Effect of Touch on Cotton and Maize

- Influence on plant morphology and feeding preference in *Spodoptera littoralis*

Anna Magnusson

Independent project • 15 credits
Swedish University of Agricultural Sciences, SLU
Faculty of Landscape Architecture, Horticulture and Crop Production Science (LTV)
Horticultural Management – Bachelor’s Programme
Alnarp 2022

Effect of Touch on Cotton and Maize

- Influence on plant morphology and feeding preference in *Spodoptera littoralis*

Beröring av bomull och majs: påverkan på växtmorfologi och födopreferens hos en skadegörare

Anna Magnusson

Supervisor:	Peter Anderson, SLU, Department of Plant Protection Biology
Assistant supervisor:	Velemir Ninkovic, SLU, Department of Ecology
Examiner:	Mattias Larsson, SLU, Department of Plant Protection Biology

Credits:	15 credits
Level:	G2E
Course title:	Independent Project – Biology
Course code:	EX0855
Programme/education:	Horticultural Management – Bachelor's Programme
Course coordinating dept:	Biosystems and Technology
Place of publication:	Alnarp
Year of publication:	2022
Cover picture:	*Egyptian Cotton Leafworm on cotton leaf.* Anna Magnusson.

Keywords:	Mechanical stimuli, cotton, maize, thigmomorphogenesis, *Spodoptera littoralis,* induced defence, plant-plant interaction

Swedish University of Agricultural Sciences
Faculty of Landscape Architecture, Horticulture and Crop Production Science (LTV)
Department of Plant Protection Biology
Chemical Ecology

Abstract

Plants are constantly exposed to touch and mechanical stimuli in natural environments, from rain, wind, animals and plant neighbours. However, current knowledge and understanding of how touch physiologically affects plants are limited. This experimental study aimed to explore whether morphological responses such as changes in growth could be expressed in cotton and maize plants as a result of a mechanical touch treatment. Previous studies have also found that mechanical touch treatments can induce increased resistance. Following these implications, effect on feeding preference in a herbivore was also examined. The treatment consisted of brief daily sweeping with a soft brush on fully mature leaves for fourteen days. Plant height and biomass distribution were measured in cotton and maize. Trichome and epidermal gland density were compared between touch-treated and untreated cotton plants. A dual choice test was conducted on cotton using the generalist Egyptian Cotton Leafworm (*Spodoptera littoralis*).

The touch treatment affected morphology in both plant species. Plant height was greater in touch-treated maize but reduced in treated cotton. Treated cotton plants allocated more biomass to leaves compared to other plant parts than in control plants, while no such difference in distribution of biomass was found in maize. Observations of trichome and epidermal gland density indicated an increase of these in touch-treated plants. The larval dual choice test did not show any significant difference in feeding preference for any of the treatments. These results suggest that touch may elicit neighbour detection-related responses, but may not be a strong enough stimulus to induce herbivore-related plant defences.

Keywords: Mechanical stimuli, cotton, maize, thigmomorphogenesis, Spodoptera littoralis*, induced defence, plant-plant interaction*

Sammanfattning

Växter utsätts ständigt för beröring och mekaniska stimuli i naturliga miljöer, från regn, vind, djur och angränsande växter. Men nuvarande kunskap och förståelse för hur beröring påverkar växter fysiologiskt är begränsad. Denna experimentella studie hade som syfte att undersöka huruvida morfologiska förändringar i tillväxt kunde uttryckas i bomulls- och majsplantor som ett resultat av en mekanisk beröringsbehandling. Vidare undersöktes effekter på födopreferens hos en växtskadegörare, då tidigare studier rapporterat inducerad resistens mot skadedjur som följd av mekanisk stimulans. Behandlingen bestod av daglig beröring med en mjuk borste på fullt utvecklade blad under två veckors tid. Planthöjd och biomassafördelning mättes i bomull och majs. Densitet av trikomer och epidermiska glander jämfördes mellan beröringsbehandlade och obehandlade bomullsplantor. Ett tvåvalstest utfördes på bomull med en generalistisk växtskadegörarlarv (*Spodoptera littoralis*).

Beröringsbehandlingen påverkade morfologin hos båda växtarterna. Plantorna var högre i behandlad majs men lägre i behandlad bomull jämfört med kontrollplantor. Behandlade bomullsplantor allokerade mer biomassa till blad jämfört med andra växtdelar än i kontrollplantor, medan ingen sådan skillnad i distribution av biomassa visades i majs. Observationer av trikomer och glander indikerade en ökning av dessa hos beröringsbehandlade bomullsplantor. Tvåvalstestet visade inte någon signifikant skillnad i födopreferens hos larven för någon av behandlingarna. Resultaten indikerar att beröring verkar kunna framkalla konkurrensliknande respons i växter, men är troligtvis inte en tillräckligt stark stimulans för att inducera växtförsvar mot herbivora skadegörare.

Keywords: Mekanisk stimuli, bomull, majs, thigmomorphogenes, Spodoptera littoralis*, inducerat försvar, växt-växtinteraktion*

Table of contents

List of tables

List of figures

Abbreviations and technical terms

ARR	Age-Related Resilience
ODT	Optimal Defence Theory
R/FR	Red/Far-red light
TA	Terpenoid Aldehydes

Allelopathy	One plant chemically inhibiting growth of others
Constitutive	Built-in, permanent structures or functions
Epidermal gland	Site of production of organic compounds on the exterior of plants
Herbivore	Used here to describe a plant-feeding insect
Imago	Adult stage of insect metamorphosis
Intraspecific	Interaction between organisms of the same species
Interspecific	Interaction between organisms of the different species
Mechanostimulation	Used here interchangeably with touch and mechanical stimuli
Morphology	Physical structure and form of plants
Natural enemies	Insects that prey or parasite on pests of plants
Petiole	Leaf stalk
Phytotoxic	Substances that cause adverse effects in plants
Plant neighbour	Plants growing adjacently in the same community
Secondary metabolites	Non-essential organic compounds produced in organisms
Volatiles	Compounds emitted by plant in biotic interactions

Introduction

Annually, biotic stress factors such as diseases and pests are estimated to be responsible for approximately 30% of staple crop losses worldwide (Ashraf et al 2012). Current plant protection practices raise a number of concerns. As food production expands, pesticide use increases, causing more damage to biodiversity, the environment and worker health. It has resulted in greater habitat loss and many ecosystems have experienced significant losses in species diversity and biomass (Beketov et al 2013; Hallmann et al 2017). These trends are not slowing down with the invention and usage of modern agrochemicals, and they inflict harm even at levels deemed safe for the environment (Beketov et al 2013). Nonetheless, a reduction in plant protection products may result in lower crop yields, greater food insecurity and threaten the viability of many farmers. The phasing out of pesticides and limited alternatives challenged by environmental, social and practical circumstances necessitates innovation and development of new and emerging crop protection practices. Implementation of integrated pest management practices, plant breeding advancements and use of biological control measures are on the rise and pave the way for a more sustainable crop production in the future (Held 2020).

Some emerging alternative plant protection methods are taking advantage of built-in defences towards pathogens and herbivores in plants (Xiaoning Zhang et al 2021; Burketova et al 2015; Walters et al 2013). Exposing plants to mild forms of stress may cause a ‘priming’ effect in the plant, similar to an immune response, which make the plants better prepared come real pathogenic or herbivoral attack (Conrath 2009). Currently, developments in abiotic priming for increased resilience for use in agricultural crop production has been focused mainly on chemical methods (Savvides et al 2016). New discoveries of physical and mechanical forms of abiotic priming agents are being examined for their applicability, with promising results (Ghosh et al 2021). However, further investigation is needed to fully understand the inner workings of plant defences in relation to external stimuli. Following this objective, this present study aimed to do just that: investigating whether touch as a physical priming agent could induce insect-related defences in plants, which would later alter a herbivore's feeding behaviour.

Plants and their environment

Plants are constantly interacting with their biotic and abiotic environment. Adverse consequences from some of these interactions, like drought or pest attack, has led to developmental adaptations to combat or persist in unfavourable conditions (Schoonhoven et al 2005). Plant survive attack from pests or pathogen through two main strategies: by tolerating or resisting the attacker (Pagán & García-Arenal 2018). Tolerance pertains to a host plant's ability to reduce the impact of the attack through developmental strategies (e.g. fast growth, delayed maturing or plant architecture) (Strauss & Agrawal 1999). Resistance, in contrast, relates to plants actively preventing or reducing attack altogether (Howe & Schaller 2008). The adoption of either of these strategies comes at different costs and are favourable under different conditions or developmental stages of the plant (Haukioja & Koricheva 2012; Boege et al 2007). Resistant defence strategies are typically divided into constitutive or inducible defences, that are either direct or indirect (Howe & Schaller 2008). Plants may possess both strategies simultaneously or adopt different strategies depending on factors such as development, conditions and resource cost strategy. Depending on life-history traits, it might be more resource friendly for the plant to possess constitutive defences in environments of high herbivore pressure, or over a longer lifetime (Dicke & Hilker 2003). On the other hand, if pest presence is low or scattered, it might make more sense to have defences on standby (Howe & Schaller 2008).

Moreover, there can be a spatial variation of defences over the plant, following the 'optimal defence theory' (ODT) (Hunziker et al 2021). ODT relates to a cost-benefit tradeoff for future plant fitness and reproduction. In a world of limited resources and time, plants cannot defend all plant parts equally. Inducing the best defences and allocating resources to the most valuable and vulnerable plant parts may reduce the pressure of biotic and abiotic stress factors (Meldau et al 2012). This results in a variation of type and strength of defences throughout the plant (Anderson & Agrell 2004). Results presented by Holeski (2007) indicated evidence of this theory further. In this experiment, simulated herbivore damage on early *Mimulus guttatus* leaves induced a stress response in later plant parts.

Defence strategies

Constitutive direct defences against herbivory concern physical barriers and chemical compounds that deter feeding (Kessler & Heil 2010). Examples of physical defences include trichomes, cuticular wax, rigid cell walls and lignification (Schoonhoven et al 2005). Trichomes reduce the speed or impede the movement of herbivores (Khan et al 1986; Shanower 2008), while rigid cell tissues inhibit grazing and wears down mouth parts (Schoonhoven et al 2005; Moore & Johnson 2017). Constitutive secondary metabolites are chemical compounds that are either toxic, bad tasting, antinutritional or digestive inhibitors to the herbivore (Howe & Schaller 2008). Some plants also have constitutive indirect defences by providing refuge, food or favourable egg laying sites that attract natural enemies of pests (Llandres et al 2019; Howe & Schaller 2008).

In the case of inducible defences, plants have a wide array of mechanisms to protect against pests. Upon pest detection, the plant may produce or direct anti-herbivore compounds that are pest-specific or generally insecticidal, either locally or systemically to reduce the attack (Howe & Schaller 2008). Timing and locality of such induced defences are believed to be of importance. Herbivorous insects are thought to prefer undamaged and unstressed plant tissues over damaged ones (Edwards & Wratten 1983; Dicke et al 2003). By waiting to induce defences at maximum levels until just before the herbivore causes the most damage, the plant can use this behaviour as a protective strategy, as it is more beneficial (resource-wise) for the herbivore to move on to an undamaged plant neighbour than to remain on the original host where maximum defences are induced (Van Dam et al 2001).

Plant volatiles is an indirect protective mechanism, that can be systemically induced as a response to herbivore damage (Arce et al 2021). In fact, the blend of volatile compounds appears to be somewhat specific to different types of Lepidoptera larvae, detected, for example, by the plant by different compounds present in the saliva and regurgitation of the feeding larvae (Arce et al 2021; De Moraes et al 1998). The concentration of some volatiles following herbivore damage can vary over the course of a day. Loughrin et al (1994) found that the concentration of volatile terpenes emitted by cotton plants upon caterpillar damage was increased up to ten times during the photoperiod compared to the dark period. They also argued that the peaks of this diurnal rhythm could be coinciding with periods of peak activity of some natural enemies of the herbivore.

Plant – insect interaction

Interaction between an insect and its host plant is governed to a large degree by chemical cues. Both plant antagonistic (pests) and beneficial insects like pollinators or natural enemies, find and recognize their host by olfactory signals (UCR n.d.). Plants, in turn, can identify an attacker by specific feeding patterns or chemical so called 'elicitors' from the pest (Arimura 2020). Host plants may attract natural enemies upon attack by induced indirect defence signals that are, through evolutionary adaptation, recognized as potential food or host location in the parasitoid or predator (Howe & Schaller 2008). Defence signals, like volatile emissions, attract intended receivers (natural enemies), but can also be picked up by unwanted visitors (pest) that read the released stress signal as an opportunity to infest an already fitness compromised host (plant) (Baldwin 2010). When a herbivore pest has located a host through either visual (general colour or shape) or olfactory signals, it must evaluate the identity and quality of the host through sensory, olfactory (smell) or gustatory (taste) cues (Schoonhoven et al 2005). Acceptance of a host by a herbivore is largely determined by the degree of specialism in the insect species, but also on the degree of physical and chemical obstacles presented by the plant (Knolhoff & Heckel 2014).

Plant-plant interaction

Plants also interact with its plant neighbours, in both beneficial (facilitation) and antagonistic ways. The main form of plant-plant interaction is perhaps through competition of space, light, water and nutrients. Plant competition shapes whole populations and community compositions and is a critical part of plant adaptation to stress (Keddy & Cahill 2012). A plant can outcompete its neighbour by altering morphological features like plant architecture, temporal modifications of maturation, or seed setting strategy (Gillet 2008). Growing tall or increasing leaf size is a combative form of competition. Allelopathy is another competitive strategy in which one plant inhibit the growth of another through release of phytotoxic compounds, usually through root exudation, but also as volatile emissions (Kong et al 2018; Blum 2011). By flowering early or late in the growing season or changing the direction of growth away from a competitor, the plant can avoid immediate competition for a resource all together (Gruntman et al 2017).

Plants can detect their neighbours by chemical and physical signals. Arguably, the first cue might be sensing a reduced availability of light (namely a reduction of red/far-red light ratio, R/FR) (Franklin 2008). There is also evidence of root exudate signalling, informing plants of a close-by neighbour (Bais et al 2006; Kong et al 2018). Volatile emissions, in similar ways to that between host plants and insects, might also be a form of communication between plant neighbours. Detection of such signals from intra- or interspecific plant transmitters may allow for the plant to instigate measures to reduce potential competition, like increasing plant height or allocate more resources to root mass (Ninkovic 2003). Furthermore, plants might detect a neighbour by physical touch between leaves of individual plants, by so-called mechanostimulation. A study on densely grown *Arabidopsis* found that two plants that touched leaves grew away from each other (de Wit et al 2012). The growth response even preceded R/FR ratio levels that otherwise trigger neighbour detection in Arabidopsis plants. This shows that plants perceive others through touch, and ultimately possess awareness of its physical surroundings.

Thigmomorphogenesis - change in developmental pattern in response to touch

Touch stimuli affect plants through animal movement, wind, raindrops falling, or between neighbouring plants. Nastic or tropic movements are quick responses dependent on the affected plant tissue or the direction of the stimulus (Braam et al 2005). Some examples include the Venus flytrap or the coiling tendrils of Cucurbitaceae. Responses to touch can also be displayed slowly over time. Many plant species respond physiologically to touch through so-called thigmomorphogenesis. This term was first used by Jaffe (1973) in his studies of mechanical stimulation of plants, and refers to a non-directional, possibly adaptive, physiological touch-induced response. The most common expression of thigmomorphogenesis is reduced stem length and reallocation of biomass to exposed plant organs like roots or leaves, resulting in reinforced and sturdier plants (Biddington & Dearman 1985; Markovic et al 2016). These responses have been reported in conifers, deciduous trees, and herbaceous plants (Telewski & Jaffe 1986; Coutand et al 2008; Ishihara et al 2017; Jaffe 1973). Other reported effects include increases in trichome density and lignin content in aboveground plant parts, delayed maturation or changes to plant hormones and secondary metabolites (Holeski 2007; Cipollini 1997; Markovic et al 2016). One of the most remarkable effects of touch is the increased pest and disease resistance indicated in various studies. A study on the effect of a touch treatment on *Arabidopsis* found that lesion size of *Botrytis cinerea* was reduced by 30 percent after 48-72 h inoculation and thus showed enhanced resistance to the fungus as a result of the touch treatment (Chehab et al 2012). The same study also found that the weight of *Trichoplusia ni* larvae that fed on touch-treated leaves was only approximately 70 percent to that of larvae fed on untreated control plants. Another study looked at real and simulated ‘footsteps’ of Lepidoptera (larvae and imago) on *Solanum lycopersicum,* and found an increase in defence transcripts (proteinase inhibitor 2) following the rupturing of leaf trichomes when stepped on (Peiffer et al 2009). Why touch may induce pest resistant responses in plants is not fully understood. Mechanical perturbation and stimulation have been shown to increase transcripts in genes relating to JA synthesis, which is a common mediator in herbivore-induced defences (Chehab et al 2008). It was made evident by touch treating JA-deficient mutant of *Arabidopsis,* which did not show thigmomorphogenetic responses (Chehab et al 2012).

Mechanical stimuli in natural settings may cause a generalized stress response. Such responses typically lead to increased production or accumulation of lignin via the phenylpropanoid pathway (Cipollini 1997). The increased lignin content in plants is associated with decreased or inhibited herbivory and pathogen infection due to increased cell wall rigidity and inhibitions in larval digestion (Moerschbacher et al 1990). There has also been some evidence relating induced pest resistance as a cross-adaptation to mechanical stress (Zhao et al 2005; Wang et al 2006), wherein attempts to reduce one stress factors increases resistance to another type of stress.

Model species

Cotton *Gossypium hirsutum* L.

Cotton (*Gossypium*) is a historically significant plant that has dominated the global fibre market for centuries. Even today, cotton is the most produced fibre crop in the world and accounts for 80 percent of the natural fibre production market. Over 26 million tonnes are produced annually, with production expected to rise by 3 million tonnes by 2030 (FAO 2021a). Four species in the *Gossypium* genus have been domesticated (*G. barbadense, G. arboreum, G. herbaceum and G. hirsutum*) and are produced commercially worldwide. However, over 90 percent of all cultivated cotton consists of *G. hirsutum* (Hu et al 2019).

Over a thousand different pests have been reported for cotton, and hence the crop's economic profitability is threatened (Hargreaves 1948). Cotton has, as a result, evolved physiological and chemical adaptations to reduce the effect of pest attacks. Trichomes are present on all aboveground plant parts of cotton, especially along the petioles and main nerves of leaves (Nawab et al 2011). Cotton cultivars with a higher trichome density proved resistant to three common pests (common spider mite, the cotton aphid and the cotton jassid), which was not the case for less hairy cotton cultivars (Kamel 1965a). Females of a Noctuidae moth appeared to abstain from ovipositing on pubescent (hairy) leaves, and larvae had a significantly increased mortality rate (60%) compared to a hairless cultivar (Kamel 1965b).

Cotton also possesses compounds that deter or otherwise directly defend against pest damage like herbivory. The most prevalent and studied compound is the terpenoid aldehyde gossypol, an antimicrobial substance present in all plant parts, especially the seed. Gossypol is produced in glands primarily found in epidermal cell layers of the shoot system as dark, oval spots on stems, petioles and leaves (CICR n.d.). This terpenoid acts as a natural insecticide against a wide array of pests, including Lepidopteran larvae, Hemiptera and fungi (Tian et al 2016). The toxic compound gives rise to antibiosis, which disrupts physiological functions like metabolism and reproduction, and slows the growth and development of pests (Ismail 2021).

Cotton emits a wide array of volatile compounds which act as attractants of both generalistic and specialized parasitoids and natural enemies (De Moraes et al 1998). Cotton plants are also able to employ and enhance the effectiveness of natural enemies by providing nectar in extrafloral nectaries on the underside of leaves (Llandres et al 2019). Upon herbivore damage to leaves, plants may increase the nectar production 12-fold to increase the recruitment of natural enemies 48 h after an attack (Wäckers et al 2001). However, the damage must be sufficiently severe to induce this response, as nectar production comes at a photoassimilate cost. Occasional feeding damage does not induce or increase nectar provision (Park et al 2019).

Maize *Zea mays* L.

Maize (*Zea mays*) is another staple crop worldwide, essential as a food, feed and biofuel source. It is the most prevalently grown cereal, with a global annual harvest surpassing 1 billion tons (FAO 2021b). Many pests like herbivorous insects and microbial pathogens infest maize crops and account for approximately 15% of annual yield losses (Oerke 2006). Examples include the now cosmopolitan Fall Armyworm (*Spodoptera frugiperda*), grain borers and spider mites (Ortega 1987). Like cotton, maize has several inducible or constitutive defences in its morphology and chemistry to fight antagonists. Physical defences include increased cell wall rigidity due to increased lignin content and silica uptake, which reduces digestibility and nutrient value in leaves (Santiago et al 2013; Moore & Johnson 2017). Crystalline silica in plant cells also wears on mouth apparatuses of chewing insects, further reducing their growth (Moore & Johnson 2017). Maize possesses trichomes on both upper and lower plant parts that aid in reducing herbivory (Moya-Raygoza 2016). A number of constitutive and inducible chemical defences are present in maize. Lipids in the cuticula of leaves inhibit herbivore growth and may thus constitute a direct and constant defence (Yang et al 1991). An increase in proteinase inhibitors was found in relation to herbivore damage on maize leaves, which reduces nutritional intake and in turn growth in grazing larvae (Tamayo et al 2000). Maize emits several volatiles as indirect inducible defence to attract natural enemies, that vary in quantity and quality depending on cultivar, pest species and level of attack (De Morales et al 1998). Interestingly, maize may also employ the service of insect-predatory nematodes during herbivoral infestation through root signalling (Rasmann et al 2005).

Egyptian Cotton Leafworm *Spodoptera littoralis* Boisd.

The Egyptian Cotton Leafworm (*Spodoptera littoralis* Boisd.) is a significant pest in many cropping systems worldwide. As of now, its current distribution is limited to Africa, Western Asia and Southern Europe, mainly due to cold winters and the species (apparent) inability to reproduce in colder climates (Sidibe & Lauge 1977). It is highly polyphagous and can cause economic damage in yield loss in plants belonging to over 40 families, including cotton and maize (CABI 2020). It has been labelled as an A2 quarantine pest (EPPO 2021). The larva typically grazes on leaves and can strip the entire plant, severely reducing the photosynthetic ability, but is also known to dig into stems or buds of cotton and maize, further reducing the market value (CABI 2020).

S. littoralis is affected by plant defences induced by herbivory or mechanical stress (Anderson & Alborn 1999; Mithöfer et al 2005). Adult moths exhibit changed mating behaviour on damaged cotton plants, with reduced male calling in females and mating events (Zakir et al 2017). Females can also be deterred from ovipositing on herbivore-damaged cotton plants (Anderson & Alborn 1999). Terpenoids in cotton are known to slow larval growth in *S. littoralis* (Zur et al 1979), and some herbivore-induced volatile compounds, like indole, constitutes a specialized volatile against *S. littoralis* in maize (Veyrat et al 2015).

Experimental aim

The present experiment investigated whether touch could cause altered physiology in two plant species, expressed through changes to morphology and induced defences against a generalist herbivore (*S. littoralis*). The physiological effect of mechanical stimuli, especially in cotton, is poorly understood. A few studies have been carried out, but more research is required to comprehend the effects and potential applications further. (Zhang et al 2013; Markovic et al 2014; Chehab et al 2012; Jaffe 1973). The stimuli have also typically been applied in the form of wounding, either herbivoral or mechanical (Anderson & Alborn 1999; Bricchi et al 2010). This study set out to explore a non-destructive stimulus and its potential to act as a priming agent with applications in plant protection. A general hypothesis was held; mechanical stimulation in the form of daily touching for 14 days simulates neighbour detection in natural surroundings and can induce physiological variation in the plants. In the following experiments, this hypothesis was further broken down and tested.

Experiment 1: physiological effects of touch on cotton and maize.

Cotton and maize plants exposed to touch-treatments are hypothesized to 1) exhibit plant height and biomass reductions, 2) allocate biomass to leaves to a greater extent, i.e. touch-treated plants develop thicker leaves, and 3) morphological properties related to defences, such as trichomes and glands, increase in density. Furthermore, morphological effects are exhibited to a greater extent in apical plant tissues compared to terminal leaves, in accordance with the optimal defence theory. Parameters to examine physiological effects on cotton and maize included measurements of stem length, aboveground (fresh and dry) biomass and leaf area, and observations of non-glandular trichomes and terpenoid gland density.

Experiment 2: effects on feeding preference in herbivorous larvae.

The potential of touch to induce herbivory-related defences in cotton was assessed by observing the behaviour of a generalist larva when presented with leaves from both a touch-treated and an untreated leaf. Touch simulates mechanical stimuli and is here hypothesized to induce pest-related defences (physical and chemical) that deter the generalist herbivore *S. littoralis* from feeding on touch-treated leaves. Larvae of *S. littoralis* have been shown to discriminate between leaves deemed to be of lesser quality and should thus prefer leaves from untreated, plants without induced defences (Anderson et al 2001; Edwards & Wratten 1983).

Furthermore, the larva is expected to exhibit a more prominent differentiating behaviour between developing, apical leaves, compared to developed, terminal leaves, in relation to the optimal defence theory of systematic induction if defences to the most valuable plant parts.

Materials and methods

Plants

Cotton *Gossypium hirsutum* L. ‘Delta Pineland 90’ and maize *Zea mays* L. ‘Sweet Nugget F1’ were grown from seed in soil with slow-release fertilizers (Hasselfors Garden pelargonjord) in 1.5 l plastic pots. The first trial batch was sown on January 3, 2022, with several batches following at two-week intervals (Table 1). Cotton seeds were soaked for 24 h before sowing. One seed was sown per pot, with 9-14 replicate pots per treatment and species. The plants were grown at 25 ± 5 °C and a 12 h light period (400W high-pressure sodium light bulbs). Watering was carried out as needed, about twice per week. The pots were treated with entomopathogenic agents (Gnatrol *Bacillus thuringiensis* subsp. *israelensis* and Entonem *Steinernema feltiae*) several times to keep fungus gnat infestation low.

Table 1. Experimental design. Column for “Treatment” describes mode of touching treatment, e.g., 1 minute brushing on 3rd true leaf on the upper leaf side.

Plant		Developmental age at treatment start	Treatment	Assay
Cotton	*Gossypium hirsutum* ’Delta Pineland 90’	6 weeks	1 min, 3rd true leaf, upper leaf side	Morphology
Cotton	*Gossypium hirsutum* ’Delta Pineland 90’	6 weeks	1 min, 3rd true leaf, upper leaf side	Feeding trial 1
Cotton	*Gossypium hirsutum* 'Delta Pineland 90'	4 weeks	1 min, 3rd true leaf, upper leaf side	Morphology
Cotton	*Gossypium hirsutum* 'Delta Pineland 90'	5 weeks	1 min, 3rd true leaf, upper + lower leaf side + petiole	Feeding trial 2
Maize	*Zea mays* L. 'Sweet Nugget F1'	4 weeks	1 min, 3rd true leaf, upper leaf side	Morphology
Maize	*Zea mays* L. 'Sweet Nugget F1'	2.5 weeks	1 min, 3rd true leaf, upper leaf side	Morphology
Maize	*Zea mays* L. 'Sweet Nugget F1'	2.5 weeks	1 min, 2nd + 3rd true leaf, upper leaf side	Morphology

Insect herbivore

Larvae of the Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae), were used in the experiment as a model generalist herbivore. The larvae were reared in 25 ± 5 °C, 65 ± 5 % relative humidity, and artificial light (L16:D8), and fed an artificial potato-based diet prior to the experiment (Hinks & Byers 1976). The Department of Chemical Ecology at SLU Alnarp continuously raises *S. littoralis*, whose culture was established in 2007 from wild specimens collected in Egypt and has been refreshed several times with wild-collected specimens.

Touch treatment

The empirical study was conducted in the spring of 2022 in Vegetum's greenhouse in Alnarp, Skåne. The experiment was designed as a two-group model, with one touch-treated and one untreated control group. The two groups were placed on a growing table in the greenhouse in the same conditions as for seedling cultivation. Plants were separated to minimize accidental touching between plants. However, due to limited space, plants occasionally brushed against neighbouring plants due to wind gusts, stems tipping over, or the growing directions of the plants. These conditions were the same for both treatment and control groups and thus deemed to be unavoidable but of minor importance. All plants used for morphological assays, as well as in the first feeding trial, were touch-treated on the upper side of the leaf disc (Table 1). The third fully developed true leaf was treated in all batches but the last maize batch, which had both the second and third true leaves touch treated. Plants in the second feeding trial received an extended touch treatment, which included, in addition to the upper leaf side, brushing the lower leaf side and the petiole.

The touch treatment was performed by briefly sweeping the fully developed leaf's adaxial (upper) side using a soft facial brush with natural bristles. The brush was held at < 45° angle to reduce the pressure of bristles on the leaf, and the entire leaf was lightly brushed in a systematic pattern for 1 minute without any other part of the plant being touched. The brush-sweeping pattern was developed through testing and video documentation to obtain uniformity in the treatment. The treatment was conducted daily for 14 days for all trial batches except the third maize batch, which was treated for 10 days.

Measurements

Morphological effects

Plant height

The height of the cotton plants was measured at the end of the treatment. The cotton stem was cut using a floral knife at the stem base (connecting stem and root tissue) and measured to the apical bud. Maize plants were cut at the stem base and measured to the tip of the longest leaf.

Biomass

After height measurements, the aboveground plant parts were weighed for fresh weight. The shoot was divided into stems, including petioles and branches, leaves, and buds (maize only). The plants were dried in an oven (70 °C) for 24 h in pre-weighed heat-tolerant paper sandwich bags. Each plant part was then weighed for dry mass. Biomass allocation was calculated as plant part dry mass to total dry mass (e.g. the dry weight of leaves / total dry weight). The root biomass was excluded in this study as it was difficult to remove the roots from the soil without damage.

Specific leaf area

Leaf area was measured as the total leaf area of each plant. Leaves from cotton were cut at the leaf disc base. Maize leaves were cut at the connection between blade and collar. Cotyledons from both cotton and maize plants were excluded from all measurements as they were often wilted or missing. Leaf area was measured using a Li-cor Li-3100 Area Meter. Any leaf with an approximate area >1 cm^2 was included. The leaves were then dried in the same method as described above. Specific leaf area (SLA) was calculated as leaf area to leaf dry weight.

Non-glandular trichomes and terpenoid glands

Epidermal terpenoid glands were counted on petioles of the apical leaves. The petioles were removed from the plant and cut to approximately 3 cm in length. Effects on trichomes were assessed through visual comparison by photographing the petioles through a stereo microscope. The glands were counted from photos by measuring out a 5 x 5 mm square and counting all visible glands within the square.

Herbivore trial

Dual choice test

To examine the effect of the treatment on the plant's defence against herbivory, a feeding selection test was performed on cotton using the generalist *S. littoralis* (early fourth instar larvae). Matching plants in size and development from the treatment and control groups were paired and constituted the two different alternatives the larva could choose. The touched-treated leaf of a treated plant and the corresponding leaf of the control plant was cut from the plant leaving approximately 6 cm of the petiole. To retain the turgor and freshness of the leaf, the petiole was placed in a small glass vial with distilled water and sealed with parafilm. The two leaves were placed in a 23.5 x 18 x 5 cm rectangular plastic box with a lid, together with an early fourth instar larva. Larvae were placed in the box facing neither of the leaves to reduce bias. The same was done for the developing apical leaf of each plant in the pair. The second feeding trial using plants with the extended touch treatment included a third sample, taking a developed and untreated fully matured leaf from each plant. The boxes were stored using the same conditions as for larval rearing. After 24 h, the leaves were photographed against a 0.25 x 0.25 cm grid. A binary test (’leaf eaten or not’), as well as calculations of leaf area eaten, was conducted at the end of the trial.

Figure 1. Dual choice test set up

Statistical analysis

Physiological effects in terms of differences in plant height, dry and fresh weight, leaf area and gland density were compared using mean values of each treatment and species. Student's t-test was used to compare the treatment and control groups in the morphological experiments. Analyses were carried out using the t-test function in the 'tidyverse' package in R (version 4.1.1) and the Rstudio Desktop application.

In the herbivore feeding choice trial, the median area of grazing on each leaf in the different leaf pairs was assessed. The feeding behaviour of larvae cannot be expected to be normally distributed (behaviour and individual dependent outcomes). Therefore, feeding choice assays were analysed in the non-parametric Wilcoxon signed-rank test, using the Wilcoxon test function in the 'rstatix' package (R version 4.1.1). Both t-tests and Wilcoxon signed-rank tests were assessed at significance level $\alpha = 0.05$.

Results

Morphological effects in cotton

Morphological effects of the touch treatment differed between plants of different developmental stages. No significant effects were detected in 6-week-old cotton plants (Table 2). Although not significant, the treated plants had higher stem height, while the total biomass (fresh and dry weight) was greater in the control plants.

The dry weight of leaves and stem did not differ and the percental biomass allocation was around 41% for stem: total mass (S:T) and 57% for leaves: total mass (L:T) for both treatment and control (Figure 2). Leaf area was slightly but not significantly less for treatment than control, and leaf area: dry leaf weight (SLA) was greater for treatment, indicating thinner leaves than in control.

Table 2. Morphological results of touch treatment on older cotton plants (6-week-old at treatment start). Student's t-test, n = 9.

Parameter		Treatment (Mean ± SE)	Control (Mean ± SE)	*t*-value	*p*-value
Stem height	cm	43.31 ± 2.01	42.83 ± 1.20	-0.186	0.546
Dry weight total	g	7.05 ± 0.57	8.05 ± 0.89	0.947	0.372
Dry weight stem	g	2.99 ± 0.25	3.30 ± 0.39	0.686	0.512
Dry weight leaves	g	4.05 ± 0.35	4.74 ± 0.51	1.088	0.308
Fresh weight	g	41.65 ± 3.69	44.56 ± 3.73	0.508	0.625
Leaf area	cm^2	1166.89 ± 94.70	1231.33 ± 98.39	0.451	0.664
SLA	$cm^2\ g^{-1}$	291.67 ± 11.45	268.03 ±14.04	-1.317	0.224

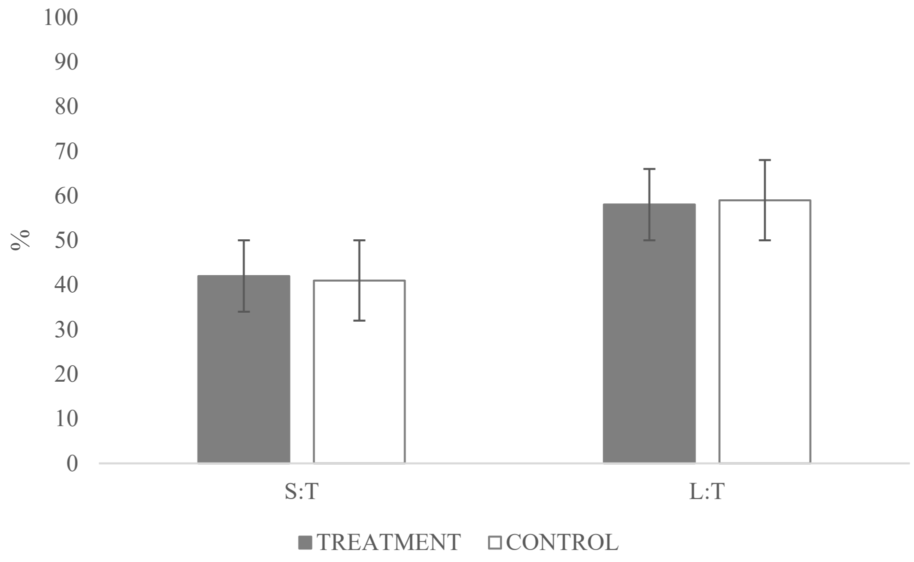

Figure 2. Biomass allocation in 6-week-old cotton. S:T stem: total biomass, L:T leaf: total biomass, (± SE). Student's t-test, n=9.

In the 4-week-old cotton plants, morphological differences were observed (Table 3). The stem height was significantly reduced in the touch-treated cotton plants (p=0.003). Total dry weight was greater in treatment, mainly due to increased leaf mass (p<0.001). Stem dry weight did not differ. Leaf area and SLA were greater for control, thus indicating that treated plants had increased leaf thickness. The treated plants seemed to allocate more aboveground biomass to leaves (57%) than control (53%) (L:T) (Figure 3). Fresh weight was not measured in 4-week-old plants.

Table 3. Morphological effects of touch treatment on younger cotton plants (4-week-old at treatment start). Student's t-test, n=14.

Parameter		Treatment (Mean ± SE)	Control (Mean ± SE)	*t*-value	*p*-value
Stem height	cm	40.45 ± 0.50	45.78 ± 1.26	3.584	0.003
Dry weight total	g	8.09 ± 0.41	6.67 ± 0.33	-3.047	0.005
Dry weight stem	g	3.44 ± 0.17	3.15 ± 0.18	-1.360	0.197
Dry weight leaves	g	4.65 ± 0.24	3.53 ± 0.16	-5.232	<0.001
Leaf area	cm^2	1013.14 ± 42.39	1118.06 ± 41.27	1.884	0.082
SLA	$cm^2\ g^{-1}$	221.14 ± 7.89	320.22 ± 10.70	7.438	<0.001

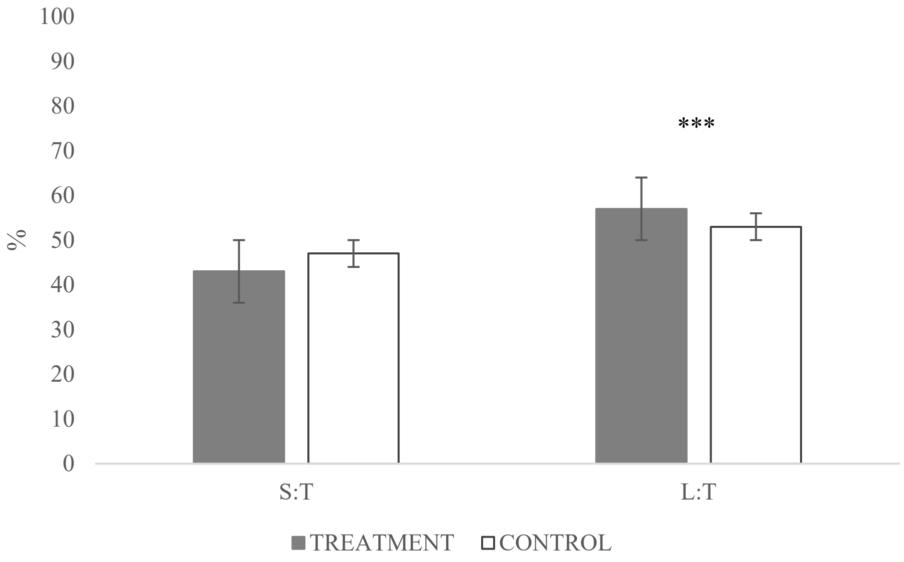

*Figure 3. Biomass allocation in 4-week-old cotton. S:T stem: total biomass, L:T leaf: total biomass, S:L stem: leaf biomass (± SE). Student's t-test, n=14. Statistical significance is indicated by *** p< 0.001.*

Morphological effects in maize

The plant length was slightly greater in touch-treated 4-week-old maize plants compared to control, although not significant (Table 4). Dry and fresh biomass was greater in control than in the treated plants in all plant parts (stem, leaves and buds), but did not differ significantly. The biomass allocation was not significantly different between the treatment and control group (Figure 4). The treated group had smaller leaves, and SLA was significantly higher (p=0.043) than the control group, indicating that the treated plants had thinner leaves.

Table 4. Morphological results of touch treatment on older maize plants (4-week-old at treatment start). Student's t-test, n = 12.

Parameter		Treatment (Mean ± SE)	Control (Mean ± SE)	*t*-value	*p*-value
Stem height	cm	120.73 ± 2.44	118.83 ± 3.46	-0.463	0.653
Dry weight total	g	5.60 ± 0.45	7.00 ± 0.73	1.683	0.121
Dry weight stem	g	1.79 ± 0.16	2.29 ± 0.27	1.702	0.117
Dry weight leaves	g	3.40 ± 0.24	4.08 ± 0.37	1.684	0.120
Dry weight flower buds	g	0.46 ± 0.07	0.62 ± 0.11	1.358	0.202
Fresh weight	g	70.52 ± 4.99	75.92 ± 6.30	0.645	0.532
Leaf area	cm^2	1363.07 ± 75.31	1459.92 ± 101.62	0.871	0.403
SLA	$cm^2 g^{-1}$	410.71 ± 15. 75	366.05 ± 10.66	-2.292	0.043

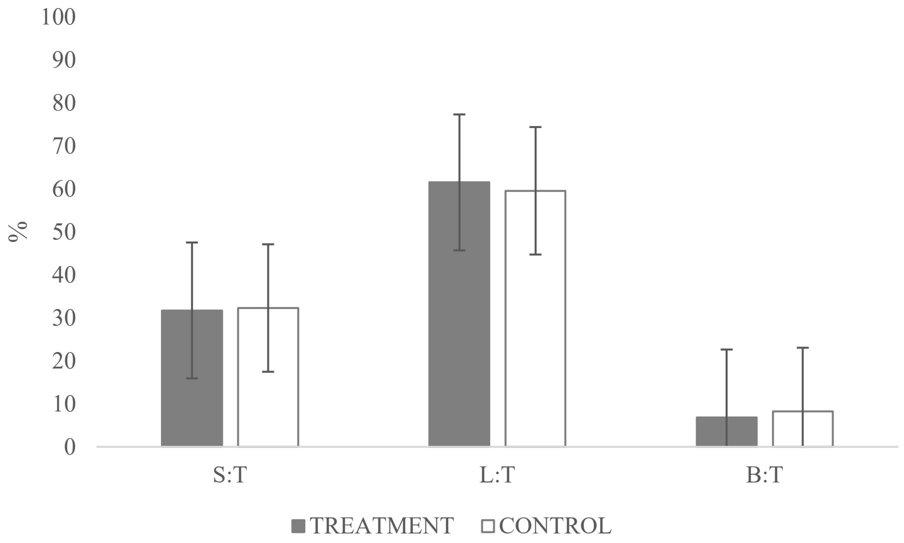

Figure 4. Biomass allocation in 4-week-old maize. S:T stem: total biomass, L:T leaf: total biomass, B:T flower buds: total biomass (± SE). Student's t-test, n=12.

Plant lengths were significantly longer in touch-treated 2.5-weeks-old maize plants (p=0.023) (Table 5). The treated group was noticeably larger and had a significantly higher fresh weight (p<0.001). The dry weight, on the other hand, was only half that of the control (p=0.002), indicating that the larger size and fresh weight of the treated plants were due to higher water content.

Table 5. Morphological results of touch treatment on younger maize plants (2.5-week-old at treatment start). Student's t-test, n = 9.

Parameter		Treatment (Mean ± SE)	Control (Mean ± SE)	*t*-value	*p*-value
Plant height	cm	90.78 ± 3.70	83.28 ± 2.71	-2.802	0.023
Fresh weight total	g	37.80 ± 4.24	19.61 ± 2.82	-5.230	<0.001
Dry weight total	g	1.24 ± 0.19	2.62 ± 0.35	4.442	0.002

The fresh and dry weight of extendedly touch treated maize plants (touch-treating 2nd and 3rd true leaf for 10 days) did not differ between treatment and control (Table 6). Plant length was significantly greater in the treatment group (p=0.026), in accordance with results from previous batches. The lack of any potentially observable effect of the intensified treatment may be due to the low number of observations.

Table 6. Morphological results of extended touch treatment on younger maize plants (2.5-week-old at treatment start). Student's t-test, n = 6.

Parameter		Treatment (Mean ± SE)	Control (Mean ± SE)	*t*-value	*p*-value
Plant length	cm	84.71 ± 2.11	77.53 ± 2.54	-2.937	0.026
Fresh weight total	g	25.14 ± 2.59	21.64 ± 2.72	-0.992	0.367
Dry weight total	g	2.01 ± 0.22	1.75 ± 0.21	-0.964	0.380

Trichome and gossypol gland count in cotton

The petioles of apical leaves on touch-treated cotton plants had a significantly greater density of glands in the epidermal cell layer compared to control within a 5 x 5 mm surface area (Figure 5). In addition, a visual inspection indicated that there was a difference between the treatment and control, with a higher trichome density on the apical petioles of touch treated plants (Figure 6). Counting number and density of trichomes requires technology that lie beyond the scope of this study.

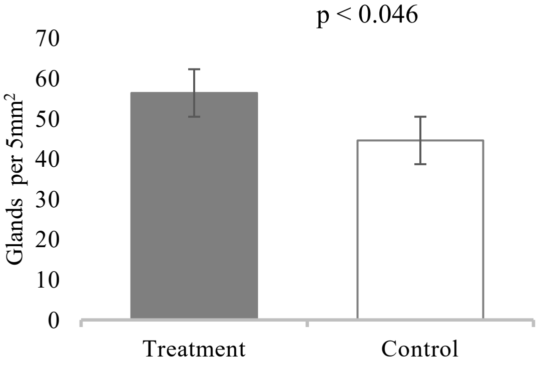

Figure 5. Terpenoid gland density, individual gland per 5 mm (± SE). Student's t-test, p<0,046, n=5.

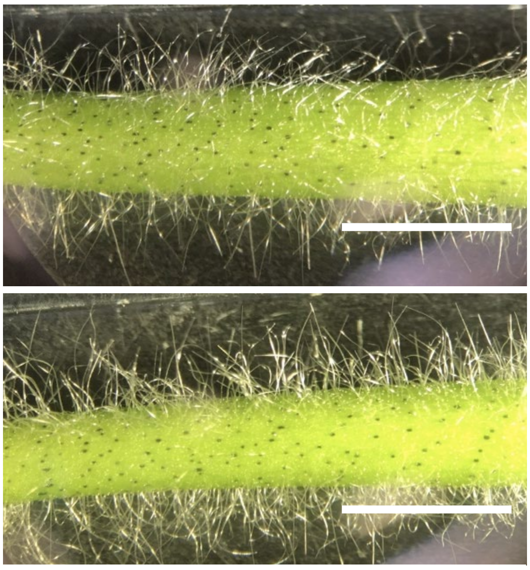

Figure 6. Trichomes on petiole of control (upper) and treatment (lower) leaves. White bar 5 mm.

Feeding trial

Effect on fully developed cotton leaves

No detectable difference was observed between treatment or control on the leaf area eaten by the larva after 24 h in 6-week-old cotton plants (Figure 7). The extended touch treatment (brushing on both upper and lower sides and petiole of the leaf) showed similar results as the previous trial. Slightly more was eaten of the treated leaves compared to control. During this trial, the larvae did not seem to 'taste test' to the same degree but rather chose to remain on one of the leaves and eat.

The second feeding trial, which used plants from the extended touch treatment, included leaf pairs of the fourth fully developed leaf of both the treatment and the control group to assess systemic effects on developed leaves. These results were similar to that of the third fully matured leaves, with no significant difference in area eaten.

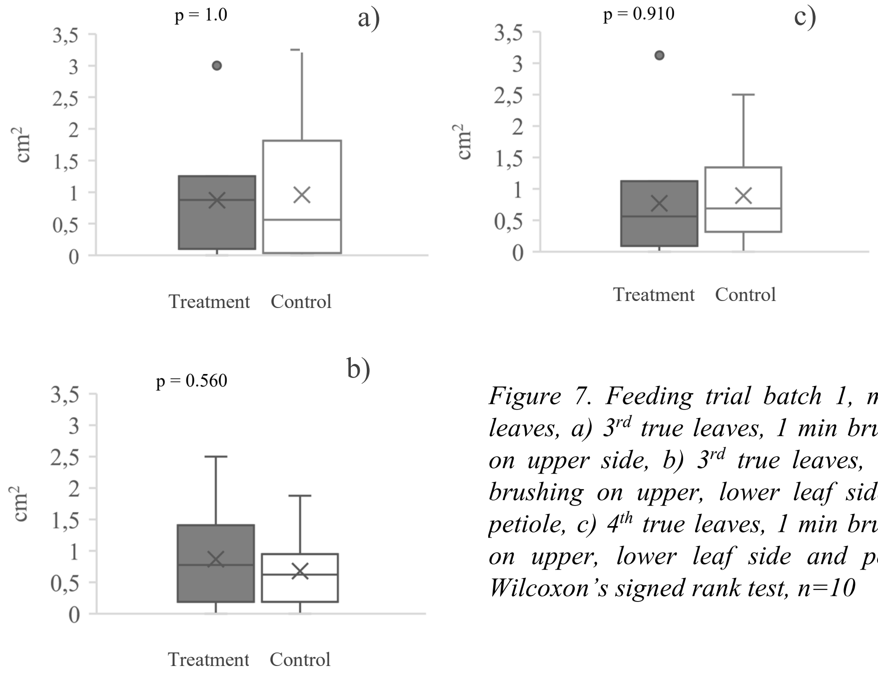

Figure 7. Feeding trial batch 1, mature leaves, a) 3rd true leaves, 1 min brushing on upper side, b) 3rd true leaves, 1 min brushing on upper, lower leaf side and petiole, c) 4th true leaves, 1 min brushing on upper, lower leaf side and petiole. Wilcoxon's signed rank test, n=10

Effect on apical, developing leaves

No significant difference was observed between leaf area fed on apical leaves from touch-treated plants and control (Figure 8). The larva tended to eat more on the treated leaves than the control leaves in the first feeding trial, and there was less variation between replicates in the treatment. In the second feeding trial, the larva fed on leaves from control group to a greater extent compared to the treatment group, but with a greater variance between the replicates. After 24 hours, it was more common for only one of the leaves to be eaten and the other to be left untouched. In comparison to fully developed leaves, the leaves of developing apical leaves were much smaller, and wilted and dried more quickly. As a result, two samples had to be discarded as no grazed leaf area could be measured in dried leaves.

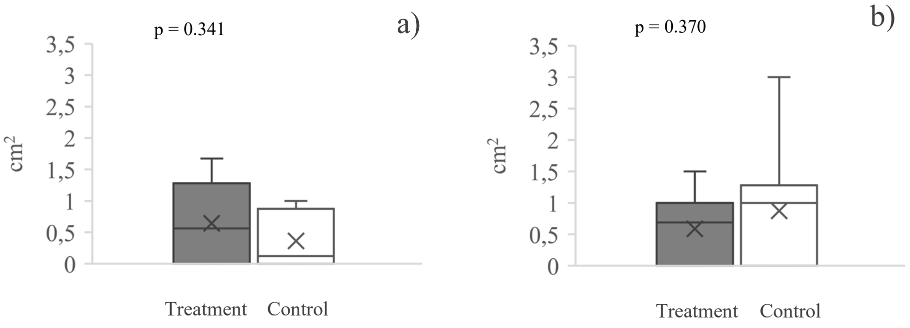

Figure 8. Feeding trial batch 2, apical leaves, a) apical leaf, 1 min brushing on upper side, b) apical leaf, 1 min brushing on upper, lower leaf side and petiole. Wilcoxon's signed rank test, n=8-10.

Discussion

Effect of touch on the morphology of cotton

The results indicate that touch stimulates physiological change in cotton plants. 4-week-old cotton plants at treatment start exhibited significant reductions in stem height, biomass and leaf area. These results are in accordance with previous studies on mechanical stimuli on cotton. Zhang et al (2013) examined the effect of mechanical stress on cotton in a field experiment. The treatment consisted of hanging identification labels commonly used in cotton experiments on the petioles of flower buds. They found that the treatment significantly reduced the plant height for all four cotton cultivars used in the trial. Cotton neighbour (plant-plant) interactions have been shown to affect plant architecture. In a study on narrow cultivation rows and increased plant density, cotton plant height was reduced by 17 percent, and bolls (fibre buds) were set much earlier than plants grown in less dense conditions (Jost & Cothren 2000). This indicates an assimilate redistribution from vegetative to generative (reproductive) plant parts in competitive plant community scenarios. The reduction in stem height can thus be explained by the cotton plant's strategic move of putting resources into reproduction rather than trying to outcompete its neighbours for limited light, water and nutrition.

The increased dry biomass was attributed to a significant leaf mass increase in touch-treated plants compared to control plants. The stem dry mass did not differ between the treatments, and thus the touch-treated plants allocated more resources to leaves. The touch-treated leaves appeared to also be thicker than control, indicated by the reduced SLA (leaf area to leaf dry mass) in treated plants. Increased leaf thickness is associated with increased chlorophyll content and photosynthesis ability (Patterson et al 1977). In greater plant densities, light becomes scarce and of lower quality. Increasing photosynthetic ability might be a competitive strategy in cotton. Moreover, increasing net photosynthesis may be a required step in the above mentioned early maturing strategy, to allocate resources needed in fruit setting (Gifford & Evans 1981). Thicker leaves can also hold more water (more vacuoles and fewer stomata per unit leaf mass), increasing the chances of survival in scarce water conditions potentially found in denser plant communities (Coneva & Chitwood 2018).

However, these morphologically expressed responses were not as evident in older cotton plants (6-weeks-old at treatment start). None of the parameters showed any significant difference, although there were some trends resembling results for the younger cotton plants.

These two batches were treated at different times. There might have been conditional differences in cultivation like watering or light (sun radiation) that could have affected growth and development. Differences in the number of replications between younger and older cotton plants (14 and 9, respectively) may have influenced the statistical outcome.

Another contributing factor may be age-related resilience (ARR) to abiotic stress (Rankenberg et al 2021). Arguably, plants are, in general, inherently more tolerant or resilient towards both abiotic and biotic stress factors in their environment with (developmental) age. As mentioned earlier, plants may also change defence strategy from resilience towards tolerance with age (Boege et al 2007). A lack of observable difference between treatment and control might then be in relation to an age-dependent resilience toward mechanical stress. However, it is unclear if a two-week difference could suffice to express increased resilience or tolerance in cotton. Future studies should include touch-treatments on young cotton seedlings to investigate if morphological responses are stronger in very young mechanically stimulated plants that might invest more resources to reduce stress in critical developmental stages.

The visually assessed increase in density of non-glandular trichomes on petioles of apical leaves in treated plants points to a physiological response to the touch stimulus, and possibly induction of defence mechanisms. Trichome density has been shown to increase with certain types of stress. One of the functions of trichomes is to protect leaves against sun radiation and prevent water loss (Karabourniotis et al 2019). The number and density of trichomes can thus be expected to increase under drought conditions, as shown in cotton by Shahzad et al (2021). Increased trichome production was also found in response to mechanical stimuli, such as touch (Marcovic et al 2016). These findings suggest that constitutive defences (such as trichomes) may have inducible properties that are triggered by stress.

A quantitative method of measurements, like counting trichomes with the method by Markovic et al (2016), could give a closer analysis of the effect of the touch treatment on trichome density. This study did not compare trichome and gland density between developing and fully mature plant tissues. According to the optimal defence theory, induced trichome, gland and defence compound production should be allocated to younger plant tissues. Markovic et al (2016) found that the trichome density was significantly greater in apical leaves in touch-treated potato plants compared to untouched plants. There was, on the other hand, no significant difference in trichome density on fully mature leaves between treatment and control. This further indicates a systemic induction of physical defences as result of touch, conforming with the optimal defence theory.

The significant increase in gland density on touch-treated leaves indicates an increase in terpenoid aldehydes (TA). The increased production of defence compounds suggests that some form of defence has been induced by the treatment. The greater the TA content, the more the herbivores are deterred from feeding on the plant (McCarty et al 1996; McAuslane et al 1997).

Some volatile compounds are believed to be stored and synthesized in terpenoid glands, as glanded cotton plants emit considerably higher amounts of volatiles than glandless cultivars (Elzen et al 1985). In light of this, it would have been interesting to perform volatile collection and olfaction profile identification, e.g., through gas chromatography. Relatively much is known about volatile emissions from cotton, and comparisons of volatile profiles between touch-treated and untreated plants can thus aid in the examination of induced chemical defences (Röse et al 1996; Huang et al 2015).

Physiological effects on maize

The reduced dry weight and leaf area in all touch-treated maize batches followed results found in previous studies (Markovic et al 2014; Beardsell 1977). The plants thus seem to be physiologically affected by touch as a mechanical stimulus. Biomass allocation did not differ between the treatment and control groups; it was instead an overall biomass reduction in the plants. However, the greater plant length in touch-treated maize plants compared to control in all batches points to a spatial biomass distribution in the vertical dimension. This stood in contrast to what was hypothesized, as other studies have found reduced stem length as a result of mechanical stimuli (Markovic et al 2014; Beardsell 1977; Jaffe 1973). Though it should be noted that the measuring methods in these studies and the one used here were not the same, and comparisons may therefore be misleading.

Inconsistent with results from Markovic et al (2014) was the greater leaf area and SLA in treatment compared to control. Treated maize plants had less leaf mass per unit leaf area than control, possibly indicating a ‘confrontational’ competitive strategy, as described by Gruntman et al (2017), outcompeting perceived neighbours for light by increasing leaf area. Such a response was found in touch-treated potato, possibly indicating a strategic move towards incoming competition (Markovic et al 2016).

The treated plants had a substantially greater fresh weight than the control in the second maize batch while only having approximately half of the dry weight. The 30-fold increase in dry weight compared to only a 12.5-fold increase in control (from values in Table 5, not shown) points to a greater water accumulation in the touch-treated plants. The touch treatment may have elicited a neighbour-detection response and 'informed' the maize plants of incoming competition. In intra- and interspecific competition, water stress is a threat plants may counteract by accumulating water and adjusting osmotic potential (Hsiao et al 1976). Since the plants were grown in separate pots (with limited nutrient supply) and did not compete for resources in a common substrate, increasing size through water uptake might allow the touch-treated plants to reach above their competitors and compete for shared, aboveground resources such as light. However, a substantial water accumulation was not found in all maize batches, and further examination is required to determine the causes for the contradictory results in maize.

Feeding choice trial

Contrasting hypothesis, the larvae did not exhibit any preference in feeding choice between leaves of touch-treated cotton plants and control. This may be explained by insufficient replication (n-value) for statistical evaluation. Increasing the number of observations might have amplified any tendency in preference. It may also be attributed to the polyphagous nature of *S. littoralis*. The larva may have found 'contentment' with the food choice it was presented, independent of which of the treatments it arrived at first in the box. If defences were induced through the touch treatment, it might not have been strong enough to deter or harm the larva. There might also be too big of a risk for the larva to switch feeding sites. *S. littoralis* is antagonized by many natural enemies that can attack exposed larva searching for better feeding cites (CABI 2020). Moreover, even though *S.littoralis* can feed on many different host plants, it does not grow or develop equally on all hosts (Dimetry 1972; Adham et al 2009). Thus, it might be the most beneficial to remain on a leaf of acceptable quality, and for the larva to perceive its food choice as 'not very tasty, but good enough'. Future studies should examine larval development (e.g. weight) when forced to feed on either touch-treated or untreated leaves. Induction of toxic compounds, like TA, as a result of mechanical stimuli, would make a touch-treated cotton plant a low-quality (or toxic) food source, and thus inhibit larval growth.

Despite the lack of evidence in this study, the exhibition of feeding selection has been shown in fourth instar *S. littoralis* larva on cotton (Dimetry 1972; Anderson & Agrell 2005). The latter study performed a similar feeding trial, testing on apical leaves from plants damaged by a herbivore on the third and fourth fully developed true leaves. The larvae fed significantly more on leaves of undamaged plants compared to damaged ones. The touch stimulus used in the present study (light brushing for 1 minute) might have been too feeble to induce herbivore-related defences in cotton. Most studies on stimulated induced defences and mechanical stimuli have been conducted with wounding or forceful stimuli (Anderson & Agrell 2005; Peiffer et al 2009; Ishihara et al 2016). The touch treatment performed here might not have been a strong enough cue to elicit a pest-resistant response.

Another theory is that the brief sweeping of a brush might not be perceived as a herbivore by the cotton plant, but instead a pathogenic attack. Cotton is exposed to a number of aboveground microbial pathogens (Cotton Research & Development Corporation 2020), and it is possible that cotton plants can perceive non-chemical cues as imminent infection (like touch stimulus resembling, for example, pressure from the appressorium of an attacking fungus) (Zhao et al 2005; Chehab et al 2012). Therefore, it would be interesting to examine infection rates of a fungal pathogen on cotton leaves exposed to a touch treatment to determine if pest resistance other than insect herbivore-related defences can be induced.

The touch-treated and untreated cotton and maize plants were grown in close proximity to each other. Some of them toppled over, brushed against other plants, or were subjected to airflow from ventilation and handled during cultivation and treatment. These are all stimuli that can resemble touch and could have interfered with results. It is also possible that untreated plants might have signalled each other or 'eavesdropped' on emitted volatiles from mechanically stressed plants subjected to the touch treatment. Thus, they induced defences of their own despite lack of physical stress. Plants are known to communicate or receive cues from other plants through root exudate and communal mycorrhiza (Elhakeem et al 2018; Gorzelak et al 2015) but may also pick up volatile signals from their neighbours (Ninkovic et al 2019; Ton et al 2006). Whether touch-treated or not, plants in the experiment might have already induced some type of defence upon start of the feeding trial, further blurring out differences for the larva in the two-way feeding choice trial. A replicating study should include spatially separated control and treatment plants to block out volatile cues to reduce the risk of undesired plant-plant communication.

Ahead lies further exploration of the possibilities of applying induced defences through mechanically stimulating plants in crop production. Developing an understanding of how, and to say the least, if, it is possible to increase pest resilience through touch allows for the development of plant protection methods that are more cost effective and safer for human and environmental health than chemical alternatives.

Conclusion

Touching fully mature leaves of cotton and maize plants for 1 minute for 14 days was intrusive enough to elicit morphological responses. Although in different ways, the two plant species exhibited physiological adjustments that resemble plant-plant interactions in competitive scenarios. This further implies that plants can detect and perceive neighbours through mechanical sensing.

However, contrasting previous studies, the touch treatment did not appear to elicit strong enough defences to deter the generalist *Spodoptera littoralis* from feeding on leaves of treated cotton plants. Inherent tolerance towards stress in the plants, or behavioural quirks of the larvae may explain why feeding preference was not shown. Whether stimulated neighbour detection through touch can induce pest-resistant properties in cotton remains to be further investigated with chemical and biological assays, in addition with ecological implications of mechanical stimulation and induced defences.

Acknowledgements

I wish to express my deepest gratitude towards my supervisors: Peter, for your patience, engagement, presence and positive, explorative approach to the experiments, and Velemir, for invaluable advice, insights and support along the way. Thank you both for taking on this at times overambitious project and for believing in me and cheering me on!

I would also like to give warm thanks to Anna-Karin, Håkan and Lotta from BT for all your help with technical assistance.

And to Anton, for your never-ending patience and support.

References

Adham F, Rashad E, Shoukry I & Nasr E (2009) Host plants shifting affects the biology and biochemistry of spodoptera littoralis (boisd.) (lepidoptera: noctuidae). Egyptian Academic Journal of Biological Sciences 2(1):63-71

Anderson P & Agrell J (2004) Within-plant variation in induced defence in developing leaves of cotton plants. Oecologia 144: 427-434. https://doi.org/10.1007/s00442-005-0095-3

Anderson P & Alborn H (1999) Effects on oviposition behaviour and larval development of Spodoptera littoralis by herbivore-induced changes in cotton plants. Entomologia Experimentalis et Applicata 92:45-51. https://doi.org/10.1046/j.1570-7458.1999.00523.x

Anderson P, Jönsson M & Mörte U (2001) Variation in damage to cotton affecting larval feeding preference of Spodoptera littoralis. Entomologia Experimentalis et Applicata 101:191-198. https://doi.org/10.1046/j.1570-7458.2001.00903.x

Arce C, Besomi G, Glauser G & Turlings T (2021) Caterpillar-Induced Volatile Emissions in Cotton: The Relative Importance of Damage and Insect-Derived Factors. Frontiers in Plant Science 12(709858). https://doi.org/10.3389/fpls.2021.709858

Arimura G-I (2020) Making Sense of the Way Plants Sense Herbivores. Trends in Plant Science 25(3):288-298. https://doi.org/10.1016/j.tplants.2020.11.001

Ashraf M, Ahmad M, Öztürk M, & Aksoy A (2012) Crop Improvement Through Different Means: Challenges and Prospects. *Crop Production for Agricultural Improvement.* London: Springer Science+Business Media 1-15. https://doi.org/10.1007/978-94-007-4116-4_1

Bais H, Weir T, Perry L, Gilroy S & Vivanco J (2006) The Role of Root Exudates in Rhizosphere Interactions with Plants and Other Organisms. Annual Review of Plant Biology 57:233-266. https://doi.org/10.1146/annurev.arplant.57.032905.105159

Baldwin I (2010) Plant Volatiles. *Current Biology* 20(9): 392-397. https://doi.org/10.1016/j.cub.2010.02.052

Beardsell M (1977) Effects of Routine Handling on Maize Growth. *Journal of Plant Physiology* 4(6):857-861

Beketov M, Kefford B, Schäfer R & Liess M (2013) Pesticides reduce regional biodiversity of stream invertebrates. *PNAS* 110(27): 11039-11043. https://doi.org/10.1073/pnas.1305618110

Biddington N & Dearman A (1985) The Effect of Mechanically Induced Stress on the Growth of Cauliflower, Lettuce and Celery Seedlings. *Annals of Botany* 55: 109-119.

Blum U (2011) *Plant-Plant Allelopathic Interactions*. New York: Springer Science+Business Media. https://doi.org/10.1007/978-94-007-0683-5

Boege K, Dirzo R, Siemens D & Brown P (2007) Ontogenetic switches from plant resistance to tolerance: minimizing costs with age?. *Ecology Letters* 10(3): 177-187. https://doi.org/10.1111/j.1461-0248.2006.01012.x

Braam J & Davis R (1990) Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-related genes in Arabidopsis. *Cell* 60(3): 357-364. https://doi.org/10.1016/0092-8674(90)90587-5

Bricchi I, Leitner M, Foti M, Mithöfer A, Boland W & Maffei M (2010) Robotic mechanical wounding (MecWorm) versus herbivore-induced responses: early signaling and volatile emission in Lima bean (*Phaseolus lunatus* L.). *Planta* 232:719-729. https://doi.org/10.1007/s00425-010-1203-0

Burketova L, Trda L, Ott P & Valentova O (2015) Bio-based resistance inducers for sustainable plant protection against pathogens. *Biotechnology Advances* 33(6):994-1004. https://doi.org/10.1016/j.biotechadv.2015.01.004

CABI (2020) Spodoptera littoralis (cotton leafworm) [Datasheet] Invasive Species Compendium. https://www.cabi.org/isc/datasheet/51070 [2022-05-04]

Chehab W, Eich E & Braam J (2008) Thigmomorphogenesis: a complex plant response to mechano-stimulation. *Journal of Experimental Botany* 60(1): 43-56. https://doi.org/10.1093/jxb/ern315

Chehab W, Yao C, Henderson Z, Kim S & Braam J (2012) *Arabidopsis* Touch-Induced Morphogenesis Is Jasmonate Mediated and Protects against Pests. *Current Biology* 22: 701-706. https://doi.org/10.1016/j.cub.2012.02.061

CICR (n.d.) Glanded and Glandless cotton. *CICR Technical Bulletin* 12. Nagpur: Central Institute of Cotton Research.

Cipollini D (1997) Wind-induced mechanical stimulation increases pest resistance in common bean. *Oecologia* 111: 84-90. https://doi.org/10.1007/s004420050211

Coneva V & Chitwood D (2018) Genetic and Developmental Basis for Increased Leaf Thickness in the Arabidopsis Cvi Ecotype. *Frontiers in Plant Science* 9:322. https://doi.org/10.3389/fpls.2018.00322

Conrath U (2009) Priming of Induced Plant Defense Responses. *Advances in Botanical Research* 51: 361-395. https://doi.org/10.1016/S0065-2296(09)51009-9

Cotton Research & Development Corporation (2020) *Cotton Pest Management Guide*. https://www.crdc.com.au/sites/default/files/pdf/CPMG%202020%20interactive.pdf

Coutand C, Dupraz C, Jaouen G, Ploquin S & Adam B (2008) Mechanical Stimuli Regulate the Allocation of Biomass in Trees: Demonstration with Young *Prunus avium* Trees. *Annals of Botany* 101: 1421-1432. https://doi.org/10.1093/aob/mcn054

De Moraes C, Lewis W, Paré P, Alborn H & Tumlinson J (1998) Herbivore-infested plants selectively attract parasitoids. *Nature* 393:570-573. https://doi.org/10.1038/31219

de Wit M, Kegge W, Evers J, Vergeer-van Eijk M, Gankema P, Voesenek L & Pierik R (2012) Plant neighbor detection through touching leaf tips precedes phytochrome signals. *PNAS* 109(36):14705-14710. https://doi.org/10.1073/pnas.1205437109

Dicke M & Hilker M (2003) Induced plant defences: from molecular biology to evolutionary ecology. *Basic and Applied Ecology* 4:3-14. https://doi.org/10.1078/1439-1791-00129

Dicke M, van Poecke R & de Boer J (2003) Inducible indirect defence of plants from mechanisms to ecological functions. *Basic and Applied Ecology* 4:27-42. https://doi.org/10.1078/1439-1791-00131

Dimetry N (1972) Further Studies on the Host Plant Preference of *Spodoptera littoralis* Boisd. (Lepid., Noctuidae). *Zeitschrift fur Angewandte Entomologie* 71(4):350-3558

Edwards P & Wratten S (1983) Wound induced defences in plants and their consequences for patterns of insect grazing. *Oecologia* 59: 88-93. https://doi.org/10.1007/BF00388079

Elhakeem A, Markovic D, Broberg A, Anten N & Ninkovic V (2018) Aboveground mechanical stimuli affect belowground plant-plant communication. *PLoS ONE* 13(5): e0195646. https://doi.org/10.1371/journal.pone.0195646

Elzen G, Williams H, Bell A, Stipanovic R & Vinson B (1985) Quantification of Volatile Terpenes of Glanded and Glandless *Gossypium hirsutum* L. Cultivars and Lines by Gas Chromatography. *Journal of Agricultural and Food Chemistry* 33: 1079-1082.

EPPO (2021) *EPPO A2 List of pests recommended for regulation as quarantine pests*. https://www.eppo.int/ACTIVITIES/plant_quarantine/A2_list#insects [2022-05-04]

FAO (2021a) *Recent trends and prospects in the world cotton market and policy developments*. Rome. https://doi.org/10.4060/cb3269en

FAO (2021b) *World Food and Agriculture - Statistical Yearbook 2021*. Rome. https://doi.org/10.4060/cb4477en

Franklin K (2008) Shade avoidance. *New Phytologist*, 179:930–944.

Ghosh R, Barbacci A & Lebland-Fournier N (2021) Mechanostimulation: a promising alternative for sustainable agriculture practices. Journal of Experimental Botany 72(8):2877-2888. https://doi.org/10.1093/jxb/erab036

Gifford R & Evans L (1981) Photosynthesis, carbon partitioning, and yield. *Annual Review of Plant Physiology* 32:485-509

Gillet F (2008) Plant Competition. *Encyclopedia of Ecology* 2783-2793. https://doi.org/10.1016/B978-008045405-4.00218-4

Gorzelak M, Asay A, Pickles B & Simard S (2015) Inter-plant communication through mycorrhizal networks mediates complex adaptive behaviour in plant communities. *AoB PLANTS* 7.https://doi.org/10.1093/aobpla/plv050

Gruntman M, Groß D, Májekova M & Tielbörger K (2017) Decision-making in plants under competition. *Nature Communications* 8(2235). https://doi.org/10.1038/s41467-017-02147-2

Hallmann C, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, Stenmans W, Müller A, Sumser H, Hörren T, Goulson D & de Kroon H (2017) More than 75 percent decline over 27 years in total flying insect biomassin protected areas. *PLoS ONE* 12(10): e0185809. https://doi.org/10.1371/journal.pone.0185809

Hargreaves H (1948) *List of Recorded Cotton Insects of the World*. Commonwealth Institute of Entomology: London.

Haukioja E & Koricheva J (2012) Tolerance to herbivory in woody vs. herbaceous plants. *Evolutionary Ecology* 14(4):551-562. https://doi.org/10.1023/A:1011091606022

Held D (2020) Nonchemical approaches to pest management. *Urban Landscape Entomology.* Auburn: Academic Press.

Hinks C & Byers J (1976) Biosystematics of the genus Euxoa (Lepidoptera: Noctuidae). V. Rearing procedures and life cycles of 36 species. *The Canadian Entomologist* 108:1345–1357

Holeski L (2007) Within and between generation phenotypic plasticity in trichome density of *Mimulus guttatus*. *Journal of Evolutionary Biology* 20: 2092-2100. http://doi.org/10.1111/j.1420-9101.2007.01434.x

Howe G & Schaller A (2008) Direct Defenses in Plants and Their Induction by Wounding and Insect Herbivores. I: Schaller A (eds.) *Induced Plant Resistance to Herbivory.* Stuttgard: Springer Science+Business Media 7-29. https://doi.org/10.1007/978-1-4020-8182-8_1

Hsiao T, Acevedo E, Fereres E & Henderson D (1976) Water Stress, Growth, and Osmotic Adjustment. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences* 273(927):479-500

Hu Y, Chen J, Fang L et al (2019) *Gossypium barbadense* and *Gossypium hirsutum* genomes provide insights into the origin and evolution of allotetraploid cotton. *Nature Genetics* 51: 739–748. https://doi.org/10.1038/s41588-019-0371-5

Huang X-Z, Chen J-Y, Xiao H-J, Xiao Y-T, Wu J, Wu J-X, Zhou J-J, Zhang Y-J & Guo Y-Y (2015) Dynamic transcriptome analysis and volatile profiling of *Gossypium hirsutum* in response to the cotton bollworm *Helicoverpa armigera*. *Scientific Reports* 5:11867. https://doi.org/10.1038/srep11867

Hunziker P, Lambertz S, Weber K & Schulz A (2021) Herbivore feeding preference corroborates optimal defense theory for specialized metabolites within plants. *PNAS* 118(47):e2111977118. https://doi.org/10.1073/pnas.2111977118

Ishihara K, Lee E & Borthakur D (2017) Thigmomorphogenesis: changes in morphology, biochemistry, and levels of transcription in response to mechanical stress in *Acacia koa*.

Canadian Journal of Forest Research 47: 583-593. https://doi.org/10.1139/cjfr-2016-0356

Ismail S (2021) Gossypol as a Natural Insecticide in Cotton Plants against Cotton Thrips and Pink Bollworm. *Progress in Chemical and Biochemical Research* 4(1): 68-79. http://doi.org/10.22034/pcbr.2021.120448

Jaffe M (1973) Thigmomorphogenesis: The Response of Plant Growth and Development to Mechanical Stimulation. *Planta* 114: 143-157.

Jost P & Cothren T (2000) Growth and Yield Comparisons of Cotton Planted in Conventional and Ultra-Narrow Row Spacings. *Crop Science* 40:430-435. https://doi.org/10.2135/cropsci2000.402430x

Kamel S & Elkassaby F (1965a) Relative resistance of cotton varieties in Egypt to spider mites, leafhoppes and aphid. *Journal of Economical Entomology* 58: 209–212.

Kamel S (1965b) Relative resistance to cotton leaf worm. *Empire Cotton Growing Rev.* 42:41-48.

Karabourniotis G, Liakopoulos G, Nikolopoulos D & Bresta P (2019) Protective and defensive roles of non-glandular trichomes against multiple stresses: structure–function coordination. *Journal of Forestry Research* 31: 1-12. https://doi.org/10.1007/s11676-019-01034-4

Keddy P & Cahill J (2012) *Competition in Plant Communities*. Oxford: Oxford University Press. https://doi.org/10.1093/obo/9780199830060-0009

Kessler A & Heil M (2010) The multiple faces of indirect defences and their agents of natural selection. *Functional Ecology* 25(2): 348-357. https://doi.org/10.1111/j.1365-2435.2010.01818.x

Khan Z, Ward J & Norris D (1986) Role of trichomes in soybean resistance to cabbage looper, *Trichoplusia ni. Entomologia Experimentalis et Applicata* 42(2): 109-117. https://doi.org/10.1111/j.1570-7458.1986.tb01010.x

Knolhoff L & Heckel D (2014) Behavioral Assays for Studies of Host Plant Choice and Adaptation in Herbivorous Insects. *Annual Review of Entomology* 59:263-278. https://doi.org/10.1146/annurev-ento-011613-161945

Kong C, Zhang S, Li Y, Xia Z, Yang X, Meiners S & Wang P (2018) Plant neighbor detection and allelochemical response are driven by root-secreted signaling chemicals. *Nature Communications* 9:3867. https://doi.org/10.1038/s41467-018-06429-1

Llandres A, Verdeny-Vilalta O, Jean J, Goebel F-R, Seydi O & Brévault T (2019) Cotton extrafloral nectaries as indirect defence against insect pests. *Basic and Applied Ecology* 37:24-34. https://doi.org/10.1016/j.baae.2019.05.001

Loughrin J, Manukian A, Heath R, Turlings T & Tumlinson J (1994) Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plants. *Proceedings of the Natural Academy of Sciences* 91: 11836-11840. https://doi.org/10.1073/pnas.91.25.11836

Markovic D, Glinwood R, Olsson U & Ninkovic V (2014) Plant response to touch affects the behaviour of aphids and ladybirds. Arthropod-Plant Interactions 8:171-181. https://doi.org/10.1007/s11829-014-9303-6

Markovic D, Nikolic N, Glinwood R, Seisenbaeva G & Ninkovic V (2016) Plant Responses to Brief Touching: A Mechanism for Early Neighbour Detection?. *PLoS ONE* 11(11): e0165742. https://doi.org/10.1371/journal.pone.0165742

McAuslane H, Alborn H & Toth J(1997) Systemic Induction of Terpenoid Aldehydes in Cotton Pigment Glands by Feeding of Larval *Spodoptera exigua*. *Journal of Chemical Ecology* 23:2861-2879.

McCarty J, Hedin P & Stipanovic R (1996) Cotton Gossypium Spp.Plant Gossypol Contents of Selected GL_2 and GL_3 Alleles. *Journal of Agricultural and Food Chemistry* 44(2): 613-616. https://doi.org/10.1021/jf9505887

Meldau S, Erb M & Baldwin I (2012) Defense on demand: mechanisms behind optimal defense patterns. *Annals of botany* 110(8):1503-1514. https://doi.org/10.1093/aob/mcs212

Mithöfer A, Wanner G & Boland W (2005) Effects of Feeding *Spodoptera littoralis* on Lima Bean Leaves. II. Continuous Mechanical Wounding Resembling Insect Feeding Is Sufficient to Elicit Herbivory-Related Volatile Emission. *Plant Physiology* 137(3): 1160-1168. https://doi.org/10.1104/pp.104.054460

Moerschbacher B, Noll U, Gorrichon L & Reisener H-J(1990) Specific Inhibition of Lignification Breaks Hypersensitive Resistance of Wheat to Stem Rust. *Plant Physiology* 93(2): 465–470. https://doi.org/10.1104/pp.93.2.465

Moore B & Johnson S (2017) Get Tough, Get Toxic, or Get a Bodyguard: Identifying Candidate Traits Conferring Belowground Resistance to Herbivores in Grasses. *Frontiers in Plant Science* 7:1925. https://doi.org/10.3389/fpls.2016.01925

Moya-Raygoza G (2016) Early Development of Leaf Trichomes Is Associated With Decreased Damage in Teosinte, Compared With Maize, by *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* 109(5):737-743. https://doi.org/10.1093/aesa/saw049

Nawab N, Khan I, Khan A & Amjad M (2011) Characterization and inheritance of cotton leaf pubescence. *Pakistan Journal of Botany* 43(1):649-658.

Ninkovic V (2003) Volatile communication between barley plants affects biomass allocation. *Journal of Experimental Botany* 54(389):1931-1939. https://doi.org/10.1093/jxb/erg192

Ninkovic V, Rensing M, Dahlin I & Markovic D (2019) Who is my neighbor? Volatile cues in plant interactions. *Plant Signaling & Behavior*. https://doi.org/10.1080/15592324.2019.1634993

Oerke EC (2006) Crop losses to pests. *Journal of Agricultural Science* 144: 31– 43.

Ortega A (1987) *Insect pests of maize: a guide for field identification.* Cimmyt.

Pagán I & García-Arenal F (2018) Tolerance to Plant Pathogens: Theory and Experimental Evidence. *International Journal of Molecular Sciences* 19(3):810. https://doi.org/10.3390/ijms19030810

Park S-H, Scheffler J, Scheffler B, Cantrell C & Pauli C (2019) Chemical defense responses of upland cotton, *Gossypium hirsutum* L. to physical wounding. *Plant Direct* 3:1-15. https://doi.org/10.1002/pld3.141

Patterson D, Bunce J, Alberte R & van Volkenburgh E (1977) Photosynthesis in Relation to Leaf Characteristics of Cotton from Controlled and Field Environments. *Plant Physiology* 59(3):384-387. https://doi.org/10.1104/pp.59.3.384

Peiffer M, Tooker J, Luthe D & Felton G (2009) Plants on early alert: glandular trichomes as sensors for insect herbivores. *New Phytologist* 184: 644-656. https://doi.org/10.1111/j.1469-8137.2009.03002.x

Rankenberg T, Geldhof B, van Veen H, Holsteens K, Van de Poel B & Sasidharan R (2021) Age-Dependent Abiotic Stress Resilience in Plants. *Trends in Plant Science* 28(7):692-705. https://doi.org/10.1016/j.tplants.2020.12.016

Rasmann S, Köllner T, Degenhardt J, Hiltpold I, Toepfer S, Kuhlman U, Gershenzon J & Turlings T (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* 434(7034):732-737. https://doi.org/10.1038/nature03451.

Röse U, Manukian A, Heath R & Tumlinson J (1996) Volatile Semiochemicals Released from Undamaged Cotton Leaves (A Systemic Response of Living Plants to Caterpillar Damage). *Plant Physiology* 111(2):487-495. https://doi.org/10.1104/pp.111.2.487

Santiago R, Barros-Rios J & Malvar R (2013) Impact of Cell Wall Composition on Maize Resistance to Pests and Diseases. *International Journal of Molecular Sciences* 14(4): 6960-6980. https://doi.org/10.3390/ijms14046960

Savvides A, Ali S, Tester M, Fotopoulos V (2016) Chemical priming of plants against multiple abiotic stresses: mission possible? Trends in Plant Science 21: 329–340. https://doi.org/10.1016/j.tplants.2015.11.003

Shahzad M, Khan Z, Nazeer W, Arshad F, Ahmad F, Farid B, Shahid R & Riaz H (2021) Effect of Drought on Trichome Density and Length in Cotton (*Gossypium hirsutum*). *Journal of Bioresource Management* 8(1): 154-167. https://doi.org/10.35691/JBM.1202.0174

Shanower T (2008) Trichomes and Insects. I: Capinera J (eds.) *Encyclopedia of Entomology.* Dordrecht: Springer. https://doi.org/10.1007/978-1-4020-6359-6_2535

Schoonhoven L, van Loon J & Dicke M (2005) *Insect - plants Biology*. 2nd edition. Oxford: Oxford University Press.

Sidibe B & Lauge G (1977) Effect of warm periods and of constant temperatures on some biological criteria in *Spodoptera littoralis* Boisduval (Lepidoptera Noctuidae). *Annales de la Societe Entomologique de France* 13(2): 369-379.

Strauss S & Agrawal A (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology & Evolution* 14(5): 179-185. https://doi.org/10.1016/S0169-5347(98)01576-6

Tamayo M, Rufat M, Bravo J & San Segundo b (2000) Accumulation of a maize proteinase inhibitor in response to wounding and insect feeding, and characterization of its activity toward digestive proteinases of *Spodoptera littoralis* larvae. *Planta* 211(1):62-71. http://doi.org/10.1007/s004250000258

Telewski F & Jaffe M (1986) Thigmomorphogenesis: Field and laboratory studies of *Abies fraseri* in response to wind or mechanical perturbation. *Physiologia Plantarum* 66(2): 211-218. https://doi.org/10.1111/j.1399-3054.1986.tb02411.x

Tian X, Ruan J, Huang J, Fang X, Mao Y, Wang L, Chen X & Yang C (2016) Gossypol: phytoalexin of cotton. *Science China Life Sciences* 59(2): 122-129. https://doi.org/10.1007/s11427-016-5003-z

Ton J, D'Alessandro M, Jourdie V, JAkab G, Karlen D, Held M, Mauch-Mani B & Turlings T (2006) Priming by airborne signals boosts direct and indirect resistance in maize. *The Plant Journal* 49(1):16-26. https://doi.org/10.1111/j.1365-313X.2006.02935.x

UCR (n.d) *Colonization of Natural Enemies.* Riverside: University of California http://www.faculty.ucr.edu/~legneref/biotact/bc-30.htm [2022-05-06]

Van Dam N, Hermenau U & Baldwin I (2001) Instar-specific sensitivity of specialist *Manduca sexta* larvae to induced defences in their host plant *Nicotiana attenuata*. *Ecological Entomology* 26(6): 578-586. https://doi.org/10.1046/j.1365-2311.2001.00366.x

Veyrat N, Robert C, Turlings T & Erb M (2015) Herbivore intoxication as a potential primary function of an inducible volatile plant signal. *Journal of Ecology* 104: 591-600 https://doi.org/10.1111/1365-2745.12526

Walter D, Ratsep J & Havis N (2013) Controlling crop diseases using induced resistance: challenges for the future. *Journal of Experimental Botany* 64(5): 1263-1280. https://doi.org/10.1093/jxb/ert026

Wang B, Wang J, Zhao H & Zhao H (2006) Stress induced plant resistance and enzyme activity varying in cucumber. *Colloids and Surfaces B: Biointerface* 48(2):138-142. https://doi.org/10.1016/j.colsurfb.2006.01.018

Wäckers F, Zuber D, Wunderlin R & Keller F (2001) The Effect of Herbivory on Temporal and Spatial Dynamics of Foliar Nectar Production in Cotton and Castor. *Annals of Botany* 87: 365-370. https://doi.org/10.1006/anbo.2000.1342

Xiaoning Zhang N, Stephan J, Björkman C & Puentes A (2021) Global change calls for novel plant protection: reviewing the potential of omnivorous plant-inhabiting arthropods as predators and plant defence inducers. *Current Opinion in Insect Science* 47:103-110. https://doi.org/10.1016/j.cois.2021.06.001

Yang G, Isenhour D & Espelie K (1991) Activity of maize leaf cuticular lipids in resistance to leaf-feeding by the fall armyworm. *The Florida Entomologist* 74(2):229-236.

Zakir A, Khallaf M, Hansson B, Witzgall P & Anderson P (2017) Herbivore-Induced Changes in Cotton Modulates Reproductive Behavior in the Moth Spodoptera littoralis. *Frontiers in Ecology and Evolution* 5:49. https://doi.org/10.3389/fevo.2017.00049

Zhang Z, Zhang X, Wang S, Xin W, Tang J & Wang Q (2013) Effect of Mechanical Stress on Cotton Growth and Development. *PLoS ONE* 8(12): e82256. http://doi.org/10.1371/journal.pone.0082256

Zhao H, Wang B-C, Zhao H-C & Wang J (2005) Stress stimulus induced resistance to Cladosporium cucumerinum in cucumber seeding. *Colloids and Surfaces B: Biointerfaces* 44(1):36-40. https://doi.org/10.1016/j.colsurfb.2005.04.014

Zur M, Meisner J, Kabonci E & Ascher K (1979) Gossypol content of cotton leaves during the growing season, and growth suppression of *Spodoptera littoralis* (Boisduval) larvae on some high-gossypol (HG) cotton strains. *Zeitschrift fuer Angewandte Entomologie* 87: 435-439.

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file. If you are more than one author, the checked box will be applied to all authors. Read about SLU's publishing agreement here:

- https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/.

☒ YES, I hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.