herbaceum* that have evolved together with pests in India. There are also cultural values in keeping old varieties and cultivars that have been grown for centuries in an area like *G. herbaceum* in India and *G. arboreum* in China (Fryxell, 1979).

Lastly, Bt-cotton might not be the ultimate solution to pest problems. As I have shown in this report inbreeding and domestication of plants lead to plants with weaker defences. Bt-cottons are intensively bred varieties that have been genetically engineered putting one or a few genes in that produce toxins that kill insect herbivores feeding on the cotton (Fryxell, 1979). There are however evidence that suggest that pests are developing resistance against these toxins (Gassmann et al., 2014). If or when the insects overcome the Bt-toxins the cultivars are just as vulnerable as any other cotton variety to the resistant insect strains. Therefore, it is important to look into more resistance traits and investigate further what makes the wild cottons more resistant. The more of these resistance traits that can be identified and bred or genetically engineered into cotton varieties the better protection they will have. However the new resistance traits might come with the price of a trade off with yield.

Conclusions

The findings in this report show that there is a clear effect that domesticated plants have a weaker defence than wild species. Dpl 90, glandless cotton, Tx269 and Tx2259 are all the same species yet they vary from the shortest development time to the longest. This knowledge could also be used by plant breeders, especially ones that do not want to use Bt-cotton for different reasons. The fact that the variance was bigger in the wild cotton varieties shows that they are more genetically diverse than the domesticated varieties. The genetic diversity within the wild strains means that within the cultivar resistance varies and different traits are expressed at different levels. The *Agriotes* two choice trial showed no significant differences but a clear trend that the larvae preferred Dpl 90 over all other cultivars except *G.herbaceum*. Also this indicates that domestication have made the plants more attractive for pests.

Reference list

Akhurst, R. J., James, W., Bird, L. J. & Beard, C. 2003. Resistance to the cry1ac delta-endotoxin of bacillus thuringiensis in the cotton bollworm, Helicoverpa armigera (lepidoptera : Noctuidae). *Journal of Economic Entomology*, 96, 1290-1299.

Bellota, E., Medina R. F. & Bernal, J. S. 2013. Physical leaf defenses- altered by Zea life-history evolution, domestication, and breeding - mediate oviposition preference of a specialist leafhopper. *Entomologia experimentalis et applicata*. 149. 185-195.

Bezemer, T. M., Wagenaar, R., Van Dam, N. M., Van Der Putten, W. H. & Wackers, F. L. 2004. Above- and below-ground terpenoid aldehyde induction in cotton, Gossypium herbaceum, following root and leaf injury. *Journal of Chemical Ecology*, 30, 53-67.

Carriere, Y., Ellers-Kirk, C., Biggs, R., Higginson, D. M., Dennehy, T. J. & Tabashnik, B. E. 2004. Effects of gossypol on fitness costs associated with resistance to bt cotton in pink bollworm. *Journal of Economic Entomology*, 97, 1710-1718.

Chung, S. H., Rosa, C., Scully, E. D., Peiffer, M., Tooker, J. F., Hoover, K., Luthe, D. S. & Felton, G. W. 2013. Herbivore exploits orally secreted bacteria to suppress plant defenses. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 15728-15733.

Fordyce, J. A. & Shapiro, A. M. 2003. Another perspective on the slow-growth/high-mortality hypothesis: Chilling effects on swallowtail larvae. *Ecology,* 84**,** 263-268.

Fryxell, P. A. 1979. *The natural history of the cotton tribe,* Collages station Texas, Texas A&M university press.

Furlan, L. 1998. The biology of Agriotes ustulatus schaller (col., elateridae). Ii. Larval development, pupation, whole cycle description and practical implications. *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie,* 122**,** 71-78.

Gassmann, A. J., Petzold-Maxwell, J. L., Clifton, E. H., Dunbar, M. W., Hoffmann, A. M., Ingber, D. A. & Keweshan R. S. 2014. Field-evolved resistance by western corn rootworm to multiple Bacillus thuringiensis toxins in transgenic maize. *Proceeding of the national academy of sciences of the United States of America*, 111,5141-5146.

Hagenbucher, S., Olson, D. M., Ruberson, J. R., Wackers, F. L. & Romeis, J. 2013. Resistance mechanisms against arthropod herbivores in cotton and their interactions with natural enemies. *Critical Reviews in Plant Sciences,* 32**,** 458-482.

Hancock, J. F. 2004. *Plant evolution and the origin of crop species,* Cambridge, Ma, USA, Cabi publishing.

Hiltpold, I. & Turlings, T. C. J. 2008. Belowground chemical signalling in maize: When simplicity rhymes with efficiency. *Journal of chemical ecology*, 34, 628-635.

Hinks, C. F. & Byers, J. R. 1976. Biosystematics of genus euxoa (lepidoptera-noctuidae) .5. Rearing procedures, and life-cycles of 36 species. *Canadian Entomologist,* 108**,** 1345-1357.

Karban, R. 2011. The ecology and evolution of induced resistance against herbivores. *Functional Ecology,* 25**,** 339-347.

Kariyat, R. R., Balogh, C. M., Moraski, R. P., De Moraes, C. M., Mescher, M. C. & Stephenson, A. G. 2013. Constitutive and herbivore-induced structural defenses are compromised by inbreeding in Solanum carolinense (solanaceae). *American Journal of Botany,* 100**,** 1014-1021.

Kariyat, R. R., Mauck, K. E., De Moraes, C. M., Stephenson, A. G. & Mescher, M. C. 2012. Inbreeding alters volatile signalling phenotypes and influences tri-trophic interactions in horsenettle (Solanum carolinense l.). *Ecology Letters,* 15**,** 301-309.

Landolt, P. J. & Heath, R. R. 1990. Sexual role reversal in mate finding strategies of the cabbage looper moth. *Science*, 249, 1026-1028

Loughrin, J. H., Manukian, A., Heath, R. R. & Tumlinson, J. H. 1995. Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *Journal of Chemical Ecology,* 21**,** 1217-1227.

Lusas, E. W. & Jividen, G. M. 1987. Glandless cottonseed - a review of the 1st 25 years of processing and utilization research. *Journal of the American Oil Chemists Society,* 64**,** 839-854.

Massei, G. & Hartley, S. E. 2000. Disarmed by domestication? Induced responses to browsing in wild and cultivated olive. *Oecologia,* 122**,** 225-231.

McDougall, G. J., Morrison, I. M., Stewart, D., Weyers, J. D. B. & Hillman, J. R. 1993. Plant fibers - botany, chemistry and processing for industrial use. *Journal of the Science of Food and Agriculture,* 62**,** 1-20.

Navea, C., Terrazas, T., Delgado-Salinas, A. & Ramirez-Vallejo,P. 2002. Foliar response of wild and domesticated Phaseolus vulgaris L. to water stress. *Genetic resources and crop evolution*, 49, 125-132.

Price, P. W., Bouton, C. E., Gross, P., McPheron, B. A., Thompson, J. N. & Weis, A. E. 1980. Interactions among 3 trophic levels - influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics,* 11**,** 41-65.

Rodriguez-Saona, C., Vorsa, N., Singh, A. P., Johnson-Cicalese, J., Szendrei, Z., Mescher, M. C. & Frost, C. J. 2011. Tracing the history of plant traits under domestication in cranberries: Potential consequences on anti-herbivore defences. *Journal of Experimental Botany,* 62**,** 2633-2644.

Schoonhoven, L. M., van Loon, J. J. A. & Dicke, M. 2005. *Insect-plant biology,* New york, Oxford University press.

Sonnemann, I., Grunz, S. & Wurst, S. 2014. Horizontal migration of click beetle (Agriotes spp.) larvae depends on food availability. *Entomologia Experimentalis Et Applicata,* 150**,** 174-178.

Stipanovic, R. D., Bell, A. A. & Obrien, D. H. 1980. Raimondal, a new sesquiterpenoid from pigment glands of Gossypium-raimondii. *Phytochemistry,* 19**,** 1735-1738.

Stipanovic, R. D., Bell, A. A., Obrien, D. H. & Lukefahr, M. J. 1978. Heliocide h-1 - new insecticidal c-25 terpenoid from cotton (gossypium-hirsutum). *Journal of Agricultural and Food Chemistry,* 26**,** 115-122.

Sunilkumar, G., Campbell, L. M., Puckhaber, L., Stipanovic, R. D. & Rathore, K. S. 2006. Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. *Proceedings of the National Academy of Sciences of the United States of America,* 103**,** 18054-18059.

Tamiru, A., Bruce, T.J.A., Woodcock, C.M., Caulfield, J.C., Midega, C.A.O., Ogol, C.K.P.O., Mayon, P., Birkett, M.A., Pickett, J.A. & Khan, Z.R. 2011. Maize landraces recruit egg and larval parasitoids in response to egg deposition by a herbivore. *Ecology letters*, 14, 1075-1083.

Thoming, G., Larsson, M. C., Hansson, B. S. & Anderson, P. 2013. Comparison of plant preference hierarchies of male and female moths and the impact of larval rearing hosts. *Ecology,* 94**,** 1744-1752.

Wackers, F. L. & Bonifay, C. 2004. How to be sweet? Extrafloral nectar allocation by Gossypium hirsutum fits optimal defense theory predictions. *Ecology,* 85**,** 1512-1518.

Weech, M. H., Chapleau, M., Pan, L., Ide, C. & Bede, J. C. 2008. Caterpillar saliva interferes with induced arabidopsis thaliana defence responses via the systemic acquired resistance pathway. *Journal of Experimental Botany,* 59**,** 2437-2448.

Wendel, J. F. & Cronn, R. C. 2003. Polyploidy and the evolutionary history of cotton. *Advances in Agronomy, Vol 78,* 78**,** 139-186.

Wiseman, B. R., Carpenter, J. E. & Wheeler, G. S. 1996. Growth inhibition of fall armyworm (lepidoptera: Noctuidae) larvae reared on leaf diets of non-host plants. *Florida Entomologist,* 79**,** 302-311.

Wu, K. M., Lu, Y. H., Feng, H. Q., Jiang, Y. Y. & Zhao, J. Z. 2008. Suppression of cotton bollworm in multiple crops in china in areas with bt toxin-containing cotton. *Science,* 321**,** 1676-1678.

Zakir, A., Bengtsson, M., Sadek, M. M., Hansson, B. S., Witzgall, P. & Anderson, P. 2013. Specific response to herbivore-induced de novo synthesized plant volatiles provides reliable information for host plant selection in a moth. *Journal of Experimental Biology,* 216**,** 3257-3263.

Zangerl, A. R. 2003. Evolution of induced plant responses to herbivores. *Basic and Applied Ecology,* 4**,** 91-103.