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Finding the right one

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Axel Rösvik

Faculty of Landscape Architecture, Horticulture and Crop Production Science
Department of Plant Protection Biology
Alnarp

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Cover: Moth on a leaf surface
(Photo: Generated by Dall-E 2)

Finding the right one: Host-plant choice and its transgenerational influence on behaviour and life-history traits in *Spodoptera littoralis*

Abstract

Herbivorous insects have an intricate relationship with their host plants, which they use as food, oviposition substrate, as well as a rendezvous site for mating. For generalist herbivorous insects that have a wide host range, finding a suitable host plant in a complex environment is of great importance for their offspring's development and survival. Most herbivorous insects use olfactory cues for host-plant location, where changes in odour profiles of plants due to extrinsic factors from both biotic and abiotic stressors must be taken into consideration when locating a suitable host. Several generalist insects are known to use odour cues experienced during their larval development to facilitate host-plant location in the adult stage, even if they undergo complete metamorphosis between the two developmental stages. This may funnel individuals onto a particular plant species, increasing the chances of their offspring ending up in a matching environment to that of the parent. The use of previous experience is thought to facilitate transgenerational acclimatization, where parents acclimatize their offspring to the environment that they experienced.

This thesis investigates how herbivory influences host-plant choice in the generalist moth *Spodoptera littoralis* (**paper I**) and the transgenerational impact of plant choice on preference and life-history traits (**papers II and III**).

I found that herbivory by conspecifics influenced host-plant location and preference hierarchies of *S. littoralis* in a sex-specific manner where the odours released from damaged plants were driving the behavioural shifts. Furthermore, I found that (previous) parental experience does not influence host-plant location in neonate larval or adult females, but that host-plant choice relies on innate preference hierarchies and within-generational phenotypic plasticity. Moreover, I demonstrated how transgenerational acclimatization could influence developmental traits while reproductive output shows no signs of transgenerational acclimatization but rather the opposite. Thus, the use of previous experience may be a way to facilitate host-plant location in adult individuals, which comes at the expense of the reproductive output of their offspring.

The findings in this thesis emphasize the complexity of host-plant choice in generalist insects and the influence the plant community have on behaviour and life-history traits within- and across-generations.

Keywords: chemical ecology, preference hierarchy, plant volatiles, herbivore-induced plant volatiles, phenotypic plasticity, transgenerational effects, transgenerational acclimatization, within-generation phenotypic plasticity, carry-over effects, insect-plant interaction

Att hitta den rätta: Värdväxtval och dess påverkan på beteende, utveckling och reproduktion över generationsgränsen hos *Spodoptera littoralis*

Abstract

Växtätande insekter har ett invecklat förhållande till sina värdväxter som agerar föda, äggläggningssubstrat och mötesplats vid parning. För generalister, som har ett brett spektrum av potentiella värdväxter, är det av stor betydelse att hitta en lämplig värdväxt i en komplex miljö för deras avkommas utveckling och överlevnad. De flesta växtätande insekter använder doftsignaler för att hitta sina värdväxter, där förändringar i växternas doftprofiler på grund av yttre biotiska och abiotiska stressfaktorer måste tas i beaktande för att lokalisera en lämplig värdväxt. Flera generalistiska insekter är kända för att använda doftsignaler som de upplevt under sin larvutveckling för att underlätta valet av värdväxt i det vuxna stadiet, även om de genomgår fullständig metamorfos mellan larv- och vuxenstadiet. Detta kan styra individer mot en viss växtart, vilket ökar chansen att deras avkomma hamnar i en miljö som motsvarar förälderns. Användningen av tidigare erfarenhet anses underlätta generationsöverskridande acklimatisering, där föräldrar tillvänjer sin avkomma till den miljö som de själva har upplevt.

Denna avhandling undersöker hur herbivori påverkar valet av värdväxt hos den generalistiska nattfjärilen *Spodoptera littoralis* (**artikel I**) och de konsekvenser värdväxtvalet har på beteende, utveckling och reproduktiv förmåga över generationsgränsen (**artikel II och III**).

Jag fann att herbivori från artfränder påverkar värdväxtval och preferenshierarkier i *S. littoralis* på ett könsspecifikt sätt där dofterna som avges från de skadade växterna driver förändringarna i beteendet. Dessutom fann jag att (tidigare) erfarenhet hos föräldern inte påverkar värdväxtvalet hos nykläckta larver eller vuxna honor, utan att värdväxtvalet är beroende av medfödda preferenshierarkier och tidigare erfarenhet inom generationen. Dessutom visar jag hur generationsöverskridande acklimatisering kan påverka utvecklingen av avkomman medan reproduktiv förmåga inte visar några tecken på generationsöverskridande acklimatisering utan snarare motsatsen. Användningen av tidigare erfarenheter kan alltså snarare vara ett sätt att underlätta värdväxtvalet hos vuxna individer på bekostnad av deras avkommas reproduktiva förmåga.

Resultaten i avhandlingen betonar den komplexitet som valet av värdväxt innebär för generalistiska insekter och det inflytande som växtsamhället har på beteende, utveckling och reproduktiv förmåga, inom och över generationsgränsen.

Dedication

To Elsie and Siri.

"Playing things too safe is the most popular way to fail. "

Elliott Smith

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I.   Rösvik, A., Gupta, S., Bengtsson, M., Bohman, B. & Anderson, P. Herbivore damage influences male and female moth host-plant choice in a within- and between-plant species context. Manuscript.

II.   Rösvik, A., Lhomme, P., Khallaf, M.A. & Anderson, P (2020). Plant-induced transgenerational plasticity affecting performance but not preference in a polyphagous moth. *Frontiers in Ecology and Evolution*, 8, 254.

III.   Rösvik, A., Gupta, S., Ericson, M., Karlsson Green, K. & Anderson, P. Parental host-plant experience and its transgenerational influence on offspring reproductive output and mortality in a generalist moth. Manuscript.

The contribution of Axel Rösvik to the papers included in this thesis was as follows:

    I.   Designed the study together with the co-authors. Collected headspace samples, performed GC-MS, identified compounds of interest, performed behavioural experiments and analysed the data. Wrote the manuscript with input from the co-authors.

    II.   Designed the study together with the co-authors. Performed behavioural and developmental experiments and analysed the data. Wrote the manuscript with input from the co-authors.

    III.   Designed the study together with the co-authors. Collected data on insect life-history traits. Performed the image- and statistical-analysis. Wrote the manuscript with input from the co-authors.

Abbreviations

AL	Antennal lobe
GC-MS	Combined gas chromatography and mass spectrometry
GLV	Green leaf volatile
GR	Gustatory receptor
HHSP	Hopkins host selection principle
HIPV	Herbivore-induced plant volatile
NMDS	Non-metric multidimensional scaling
OR	Olfactory receptor
OSN	Olfactory sensory neuron
PCA	Principal component analysis
PERMANOVA	Permutational multivariate analysis of variance
RH	Relative humidity
TGP	Transgenerational phenotypic plasticity
VOC	Volatile organic compound
WGP	Within-generation phenotypic plasticity

1. Introduction

Insects and plants have co-existed for over one hundred million years and the interactions between the two are both mutualistic and antagonistic (Schoonhoven *et al.* 2005). Their co-evolution has been postulated to be an important underlying force giving rise to the great diversity of terrestrial life forms (Ehrlich & Raven 1964). As for insects, this group makes up half of all the species described worldwide (Grimaldi & Engel 2005; Mayhew 2007), whereas herbivorous insects make up a third (approximately 500 000) of all described eukaryotes (Hardy *et al.* 2020). Two-thirds of herbivorous insects belong to the two orders Coleoptera (beetles) and Lepidoptera (butterflies and moths), where the larvae and adult beetles and the larval stage of butterflies and moths devour plant tissue by chewing, tearing or snipping with their mouthparts (Schoonhoven *et al.* 2005). In agroecosystems, herbivorous insects pose a serious threat, with estimates of around 15 % yield reduction due to the damage they cause to the plants (van der Meijden 2014; Savary *et al.* 2019). This makes insect-plant interactions not only interesting from an ecological and evolutionary perspective but also from an applied aspect due to their impact on food availability (Schoonhoven *et al.* 2005).

In Lepidopterans, which are holometabolous insects, a pupal stage separates the larval and the adult stage (Wiegmann *et al.* 2009). Resource acquisition is predominantly limited to the larval stage, while the adult stage is responsible for mating and host-plant location (García-Robledo & Horvitz 2012). For adult females, utilizing host-plant chemosensory cues to find a suitable oviposition substrate for larval development is therefore of great importance (Bruce *et al.* 2005; Bruce & Pickett 2011). For males, host-plant chemosensory cues in combination with female pheromones are crucial for finding a mating partner (Reddy & Guerrero 2004).

In this thesis, the focus lies on the herbivorous moth *Spodoptera littoralis*, a highly polyphagous insect herbivore, which is considered a quarantine pest in Europe and a phytosanitary problem on several continents (CABI 2022; EPPO 2023). This species utilizes odour cues to locate its host plant and is known to use previous experience to facilitate host-plant choice (Anderson *et al.* 2013; Thöming *et al.* 2013; Borrero-Echeverry *et al.* 2015; Proffit *et al.* 2015). In species that utilize previous experience to guide behavioural decisions, the chances of parent-offspring diets to match increase. This has been proposed to facilitate transgenerational acclimatization, where parental generations could preadapt their offspring to an expected environment (Cahenzli *et al.* 2015). In this thesis, I investigated how changes in plant-odour cues due to herbivory affect male and female host-plant choice and how host-plant experience influences behaviour, development and reproduction in a transgenerational context.

2. Research background

2.1 Insect plant interactions

Herbivorous insects show various feeding strategies, in which certain species feed throughout their lifetime, while other species have particular life stages where feeding takes place and other life stages where mating and mate location occur (Pierce & Berry 2011). In Lepidopterans, which are holometabolous and, thus, undergo a complete metamorphosis, the larval stage is responsible for most of the resource acquisition that will influence the adult individual resource allocation, e.g. egg production (Jiménez-Pérez & Wang 2004; Rhainds 2015). However, adult individuals can replenish themselves by feeding on nectar, which may increase their flight ability and fecundity (Levin *et al.* 2017). The adult stage is responsible for reproduction, such as locating a rendezvous site for mating and finding a suitable oviposition substrate for larval development (Gripenberg *et al.* 2010). Finding a suitable oviposition substrate is a complex task where the adult female must consider several aspects of plant suitability. The adult female must be able to discriminate between plants of different suitability in terms of larval development (Jaenike 1978; Thompson & Pellmyr 1991) and competition from conspecifics (De Moraes *et al.* 2001; Allmann *et al.* 2013). Furthermore, she must also be able to find a plant with a low abundance of natural enemies or a plant that, due to its architecture, further complicates the host location by the natural enemies (Bernays & Graham 1988; Thompson & Pellmyr 1991; Jones 2022). However, many empirical examples of host-search gone wrong have coined the term 'optimal bad motherhood' (also known as 'parent-offspring conflict'), where the mothers choose suboptimal host plants for their offspring to increase their longevity

(Mayhew 2001; Garcia-Robledo & Horvitz 2012). Although less mobile than their winged adult counterpart, host-plant location, or relocation, does occur in larvae. Young larvae in the Lepidoptera order are known to use ballooning behaviour, using a silk thread they spin to migrate within dense plant communities (Zalucki *et al.* 2002; Bell *et al.* 2005). Furthermore, later larval instars are more mobile and individuals can migrate towards neighbouring plants by crawling (Mitra *et al.* 2021).

2.1.1   Chemosensation in insects

For host-plant location to occur, the adult insects must be able to sense the world around them. This is accomplished from afar through olfaction using their primary olfactory organ, the antennae (Hansson 1999). The antennae contain hair-like sensilla which house olfactory receptors (ORs) and ionotropic receptors which can be activated by ligands that bind to the receptor surface (Sachse & Krieger 2011). For herbivorous insects, ligands of importance are volatile organic compounds (VOCs) released from plants in the surrounding environment or pheromones released by conspecifics. VOCs released from plants are composed of organic compounds with low molecular weight and high vapour pressure at ambient temperatures, causing them to easily volatilize (Pichersky *et al.* 2006; Dudareva *et al.* 2013). The receptors detecting VOCs released from plants can be both narrowly or broadly tuned, while pheromone receptors are directly tuned to the pheromone in question (Andersson *et al.* 2015). When a VOC is detected by the OR, olfactory sensory neurons (OSNs) forward the information towards the antennal lobe (AL), where OSNs expressing the same receptors coincide at a single glomeruli (Sachse & Krieger 2011). Multiple glomeruli present in the AL acts as coding units where the combinatorial activation underlie the olfactory discrimination (Galizia *et al.* 1999; Wang *et al.* 2003). The information is then forwarded through projection neurons to the higher brain centres in the protocerebrum of the insect containing the lateral horn and the mushroom bodies, where odour perception takes place (Hansson 1999; Sachse & Krieger 2011). The outcome of processed stimuli induces behaviours, such as upwind flight towards the source of the stimuli, where odour cues from plants create an odour plume that can be followed by the receiver to reach the emitter, much like that of a 'path' in a physical landscape (Conchou *et al.* 2019).

When in physical contact with the host plant, insects also utilize contact cues (Schoonhoven *et al.* 2005). These are detected by gustatory receptors (GRs) that have a similar function as ORs although they sense chemosensory contact cues (Agnihotri *et al.* 2016). The GRs are located on the mouthparts, antennae, tarsi, wings and ovipositor of insects (Benton 2015; Agnihotri *et al.* 2016). Ligands such as water, sugars, sugar alcohols, salt, amino acids, and bitter substances bind to the GR, which activates downstream signalling of information to the central nervous system of the insect, eliciting a behavioural response, e.g. feeding or deterrence (Tang *et al.* 2014).

2.1.2 Plant volatile organic compounds

All plants continuously release VOCs from most of their tissues (Penuelas & Llusià 2001; Dudareva *et al.* 2006) and the composition of the released VOCs varies greatly among plant genera (Vivaldo *et al.* 2017). VOCs are secondary metabolites, which impose that they are not responsible for the direct metabolism of the plant (Crozier *et al.* 2006; Picazo-Aragonés *et al.* 2020). However, the production of VOCs by the plant requires carbon, nitrogen, sulphur and energy provided by primary metabolism (Dudareva *et al.* 2013). This implies that the production and release of VOCs are directly influenced by the primary metabolism.

Almost all VOCs released from plants originate from four metabolic pathways. Terpenoids, which are the most numerous and structurally diverse group of compounds, originate from the methylerythritol phosphate and mevalonic acid pathways (Cheng *et al.* 2007). Phenylpropanoid and benzenoid compounds, the second largest class of plant volatiles (Knudsen et al 2006), are produced from the shikimate pathway (Maeda & Dudareva 2012). Green leaf volatiles, which consists of saturated or monosaturated six to nine-carbon aldehydes and alcohols, originate from the lipoxygenase pathway (Dudareva *et al.* 2013). Other metabolic pathways do exist, although these are generally associated with species-specific compounds (Dudareva *et al.* 2013).

In plants, VOCs are considered to be the most important secondary metabolite due to their interaction with the surrounding environment (Picazo-Aragonés *et al.* 2020). It is responsible for several ecological functions, such as the attraction of mutualistic organisms including pollinators (Muhlemann *et al.* 2014; Schiestl 2015), seed dispersers (Hodgkison *et al.* 2007; Borges *et al.* 2008; Youngsteadt *et al.* 2008),

microorganisms (Schulz-Bohm *et al.* 2018) and natural enemies of herbivores (Dicke 2009; Dicke & Lucas-Barbosa 2020). For herbivorous insects, the use of VOCs released from plants can be used to differentiate between host and non-host, but also hosts of different suitability (Bruce *et al.* 2005; Bruce & Pickett 2011; Silva & Clarke 2020). VOCs are also important in plant-to-plant communication (Baldwin *et al.* 2006; Heil & Karban 2010; Ninkovic *et al.* 2021) and tolerance towards abiotic stressors, such as high temperatures (Jun-Wen & Cao 2005).

2.1.3 Extrinsic factors influencing the release of VOCs

Although plants show a continuous release of VOCs, the odour profile of a particular plant is not always constant. The volatiles released from a plant vary due to the ontogenetic state of the plant (Bracho-Nunez *et al.* 2011) and due to the time of day or night (Badra *et al.* 2021). Furthermore, abiotic factors such as drought, temperature, ozone and CO_2 (Dicke & Loreto 2010; Conchou *et al.* 2019), as well as biotic factors, e.g. insect herbivory and oviposition (Dicke & Van Loon 2000; Dicke 2009), are important factors that influence the release of VOCs.

During herbivory by larvae, the tearing of the leaf by the insect mouthparts creates open wounds that cause a direct release of already stored compounds (Loughrin *et al.* 1994; McCall *et al.* 1994). Moreover, elicitors from the feeding larva and ovipositing adult, e.g. saliva and secretion respectively, can cause phytochemical changes in the plant, initiating changes in metabolic pathways (Mumm & Dicke 2010; Hilker & Fatouros 2015). This influences the production of *de-novo* synthesized compounds, which can be produced by undamaged parts of the plant (Turlings & Tumlinson 1992; Pare & Tumlinson 1997; Röse & Tumlinson 2005). Natural enemies of herbivores are known to eavesdrop on the released VOCs from herbivory, often referred to as herbivore-induced plant volatiles (HIPV), as a way of locating their prey (Dicke & Van Loon 2000; Dicke & Lucas-Barbosa 2020). The HIPVs released during damage from herbivores belong to the three major classes of compounds; terpenoids, aromatic compounds and green leaf volatiles (Dicke 2009; Dicke & Lucas-Barbosa 2020) (figure 1). Directly after herbivore damage, constitutively stored volatiles are released from the damaged plant part, influencing the odour blend composition (Jansen *et al.* 2011). Furthermore, variations in odour blend composition take place days after herbivore attack, with different compounds

having a peak in release at different time points (Holopainen & Blande 2013).

The influence of extrinsic factors on the volatile profiles of plants increases the complexity of the olfactory environments experienced by insects. In a natural context, multitrophic interactions influence the release of VOCs, where soil microbiota (Farré-Armengol *et al.* 2016), the type of insect feeding on the plant (Mann *et al.* 2021), neighbouring plant community (Ninkovic *et al.* 2021) and interaction between herbivory and plant community complexity (Kigathi *et al.* 2019) play influential roles on plant-odour profiles.
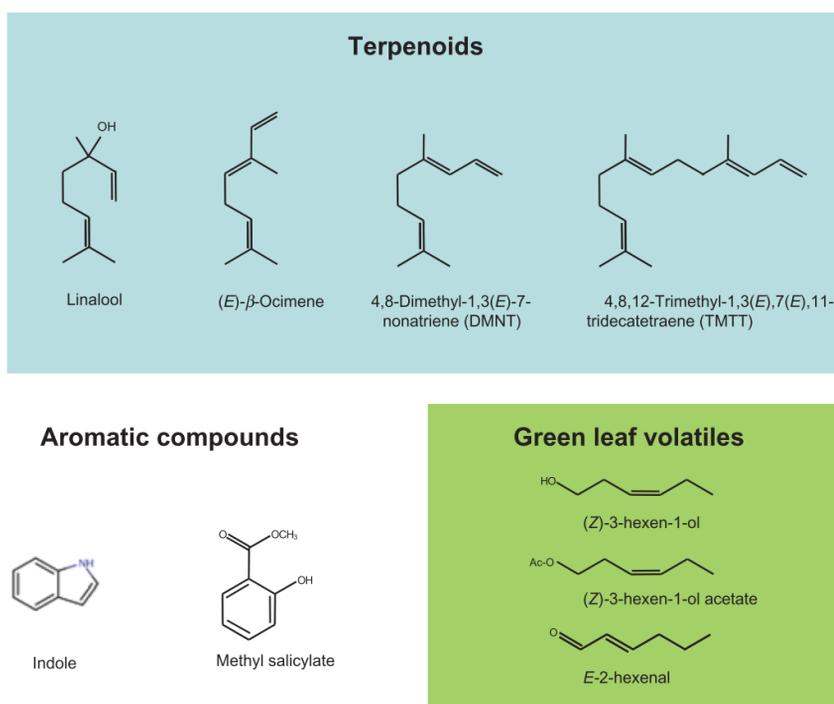
Figure 1. Examples of common HIPVs from the three major classes of compounds originating from the four metabolic pathways mentioned in the 'plant volatile organic compound' section 2.1.2. Modified from Dicke (2009) with the publisher's permission.

2.1.4   Making sense of the olfactory environment

Insects use their primary olfactory organ, the antennae, to make sense of their surrounding environment and the volatiles it contains. Odours emitted from

potential host plants need to be distinguished from those of non-hosts in an environment with a constant odour background (Beyaert & Hilker 2014; Conchou *et al.* 2019). As if this was not a complex task on its own, the VOCs released form odour bouquets that differ both quantitatively and qualitatively, where hundreds of different compounds in different combinations and concentrations can be released from a single plant (Bruce & Pickett 2011).

Some species use taxonomically specific cues, in which a plant is identified based on one particular compound or a small set of specific compounds (Bruce *et al.* 2005). The *Brassicaceae* specialists cabbage aphid *Brevicoryne brassicae* (Nottingham *et al.* 1991) and the cabbage seed weevil, *Ceutorhynchus assimilis* (Blight *et al.* 1995), have been shown to utilize *Brassicaceae* species-specific isothiocyanates as key compounds for host-plant location. However, the vast majority of insects are dependent on a wider range of more ubiquitously occurring compounds independent of their diet range, i.e. whether they are specialists, oligophagous or generalists (Bruce *et al.* 2005). This implies that the different combinations of compounds and ratios play an important role, with the variation in compound composition and ratios creating endless possibilities of combinations (Bruce & Pickett 2011). In the moth *Manduca sexta*, which is a good example of this, it has been shown that individuals were not attracted to single compounds from its host plant, but to a blend of synthetic compounds, where nine out of the sixty compounds released by the plant were sufficient to mimic the plant (Riffell *et al.* 2009). Interestingly, it has also been shown that compounds present in a blend eliciting attraction could be repellent if presented alone (Webster *et al.* 2010).

Although most specialists do not use species-specific cues, the narrow host range can facilitate host-plant location due to the fine-tuning towards a particular blend of cues (Bruce *et al.* 2005; Bruce & Pickett 2011; Silva & Clarke 2020). Contrarily, for generalists, the vast range of odour cues released from the wide range of potential hosts upon which they must discriminate can make host-plant choice difficult due to neural limitations (Bernays 2001). This has been shown to influence host-plant choice where generalists are slower to locate a host than specialists in general (Bernays 1999; Bernays & Funk 1999; Janz 2003). To unravel how generalists recognize their host plants remains a major challenge that requires further studies on insect-plant systems (Bruce & Pickett 2011; Cunningham 2012).

2.2 Phenotypic plasticity

Phenotypic plasticity is the ability of a single genotype to achieve different phenotypic endpoints due to environmental influence (West-Eberhard 1989; Whitman & Agrawal 2009). Plasticity can include changes in biochemical, physiological, behavioural and developmental traits, in which the first three are reversible throughout the lifetime of an individual, influencing the phenotypic endpoint. Contrarily, developmental plasticity may cause irreversible changes during early development that cannot be changed (Pigliucci *et al.* 2006; Sheehy & Laskowski 2023). It has been proposed that organisms experiencing heterogeneous environments, where environmental fluctuations are somewhat predictable and the cost of plasticity is low, should be favoured by phenotypic plasticity (Levins 1968; Scheiner 1993; Uller 2008). Furthermore, since evolution acts on a longer timescale, plasticity could allow for resilience to environmental change in populations on a short timescale (Diamond & Martin 2021). Although heavily debated, it has also been proposed that phenotypic plasticity could have an impact on evolution through genetic assimilation where previously expressed phenotypes are subjected to selection, causing canalization where the new phenotypes are established in the population (Pigliucci *et al.* 2006; Levis & Pfennig 2016).

2.2.1 Phenotypic plasticity influencing behaviour in insects

Many insects follow innate behaviours during e.g. host-plant choice, where the behavioural outcome follows a hierarchical ranking of hosts based on host cues (Dukas 2008). To facilitate behavioural decisions in heterogeneous environments, plasticity is of great importance (Whitman & Agrawal 2009). Behaviour, being one of the more plastic phenotypic traits, has been proposed to be easily influenced by environmental cues through within-generation phenotypic plasticity (WGP) (West-Eberhard 1989). Many examples of WGP and its influence on behaviour exist, where an emphasis has been made on holometabolous insect species (Wright & Schiestl 2009) in the orders Hymenoptera (Turlings *et al.* 1993; Menzel & Müller 1996), Diptera (Busto *et al.* 2010), and Lepidoptera (Anderson & Anton 2014). In generalist insects, the use of previous experience could facilitate behavioural decisions such as host-plant location (Bernays 2001). In Lepidopterans, both males and females are influenced by previous experience (Anderson & Anton 2014; Silva & Clarke 2020). Females utilize previous experience to find a suitable host for oviposition (Olsson *et al.* 2006; Anderson *et al.* 2013;

Thöming *et al.* 2013; Proffit *et al.* 2015; Kemp 2019) and males for locating a mating rendezvous site (Li *et al.* 2005; Anderson *et al.* 2013; Thöming *et al.* 2013; Proffit *et al.* 2015; Kemp 2019). Thus, previous experience could cause association with a particular plant species, causing the generalist insect to act more as a specialist on that particular plant (Mechaber & Hildebrand 2000; del Campo *et al.* 2001).

2.2.2   Transgenerational phenotypic plasticity

The phenotype may not only be influenced by the genotype and the current environmental state but also by the environment experienced in previous generations (Mousseau & Fox 1998a). This is referred to as transgenerational phenotypic plasticity (TGP), which is a wide concept that includes various mechanisms of transfer, e.g. epigenetics (Youngson & Whitelaw 2008), vertical transfer of symbionts (Paniagua Voirol *et al.* 2018) and resource transfer (Fox & Czesak 2000). Transgenerational phenotypic plasticity has a plethora of different names in the literature, depending on its mechanism of transfer and where it originates from, i.e. mother, father or both (Mousseau & Fox 1998b; Mousseau & Fox 1998a; Marshall & Uller 2007; Engqvist & Reinhold 2016; Bonduriansky & Crean 2018). It has been shown that for invertebrates and annual plants, TGP is more common compared to vertebrates and perennial plants (Yin *et al.* 2019). It has also been proposed that TGP is more important than previously anticipated and that individual organisms might benefit from TGP to a similar extent as WGP (Salinas *et al.* 2013). In some systems, the use of TGP might even be more optimal compared to WGP, since parents could react to environmental cues and create an optimal offspring phenotype if they anticipate the environmental cues correctly (Jablonka *et al.* 1995).

In insects, several phenotypic traits could be influenced by TGP, where more evidence exists on morphological and physiological traits compared to that of behavioural traits (Woestmann & Saastamoinen 2016 and references therein; Donelson 2018; Gowri & Monteiro 2021). Although some examples of the influence of TGP on host-plant choice behaviour in insects do exist within Lepidoptera where either nutritional qualities of food (Cahenzli *et al.* 2015) or odour cues (Gowri 2019) increase the salience for the plant experienced by the parent in ovipositing females and food searching larvae respectively. The use of previous experience from parental generation guiding behaviours could play an important role in rapid adaptation to novel

environments (Ledón-Rettig *et al.* 2013). When TGP influences developmental traits, this could be accomplished through transgenerational acclimatization, where the parental generation predicts the environment of the offspring based on environmental cues experienced and adapts its offspring to the experienced environment (Mousseau & Fox 1998b; Marshall & Uller 2007; Munday 2014). This causes the offspring to have an increased fitness when parent-offspring environments are matched (Uller 2008; Engqvist & Reinhold 2016). Some examples of this in Lepidopterans do exist, although they are scarce. In the butterflies, *Pieris rapae* and *Coenonympha pamphilus*, parents acclimatize their offspring to a plant of the same nutritional qualities as they fed on, increasing their offspring's fitness (e.g. pupal weight and wing size) when parent and offspring diets match (Rotem *et al.* 2003; Cahenzli & Erhardt 2013; Cahenzli *et al.* 2015). However, in another study, *P. rapae*, were unable to transgenerationally acclimatize its offspring when parent and offspring were reared on similar plant species (Petrén *et al.* 2021). This could infer that different populations of insects react differently, or that transgenerational acclimatization could act on within-species variation in terms of nutrition. Interestingly, it has been shown that differences in epigenetic markers between populations do exist, which is one potential mechanism that could have an influence on phenotypic expression through TGP (Herrera & Bazaga 2010).

Carry-over effects, where a lack or surplus of e.g. nutrients in one generation influence upcoming generations is another example of a transgenerational effect (Marshall & Uller 2007; Uller *et al.* 2013). These are not anticipatory and influence the offspring regardless of their environmental context, although they could coexist with transgenerational acclimatization (Engqvist & Reinhold 2016). Such effects are common and many examples exist in a wide range of organisms (Lindström 1999; Qvarnström & Price 2001; Taborsky 2006; van de Pol *et al.* 2006; Krist 2011; Segers & Taborsky 2011; Franzke & Reinhold 2013). In insects, carry-over effects based on food suitability, i.e. nutrients and secondary metabolites, have shown positive and negative influence on offspring fitness in terms of development, survival and fecundity (Valtonen *et al.* 2012; Zizzari *et al.* 2016; Wang *et al.* 2020; Zeender *et al.* 2023). Therefore, when investigating parental effects, in particular transgenerational acclimatization, it is important to take carry-over effects into perspective (Engqvist & Reinhold 2016).

2.2.3 Behavioural plasticity as a facilitator for transgenerational acclimatization

Both WGP and TGP are favoured by environmental heterogeneity which should benefit both types of plasticity in organisms that inhabit heterogeneous environments (Uller 2008). Furthermore, mathematical models have suggested the co-occurrence of the two for a particular trait and that information between TGP and WGP could be integrated to achieve an optimal phenotypic endpoint (Leimar & McNamara 2015). This has been supported in various taxa (Agrawal *et al.* 1999; Sultan *et al.* 2009; Mikulski & Pijanowska 2010).

In insects, with an emphasis on Lepidopterans, it has been suggested that their ability to express WGP should favour TGP (Woestmann & Saastamoinen 2016). In herbivorous insects, specifically in individuals that use WGP for host-plant choice, the increased chance of a matching parent-offspring environment could favour the ability to transgenerationally acclimatize the offspring to the environment experienced by the parent (Cahenzli *et al.* 2015; Petrén *et al.* 2021). This would link the two types of plasticity, emphasising the importance of WGP for TGP (Kuijper & Hoyle 2015).

2.3 *Spodoptera littoralis*

The Egyptian cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera, Nuctuidae), is a generalist herbivore that is widespread in Africa, the Middle East, and Southern Europe (CABI 2022). Due to its wide host range, utilizing plants from more than 40 plant families, with more than 87 plant species of economic importance, and its ability to spread into temperate regions, it's been considered a quarantine pest and phytosanitary problem (EPPO 2015; CABI 2022). Many generalist insect pests belong to the *Spodoptera* genus, where *Spodoptera frugiperda*, the fall armyworm, native to South America has gained fame for its recent expansion to Africa and Asia, threatening food security (Mendesil *et al.* 2023). The problematic pests within the *Spodoptera* genus and the polyphagous lifestyle of *S. littoralis* make it an interesting model to study host-plant selection, both from an evolutionary and an agricultural perspective.
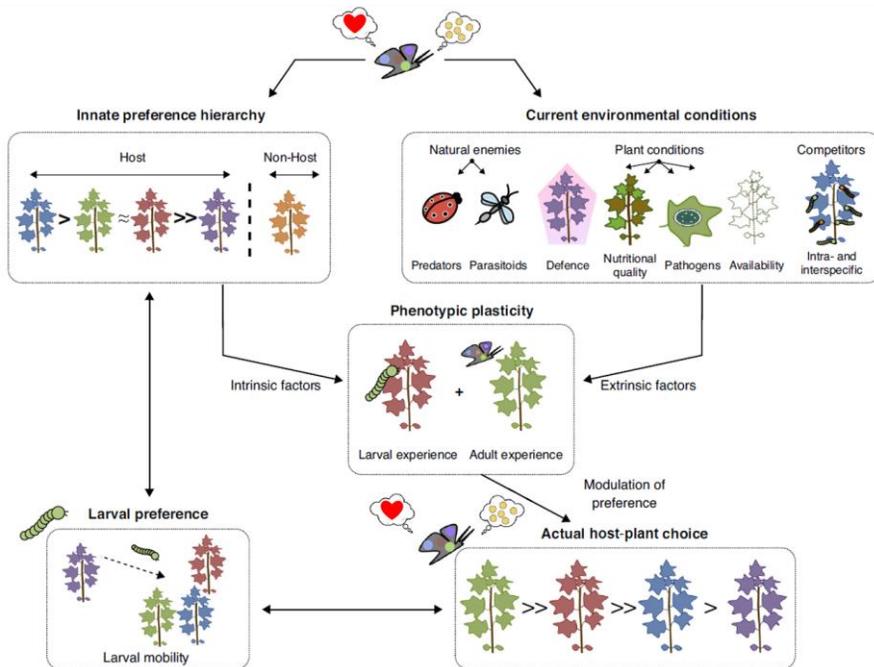
Figure 2. Conceptual representation of known intrinsic and extrinsic factors influencing host-plant choice during mate location and oviposition in male and female *S. littoralis*. Modified from Carrasco *et al.* (2015) with the publisher's permission.

Due to its wide host range, the challenges *S. littoralis* faces during host plant search could give an insight into other closely related species or species with a wide host plant range. Behavioural studies have shown that *S. littoralis* is influenced by both intrinsic (innate preference hierarchy) and extrinsic factors (environmental conditions) during host-plant choice (Anderson *et al.* 2013; Thöming *et al.* 2013; Proffit *et al.* 2015) (figure 2). It has also been demonstrated plant volatile cues are important for host-plant choice and the phenotypic plasticity in *S. littoralis* (Lhomme *et al.* 2018a). Plants present in the preference hierarchy show species-specific odour profiles of the VOCs that are sensed by *S. littoralis* antennae (Conchou *et al.* 2017). The odour profile can change due to previous damage, causing the plant to produce HIPVs influencing behavioural decisions in both males and females which could cause associational resistance in neighbouring plants (Zakir *et al.* 2013a; Zakir *et al.* 2013b).

In this thesis, based on the importance of VOCs for host-plant choice in *S. littoralis*, changes in odour profiles due to conspecific herbivory and its influence on the preference hierarchy in several plant species were studied in **paper I**.

The innate preference hierarchy of *S. littoralis* is influenced by previous experience through WGP (Anderson *et al.* 2013; Thöming *et al.* 2013; Proffit *et al.* 2015) (figure 2). During oviposition, this increases the chance of individuals being placed on the same plant as experienced by the mother. Due to its plastic behaviour through WGP and its possibility to facilitate TGP, *S. littoralis* was used to study whether both behaviour and life-history traits were influenced through TGP in **papers II and III**.

3. Aim and objectives

This thesis aimed to investigate how changes in environmental cues (host-plant odours) influence the host-plant choice of a generalist insect and how host-plant shifts influence behaviour and life-history traits in a transgenerational context.

The first part of the thesis (**Paper I**) investigates how changes in host-plant odour emission due to herbivory from conspecifics influence host-plant discrimination of *S. littoralis* within- and between-plant species. The specific objectives were:

- o Identify whether male and female within-species host-plant choice is influenced by the physiological changes in the plant due to herbivory.
- o Assess the consequences of damage-induced host plants on the preference hierarchy in males and females (between-species host-plant choice).
- o Analyse the odour emission of the host-plant states (damaged and undamaged) to identify key compounds responsible for the potential behavioural shifts.
- o Evaluate key compounds and assess their influence on the behaviour of males and females.

The second part of this thesis (**paper II**) explores whether TGP influences host-plant choice behaviours in *S. littoralis*. The following objective was:

- o Investigate whether TGP influences host-plant choice, increasing the salience for the plant experienced by the parent, in first-instar larvae and ovipositing females.

The third part of this thesis (**papers II and III**) investigates the influence of transgenerational effects on offspring development and reproductive output. The following objectives were:

- o Investigate whether the influence of parental diet, through transgenerational acclimatization, influences offspring development.
- o Investigate whether reproductive output and survival are influenced by transgenerational acclimatization and interpret the role of the parental diet and its qualities (nutrition and secondary metabolites).

4. Methods

The following chapter will give a brief overview of the methods used in the compiled studies (**papers I-III**). For a more detailed methodology, check the corresponding paper.

4.1 Rearing procedures of model organisms

4.1.1 *Spodoptera littoralis*

The generalist insect *S. littoralis* studied in this thesis was reared in the laboratory at the facilities in SLU Alnarp. The colony originated from the vicinity of Alexandria, Egypt where pupae were collected in 2008. The colony was reinvigorated throughout the years with new individuals originating from the same geographical area. Insects in the general rearing were reared on a potato-based artificial diet (Hinks & Byers 1976) at $25 \pm 2°C$, RH $65 \pm 2\%$ with 17:7 h L:D cycles in their larval stage and $25 \pm 2°C$, RH $50 \pm 2\%$ with 16:8 h L:D cycles in their adult stage (**paper I**). In **papers II and III**, RH in the adult chamber was increased to $65 \pm 2\%$. Males and females were separated based on pupal sexual dimorphism.

4.1.2 Host plants

Four host plants of *S. littoralis* were used throughout the thesis (table 1). All the plants were grown in a greenhouse for 5-6 weeks at $25 \pm 2°C$, $70 \pm 5 \%$ RH supplied with artificial light at 16:8 h L:D cycles. Damaged plants that were used in **paper I** had been subjected to herbivory by adding five larvae in their third instar to one of the leaves of the plant for 24 hours. Plants subjected to damage were used for experimental purposes six days after initiation of herbivory. The particular plant species were chosen on the premise that they had different nutritional value as a host, regarding insect

development, but also due to the plant's position in the preference hierarchy of *S. littoralis*. To elaborate on this:

- In **paper I**, the three plants cotton, cowpea and cabbage were used as they are found on different levels of the preference hierarchy. The most preferred plant is cowpea, followed by cotton and lastly cabbage (Thöming *et al.* 2013). This allowed us to test whether herbivory by *S. littoralis* affected the preference among plants and thus the preference hierarchy.
- In **paper II**, the two plants cotton and cowpea were used in oviposition bioassays to test for transgenerational effects influencing behaviour. The plants were used on the premise that cowpea is preferred over cotton when individuals are reared on an artificial diet but if individuals are reared on cotton the preference is shifted to cotton (Thöming *et al.* 2013). Cotton was therefore used to test if it was possible to induce a preference for this plant transgenerationally. Furthermore, maize and cotton were used on the same premise for larval behaviour, both plants can be elevated in the preference hierarchy within species. *S. littoralis* also show differences in development on the two plants.
- In **paper III**, the three plants cotton, maize and cabbage were used. The first two were chosen based on previous experiments where cotton had shown transgenerational effects influencing larval development (**Paper II**, Rösvik *et al.* 2020). In addition, a third plant, cabbage, was added since *S. littoralis* does not prefer it as a host. This gave us three plants that were ranked differently in the preference hierarchy of *S. littoralis*.

Table 1. Table of host plants and their respective cultivar or subspecies used throughout the thesis. A ticked box indicates the species and cultivar or subspecies used in that particular paper.

Plant species	Cultivar/subspecies	Paper I	Paper II	Paper III
Cabbage (*Brassica oleracea*)	cv. Sunta	✓	-	✓
Cotton (*Gossypium hirsutum*)	cv. DeltaPineland 90	✓	✓	✓
Cowpea (*Vigna unguiculata*)	subsb. Unguiculata	✓	✓	-
Maize (*Zea mays*)	cv. Golden nugget	-	-	✓
	cv. Sweet nugget	-	✓	-

4.1.3 Artificial diets

The lab colony of *S. littoralis* was maintained on a potato-based artificial diet (Hinks & Byers 1976). In previous studies on *S. littoralis*, this artificial diet has been modified by adding secondary metabolites or removing nutrients e.g. protein, causing direct effects on insect development (Lhomme *et al.* 2018a). In **paper III**, three artificial diets from Lhomme *et al.* (2018a); (Lhomme *et al.* 2018b) were used to investigate transgenerational effects in *S. littoralis* since they represent modified nutritional qualities and the presence of secondary metabolites of host plants.

4.2 Behavioural assays

4.2.1 Larval behaviour

Arena-type approaches for testing insect response have been used extensively within chemical ecology research (e.g. Lelito *et al.* 2009; Wright *et al.* 2012; Kandasamy *et al.* 2019). In *S. littoralis*, the use of Petri dishes is a suitable arena, specifically for small neonate larvae (de Fouchier *et al.* 2018; Ljunggren *et al.* 2019). In **paper II**, behavioural transgenerational effects in neonate *S. littoralis* larvae were tested by using a Petri dish arena. In short, the first instar larvae were put in the centre of a Petri dish with two leaf discs on either side (cotton or maize). The larvae were then monitored for four hours and the first choice was noted.

Since *S. littoralis* females oviposit directly on the leaf surface, neonates hatch and are directly in contact with the host plant chosen by the mother. Although the female decides where the egg batches are put, larvae can make up for an erroneous choice through migration (Zalucki *et al.* 2002; Carrasco *et al.* 2015). In the case of *S. littoralis*, neonate larvae can use a ballooning behaviour, spinning a silk-like thread from which they can hang from the plant to become more mobile (Zalucki *et al.* 2002). In **paper II**, apart from the first choice of the larvae, the influence of transgenerational effects on migration behaviour was also tested to create a more natural setting compared to the petri dish arena. In short, a rectangular arena was used with two plant leafs (cotton or maize) provided with water to avoid drying out. The two leaves were put on opposite sides and a group of twenty larvae from different treatment backgrounds were put on one of the leaves. After three days, the number of larvae on each leaf was noted.

4.2.2 Adult male behaviour

Adult male *S. littoralis* utilize plant-odour cues in combination with pheromone from the conspecific female to locate suitable mating rendezvous sites (Borrero-Echeverry *et al.* 2018). The response towards various volatile cues has previously been tested through different methodologies in moths such as wind-tunnel experiments (Thöming *et al.* 2013; Borrero-Echeverry *et al.* 2015; Proffit *et al.* 2015; Borrero-Echeverry *et al.* 2018) and olfactometers (Hern & Dorn 1999; Vallat & Dorn 2005; Liu *et al.* 2022).

In **paper I**, to test male mating rendezvous site selection, a two-choice bioassay was developed using a y-tube olfactometer based on a previous setup by Jönsson (2005). In short, the males were exposed to two host-plant odours in combination with a synthetic pheromone solution based on two compounds released from females (estimated release 880pg/min *(Z,E)*-9,11-tetradecadienyl acetate and 9pg/min *(Z,E)*-9,12-tetradecadienyl acetate). These two compounds are important components in the Egyptian populations of *S. littoralis* pheromone blend (Saveer *et al.* 2014; El-Sayed 2023 and references therein). Charcoal-filtered air was pumped through the olfactometer, passing containers with intact plant and the pheromone wick-bait dispensers (see 'synthetic blend bioassays' section 4.2.4 for elaboration) and then into the glass y-tube where the odour plumes were intermixed at the conjunction where the two arms met. The male choice between the two odours (arms) was then monitored and a choice was considered once the male had entered one of the arms.

4.2.3 Adult female behaviour

Locating a suitable oviposition site is key for offspring survival and development in herbivorous insects such as *S. littoralis* (Jaenike 1978). From afar, they use olfactory cues released from plants and when nearby other types of information such as visual, mechanosensory and gustatory also influence whether the female accepts the potential host or keeps searching (Bernays 2001; Schoonhoven *et al.* 2005). This implies that in oviposition bioassays, olfactory cues alone are not solely representative of the oviposition choice of the female, but rather a combination of sensory information. In **paper I**, an oviposition cage assay was used to test for female host-plant acceptance (oviposition) in response to changes in the plant's physiological state caused by herbivory. In short, two plants of each treatment were put on either side of the cage (four plants in total). Five females and five males were then introduced to a mesh cylinder in the bigger cage to mate. After 24 hours the moths were introduced to the larger cage by opening the mesh cylinder. After 72 hours, the number of egg batches oviposited on either option was counted and weighed.

In **paper II**, to test for transgenerational effects of parental diet on offspring oviposition, a detached leaf bioassay was used. Previous studies in *S. littoralis* have shown that detached leaves give a similar oviposition response to that of whole plants (Sadek *et al.* 2010) and this assay has been commonly used in *S. littoralis* since (Anderson *et al.* 2013; Thöming *et al.* 2013; Proffit *et al.* 2015; Lhomme *et al.* 2020). In short, detached plant leaves were put in water-filled vials and put in opposite corners of the cage (two leaves per cage). A mated female was then introduced to the cage containing the detached leaves and allowed to oviposit for 72 hours. After that, the collected eggs were weighed.

The type of oviposition assay in **papers I and II** was selected due to the nature of the experimental question asked. In **paper I**, since the physiological state and influence of herbivory were investigated, a detached leaf assay was considered inappropriate since detaching causes lesions to the leaf. In **paper II**, a full cage bioassay seemed excessive since detached leaf assay studies had previously been used to check for host-plant preference between plant species and have been proven comparable to those of intact plants (Sadek *et al.* 2010).

4.2.4 Synthetic blend bioassays

To test the influence of olfaction on adult behaviour to fully understand which chemical cues influence the behavioural output, bioassays based on the addition of synthetic chemical compounds to plants are key within the field of chemical ecology (Thöming 2021). In the male and female behavioural experiments in **paper I**, synthetic compounds representative of damaged cotton plants were released using wick-bait dispensers. An undamaged cotton plant was used as a background odour source with a synthetic mixture of compounds representing the volatiles from the damaged plant released from the dispensers. The experiments were otherwise carried out as described in the 'adult male bioassay' section 4.2.2 and 'female bioassay' section 4.2.3 above.

Wick-bait dispensers, initially described in Lejfalk and Birgersson (1997) have been used widely to verify the influence of chemical compounds on behavioural output (Zakir *et al.* 2013a; Karlsson *et al.* 2017; Khan *et al.* 2023). In short, single compounds or a combination of compounds are dissolved in a solvent, placed in a dispenser (vial), and then emitted through a wick. The release rate of the compound or compounds present in the solution can then be modified by changing the concentration of the synthetic components added.

4.3 Plant-odour collection and analysis

Plants produce metabolites some of which are volatile (Pichersky *et al.* 2006). These are released from the plant tissue and provide information to the surrounding environment that can be used by other organisms (Nordlund & Lewis 1976; Dicke & Sabelis 1988). Identification of such VOCs is key in the field of chemical ecology to understand the complex interplay between plants and other organisms in the environment such as insect-plant interactions (Bruce *et al.* 2005). Different methodologies on how to entrap, identify and quantify the release of VOCs exist with various benefits and limitations (Tholl *et al.* 2006). In **paper I**, dynamic headspace extraction of VOCs released from *S. littoralis* host plants of various physiological stages was used. Plant foliage was enclosed in an oven bag and clean air was sucked through a charcoal filter into the bag containing the plant foliage and through an adsorbent (Porapak Q). Plant odours were collected for 15 hours and the volatiles collected were then desorbed using pentane. The headspace extracts obtained were injected on a gas chromatograph coupled with mass spectrometry (GC-MS) were the volatiles released were tentatively identified. The GC columns separated the compounds present in the sample by their affinity to the stationary phase that the GC column is coated with. As a first step, this was done on a polar GC column (DB-WAX) and key compounds of interest for the synthetic bioassays were further identified using a non-polar GC column (HP-5) to fully verify the identifications made. The VOCs were first putatively identified by calculating retention indices based on known alkanes and the electron impact fragmentation pattern received from the MS, which are compared through NIST, Wiley or in-house libraries. Verification of the tentatively identified compounds were done through co-injection. Due to the wide array of compounds released from plants (Knudsen *et al.* 1993; Bruce *et al.* 2005), **paper I** focused on compounds known to be electrophysiologically active in *S. littoralis* (see appendix table A1 for reference list, **paper I**), i.e. compounds detectable by the insect.

4.4 Insect performance

Phytophagous insects are heavily dependent on the nutritional and chemical composition of their host plants for their development and fitness (Coley & Bateman 2006; Behmer 2009). In *S. littoralis* and other holometabolous moth species, the larval stage is responsible for acquiring food which not only influences the life-history traits of the larvae but also that of the adult (Pierce & Berry 2011; García-Robledo & Horvitz 2012). In the insect performance experiments, insects were kept in rearing chambers in the same

conditions mentioned in section 4.2.1 above. In both **papers II and III**, individuals were reared in a fully factorial design, creating four parent-offspring combinations in **paper II** (two had matching parent-offspring diets and two had mismatched diets). As for **paper III**, the factorial design created nine parent-offspring combinations in **paper III** (three had matching parent-offspring diets and six had mismatched diets). In **paper II**, insects were reared individually in cups while in **paper III** insects were reared in groups of ten in boxes. Additionally, in the mortality experiments in **paper III**, insects on plant diets were also reared singly in cups to decrease cannibalism on some plant diets. For insects reared on both plant diet and artificial diets, the individuals monitored throughout their development or used for experimental purposes were fed *ad libitum*.

4.4.1 Larval performance and survival

Survival, larval development time (time from hatching until pupation) and pupal weight are common proxies for fitness (Rieger *et al.* 2004; Beukeboom 2018). A fast development time could reduce the time exposed to e.g. natural enemies and parasitoids (Feeny 1976; Williams 1999) while a higher pupal weight is known to be positively correlated with reproductive output (Jiménez-Pérez & Wang 2004; Rhainds 2015; Beukeboom 2018). In **paper II**, the influence of transgenerational effects, due to parental and offspring diet, on larval development time and pupal weight were monitored. In **paper III**, individuals were followed from egg hatching until pupation and the number of surviving individuals was noted.

4.4.2 Reproductive output

The reproductive output is an important aspect of fitness in adult individuals. In **paper III**, the influence of transgenerational effects on reproductive output i.e. fecundity and fertility were monitored in adult individuals from different dietary exposures in the parental and offspring generations. This was accomplished by pairing adult males and females from the same parent-offspring diet combinations and of similar pupal weights in a mating box covered in paper. The mating boxes were then left for 72 hours before the eggs oviposited were removed and weighed. The weight of the eggs (mg) where then multiplied by 20 since previous studies have shown that 1 mg egg batch corresponds to 20 eggs (fecundity) (Sadek *et al.* 2010; Zakir *et al.* 2013a; Zakir *et al.* 2013b). All eggs were then left in individual cups for hatching. Approximately a week after the weighing, all the hatched larvae were dead and the cup containing the dead larvae was photographed. The

number of larvae (fertility) was then quantified using the image analysis platform Fiji (Schindelin *et al.* 2012) and hatching success was calculated.

5. Results and discussion

5.1   Part I: Influence of herbivory on adult male and female *S. littoralis* host-plant preference hierarchies.

Both abiotic and biotic stressors impact the release of VOCs from plants, which serve as important indicators of plant suitability (Dudareva *et al.* 2013). During host-plant location, insects must be able to differentiate between odour profiles of hosts and non-hosts, as well as hosts of different suitability (Bruce *et al.* 2005; Bruce & Pickett 2011; Silva & Clarke 2020).

In **paper I**, we focused on the changes in odour profiles of *S. littoralis* host plants due to conspecific herbivory and its influence on adult behaviour. We found that, when tested within-plant species, males discriminated among damaged and undamaged plants in cotton and cowpea, but not in cabbage plants (figure 3a, **paper I**). On the other hand, females discriminated between the two states in cotton but not in cowpea or cabbage (figure 3bc, **paper I**). Interestingly, male and female behaviour towards the damaged and undamaged states differed, with males being attracted to damaged cotton and cowpea while females were deterred by damaged cotton (figure 3abc, **paper I**). To our knowledge, this is the first time that *S. littoralis* males show attraction towards plants subjected to herbivory. Sex-specific differences in behavioural output to damaged and undamaged host plants have been shown previously in other plant-insect systems with an attraction to damaged plants in males and a deterrence in females (Ballhorn *et al.* 2013).
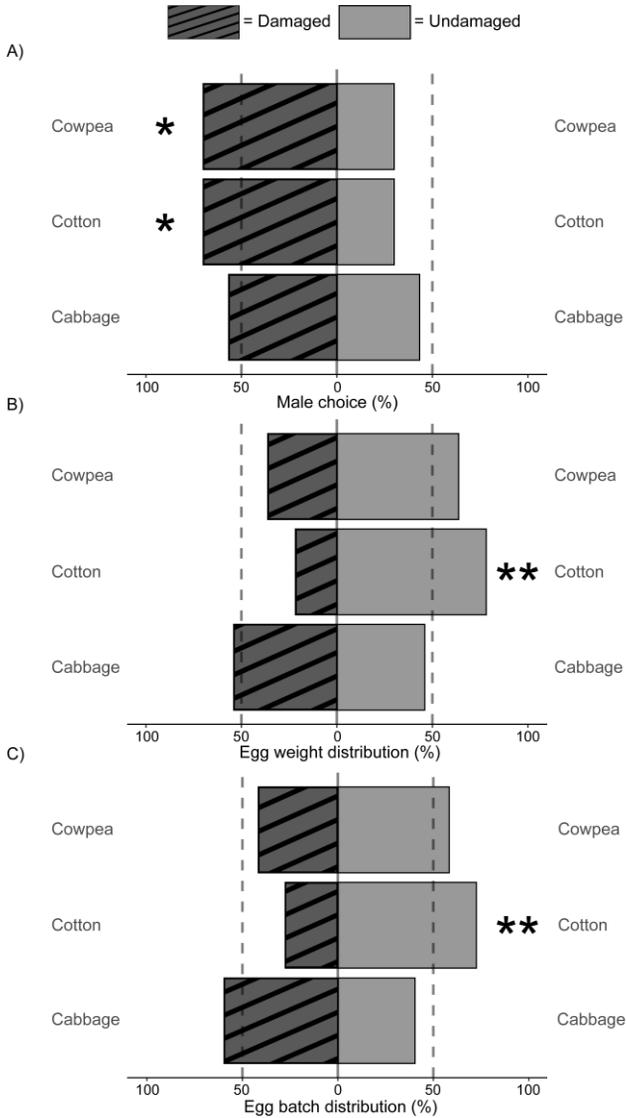
Figure 3. Within-species choice of male and female *S. littoralis* between damaged and undamaged plants of three host-plant species. (A) Horizontal bar chart showing the percentage of males entering arms supplied with odour from damaged (striped) or undamaged (unstriped) plants in a two-choice olfactometer. (B) Horizontal bar chart showing the percentage of egg batch weight distribution and (C) number of egg batches on damaged (striped) or undamaged (unstriped) plants in two-choice oviposition experiments. Asterisks indicate a preference for a particular host-plant state (damaged or undamaged) when compared against no preference (50-50 distribution) (*P < 0.05, **P <0.01, ***P < 0.001). Figure from **paper I**.

The model insect *S. littoralis* has been found to rank host plants based on an innate preference hierarchy (Thöming *et al.* 2013) that can be modified by previous experience with plants and guide upcoming behavioural decisions (Thöming *et al.* 2013; Proffit *et al.* 2015). Based on this, we tested whether within-species changes in odour profiles due to herbivory influenced the preference hierarchy of the insect. By inflicting damage on high-ranked plants (cotton and cowpea) and testing male and female choice between a preferred host subjected to damage (cotton and cowpea) and an undamaged non-preferred host (cabbage). We found that males continued to show a strong attraction towards the high-ranked plant in both cotton and cowpea after damage (figure 4a, **paper I**). In females, however, the preference for cotton was reduced in terms of the number of egg batches laid (figure 4c, **paper I**) but not the total egg weight (figure 4b, **paper I**). When testing damaged cowpea against undamaged cabbage, the preference for cowpea was retained (figure 4bc, **paper I**). This demonstrates that the response to damage induction in the between-species experiments was host-plant dependent.
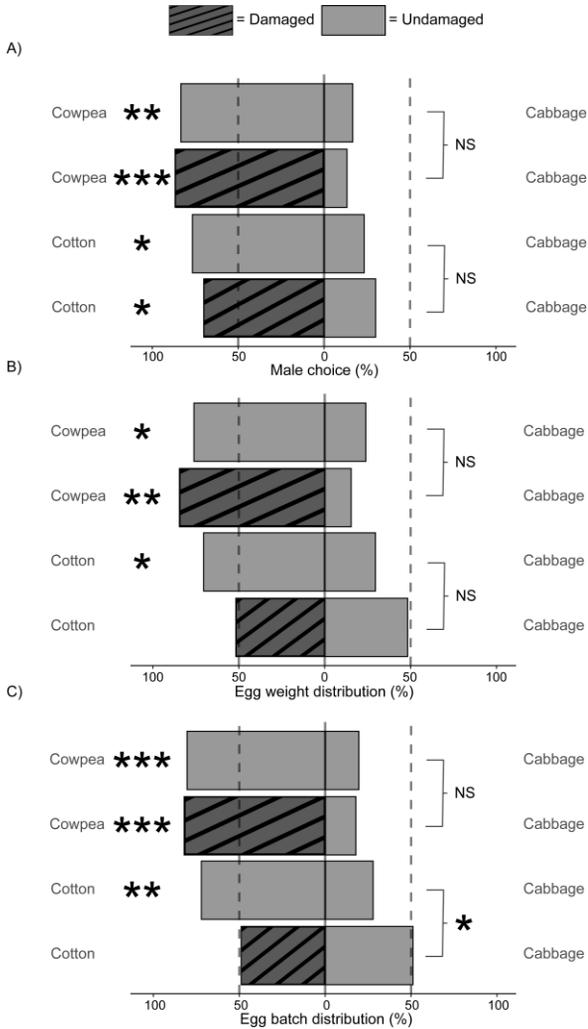
Figure 4. Between species choice of male and female *S. littoralis* between damaged plants high up in the preference hierarchy (cotton and cowpea) against undamaged cabbage (bottom of preference hierarchy). (A) Horizontal bar chart showing the percentage of males entering arms supplied with odour from damaged (striped) or undamaged (unstriped) plants in a two-choice olfactometer. (B) Horizontal bar charts showing the percentage of egg batch weight distribution and (C) number of egg batches on damaged (striped) or undamaged (unstriped) plants in two-choice oviposition experiments. Asterisks on the left side of the boxes indicate a preference for one of the plants and its state (damaged or undamaged) when compared against no preference (50-50 distribution) (*P < 0.05, **P <0.01, ***P < 0.001). Asterisks on the right side of the boxes indicate significant changes in the preference hierarchy when comparing how damage on a plant high up in the preference hierarchy influences behavioural outcome (*P < 0.05, **P <0.01, ***P < 0.001). Figure from **paper I**.

The next step was to verify whether differences in olfactory cues influenced the behaviours towards the damaged and undamaged plants. Volatile organic compounds known to be electrophysiologically active in *S. littoralis* (see appendix table A1 for reference list, **paper I**) were identified in the damaged and undamaged plants. In the cotton and cowpea plants, differences in the proportional abundance of VOCs between the damaged and undamaged states were found while this was not the case in cabbage (figure 5, **paper I**).
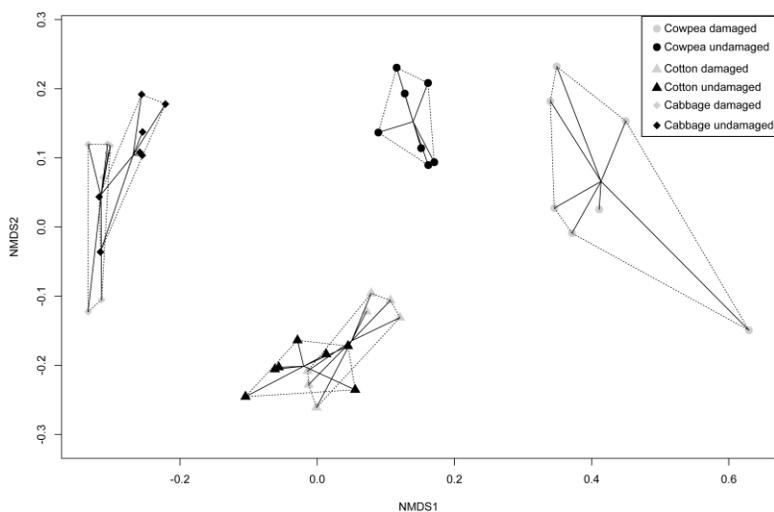
Comparison	N	Stress (%)	df	F	P
Global	42	10.82	5,36	20.46	0.001
Cotton undamaged vs cotton damaged	14	6.67	1,12	18.09	0.003
Cowpea undamaged vs cowpea damaged	14	6.02	1,12	5.84	0.003
Cabbage undamaged vs cabbage damaged	14	4.43	1,12	2.61	0.065

$P < 0.05$ are significant values

Figure 5. Non-metric multidimensional scaling (NMDS) ordination of the volatile chemical composition of undamaged plants or plants subjected to herbivory based on Bray-Curtis distance, rotated by principal component analysis (PCA). Comparisons are based on permutational multivariate analysis of variance (PERMANOVA) on plant volatiles released from undamaged plants or plants subjected to herbivory ($P<0.05$ indicate significant values). Modified from **paper I**.

The change in odour profile between the damaged and undamaged plants did reflect the behavioural output where changes in behaviour and odour profiles were shown in cotton and cowpea but not cabbage for both males and females. The volatile profiles were then further analyzed using a Random Forest algorithm to identify a set of predictor compounds of a particular plant species and state. Out of the 18 compounds in cotton and the 24 compounds in cowpea, the Random Forest algorithm selected 4 compounds in cotton (α-humulene, β-caryophyllene, (*E,E*)-TMTT and linalool) and 1 compound in cowpea ((*E*)-2-hexenal) as predictor compounds for the damaged state (table 2, **paper I**).

Table 2. Predictor volatiles from the Random Forest analysis for the two plants that show differences in their odour profiles due to herbivory. The text in bold represents the compounds with higher proportional abundance in the damaged state. The absence of a compound e.g. lack of compound A in the damaged state compared to the undamaged state could also be a predictor for the damaged state compared to the undamaged state. Modified from **paper I**.

Plant	Predictor VOCs	State with a higher proportional abundance	
		Damaged	Undamaged
Cotton	**α-humulene**	✓	-
	β-caryophyllene	✓	-
	(*E,E*)-TMTT [a]	✓	-
	linalool	✓	-
	p-cymene	-	✓
	α-pinene	-	✓
	p-cymene	-	✓
Cowpea	**(*E*)-2-hexenal**	✓	-
	limonene	-	✓
	α-pinene	-	✓
	(*Z*)-β-ocimene	-	✓
	β-pinene	-	✓
	myrcene	-	✓

[a] *(E,E)*-TMTT: *(E,E)*-4,8,12-trimethyl-1,3,7,11-tridecatetraene

To verify whether the volatiles representative of the damaged plant could explain the behavioural shifts seen in males and females, a synthetic blend containing the compounds representative of the damaged plants was emitted through wick-bait dispensers in behavioural assays with undamaged plants as a background. Undamaged plants were used with odours added to make them mimic damaged plants. This was done for the cotton system since we observed a behavioural shift in both males and females in this system. Interestingly, these experiments mimicking plant damage using a synthetic blend, showed a similar response to that of the plant experiments. Males showed an increased attraction to plants with the additional four compounds representing the damaged state (figure 6a, **paper I**). Contrarily, females showed a reduced number of egg batches oviposited (figure 6c, **paper I**) while a trend was shown for the egg batch weight (figure 6b, **paper I**) when the four compounds representing the damaged state were added.
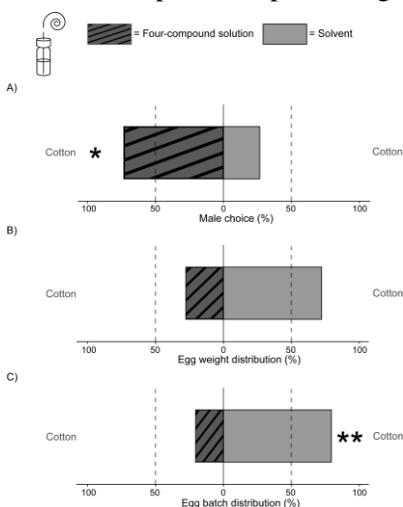

Figure 6. Behavioural response of *S. littoralis* to undamaged cotton plants supplied with a four-compound solution representative of the damaged cotton state or a solvent (control). (A) Horizontal bar chart showing the percentage of males entering arms supplied with the four-compound solution (striped) or solvent (unstriped) in a two-choice olfactometer. Asterisks indicate a preference for a specific odour source (Exact binomial test, *$P < 0.05$). (B) Horizontal bar charts showing the percentage of egg batch weight distribution and (C) number of egg batches on plants supplied with the four-compound solution (striped) or solvent (unstriped) in two-choice oviposition experiments. Asterisks indicate a preference for one of the plants (supplied with the four-compound solution or solvent) when comparing towards no preference (50-50 outcome) (*$P < 0.05$, **$P < 0.01$). Figure from **paper I**.

Altogether, these findings show that males and females perceive differences in odour profiles due to previous herbivory in different ways, showing contrasts in behavioural output. Furthermore, the female preference hierarchy is also influenced due to previous herbivory, causing her to adjust how she distributes her egg batches, laying fewer egg batches on cotton plants subjected to herbivory while allocating more egg batches on cabbage, the plant that is generally not preferred by ovipositing females. The identification of the four predictor compounds that elicited the male and female behaviours could serve as semiochemicals where their potential in behavioural manipulation should be investigated.

5.2   Part II: Innate preference and the influence of previous experience on behaviour in a transgenerational context.

In insects, it has been suggested that previous experience within a generation could act as a facilitator for complex behaviours such as host-plant choice (Bernays 2001). Often referred to as 'Hopkins host-selection principle' (HHSP), it implies that larval experience is retained through metamorphosis and influences adult behaviour (Hopkins 1917; Barron 2001). Although HHSP has been widely debated since it was first postulated, evidence of insects utilizing previous experience to facilitate behavioural decisions does exist (Anderson & Anton 2014). Behavioural plasticity in insects makes them an interesting group of organisms not only from a within-generation perspective but also from a transgenerational perspective (Gowri & Monteiro 2021). Studies in other insect systems have emphasised the use of TGP, where information from host plants in one generation could influence the behavioural traits of upcoming generations (Gowri 2019). We therefore ventured out to test whether parental experience influenced both larval and adult behaviour in *S. littoralis*.

In the neonate larval experiments, we hypothesized that individuals would have an increased preference for the plant experienced by their parents. We found that neonate larval behaviour was not influenced by transgenerational effects in the first choice or migration experiments (figure 7ab, **paper II**). However, migration bioassays showed that the first instar larvae were able to discriminate between the two host plants based on their innate preference hierarchy (figure 7b, **paper II**). When put on maize, a high proportion of the larvae migrated towards cotton. Contrarily, when larvae were put on cotton, most individuals tended to stay.
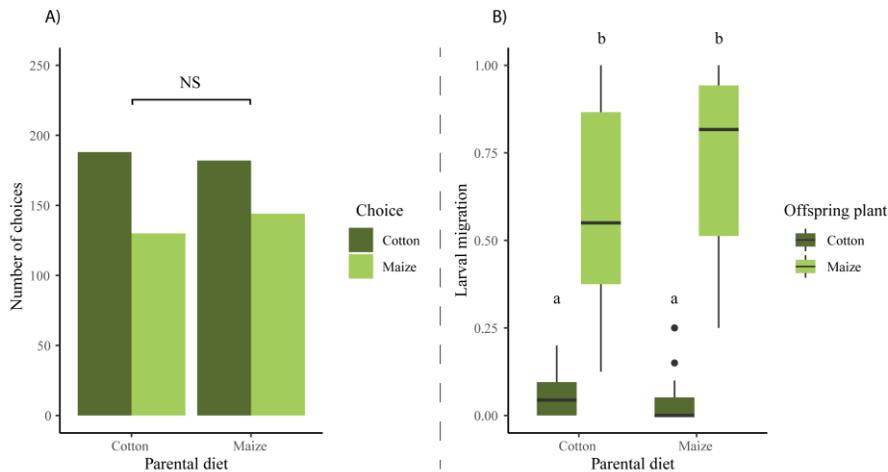
Figure 7. Host-plant choice in first instar larvae of *S. littoralis*. (A) Bar chart showing first instar feeding preferences between maize (light green bar) and cotton (dark green bar) based on parental dietary background. (B) Box plot showing indices of first instar migration from plant material ranging from 0 (no migration) to 1 (migration of all the larvae) between maize (light green bar) and cotton (dark green bar) based on parental dietary background. Different letters indicate significant between treatment differences (P<0.05), NS indicate non-significant. Figure modified from **paper II**.

In the adult oviposition bioassay, we hypothesized that if TGP were guiding adult oviposition behaviour, a shift in preference towards the food plant experienced in previous generations would occur. We therefore reared larvae in the first (F0) generation on an artificial diet, which shows *S. littoralis* innate preference for cowpea in comparison to cotton. From the second until the fourth generation (F1-F3), we reared *S. littoralis* on cotton, hypothesising that this would influence oviposition behaviour transgenerationally and shift preference from cowpea to cotton. In the fifth generation (F4), individuals from the rearing line were put on either an artificial diet or cotton. The oviposition preference of each generation was then tested in comparison to the F0 generation. Since there was no difference in the fifth (F4) generation reared on an artificial diet compared to the first (F0) generation, but the oviposition preference of all generations (F1-F4) reared on cotton differed significantly, the interpretation of this was that adult oviposition behaviour is guided by WGP rather than TGP (figure 8, **paper II**).

These results suggest that *S. littoralis* use their innate preference hierarchy in combination with WGP rather than TGP to guide host-plant location.
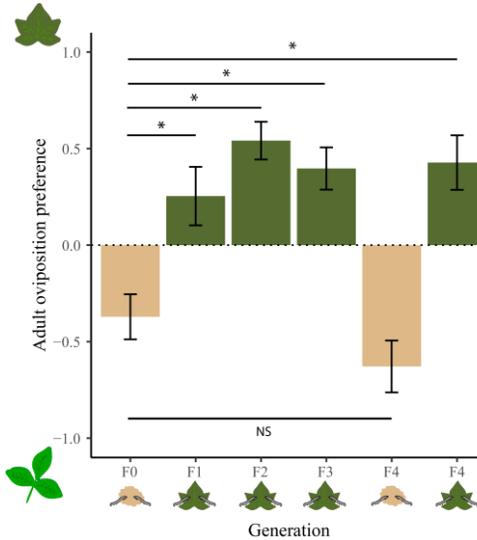

Figure 8. Effects of larval diet experience in a transgenerational context on oviposition preference of the five generations (F0–F4) reared on either the artificial diet (beige bars) or cotton (dark green bars) when given a choice of cotton (> 0) or cowpea (< 0) plants. Asterisks indicate significant difference to F0 (P < 0.05), NS indicate non-significant. Figure modified from **paper II**.

5.3 Part III: Diet-mediated transgenerational effects on larval survival, development and adult reproductive output.

Previous studies have emphasised the important connection between WGP and that of TGP where the two are believed to be of particular importance in Lepidopterans (Woestmann & Saastamoinen 2016). It has been proposed that TGP should be favoured in insects that could use previous experience within a generation to facilitate host-plant choice since this would increase the chances of parent-offspring dietary environment to match (Cahenzli *et al.* 2015). The increased chance of a matching host-plant environment between parent and offspring could hence facilitate transgenerational acclimatization, where parents adapt their offspring to the experienced environment of the parent (Mousseau & Fox 1998b; Marshall & Uller 2007; Munday 2014). In **papers II and III**, we investigated whether transgenerational acclimatization influenced life-history traits in *S. littoralis*.

In **paper II**, the larval development experiments showed a transgenerational effect influencing larval pupal weight when parent-offspring diets were matched compared to unmatched in cotton (figure 9a **paper II**). In individuals reared on maize with a matching parental diet, we found no increase in pupal weight compared to individuals on a mismatched

diet (figure 9a, **paper II**). No influence on larval development time was seen (figure 9b, **paper II**). Furthermore, larvae reared on cotton had a faster development time and higher pupal weight compared to larvae reared on maize, irrespective of parental diet (figure 9ab, **paper II**). The lower pupal weight and delayed development time on maize compared to cotton indicate that cotton is the more optimal diet of the two. In addition, the increased migration of larvae from maize to cotton, in comparison to the migration from cotton to maize, further points towards differences in suitability as hosts. This indicates that transgenerational acclimatization and its effect on development is context-dependent in *S. littoralis*. Moreover, traits related to host-plant suitability, e.g. nutritional, and secondary metabolites, might be an influencing factor.
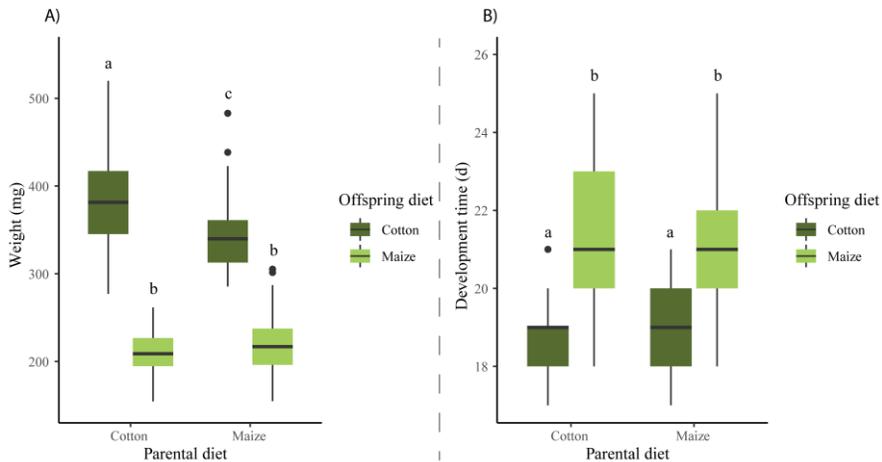

Figure 9. Transgenerational effect on development in *S. littoralis*. (A) Boxplots showing pupal weight and (B) development time in days of individuals reared on maize (light green boxes) or cotton (dark green boxes) with parents reared on either matching or mismatching diets. Different letters indicate significant differences (P<0.05). Figure modified from **paper II**.

In **paper III**, we further investigated the role of transgenerational effects but this time on reproductive output and survival, with an emphasis on transgenerational acclimatization in the offspring when reared on a matching diet to that of the parent. We found no evidence of transgenerational acclimatization influencing reproductive output. Moreover, individuals who were reared on a matching diet to that of the parent suffered a decrease in fecundity compared to those reared on a mismatched diet, although this was only the case in plant diets (Figure 10, grey boxes, **paper III**), but not for artificial diets (figure 11, grey boxes, **paper III**). Furthermore, no signs of

transgenerational acclimatization were seen in mortality for either plant or artificial diets (figure 12b and d, grey boxes, **paper III**), although, in the plant diets, individuals on maize had high mortality both in the parental generation and offspring generation (figure 12a and b, **paper III**).

Figure 10. Transgenerational influence on reproductive output in offspring generation (F1) on plant diets where (A) denotes the number of eggs laid, (B) hatching success and (C) total number of hatched larvae for the three respective diets cabbage, cotton and maize in a fully factorial design. Different lowercase letters indicate significant differences among parent dietary backgrounds within each F1 diet (P<0.05). Different uppercase letters indicate significant differences within a specific parent's dietary background across each F1 diet (P<0.05). Numbers in boxes represent the number of replicates. Grey boxes to the right correspond to contrasts between matching and mismatching diets, where "no" represents no transgenerational acclimatization and "opposite" represents an increased fitness on a mismatched diet. Modified from **paper III**.
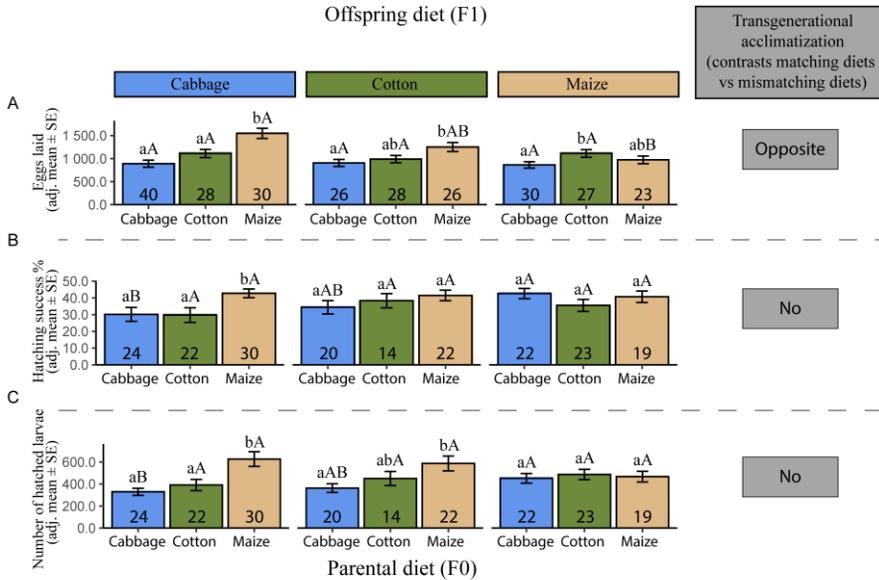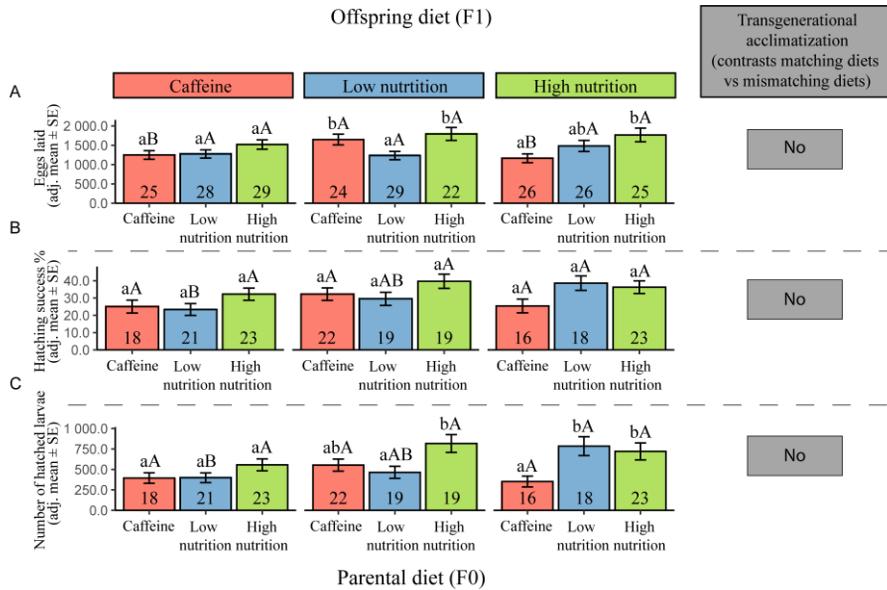
Figure 11. Transgenerational influence on reproductive output in offspring generation (F1) on artificial diets where (A) denotes the number of eggs laid, (B) hatching success and (C) total number of hatched larvae for the three respective diets caffeine diet, low-nutrition diet and high-nutrition diet in a fully factorial design. Different lowercase letters indicate significant differences among parent dietary backgrounds within each F1 diet (P<0.05). Different uppercase letters indicate significant differences within a specific parent's dietary background across each F1 diet (P<0.05). Numbers in boxes represent the number of replicates. Grey boxes to the right correspond to contrasts between matching and mismatching diets, where "no" represents no signs of transgenerational acclimatization. Modified from **paper III**.
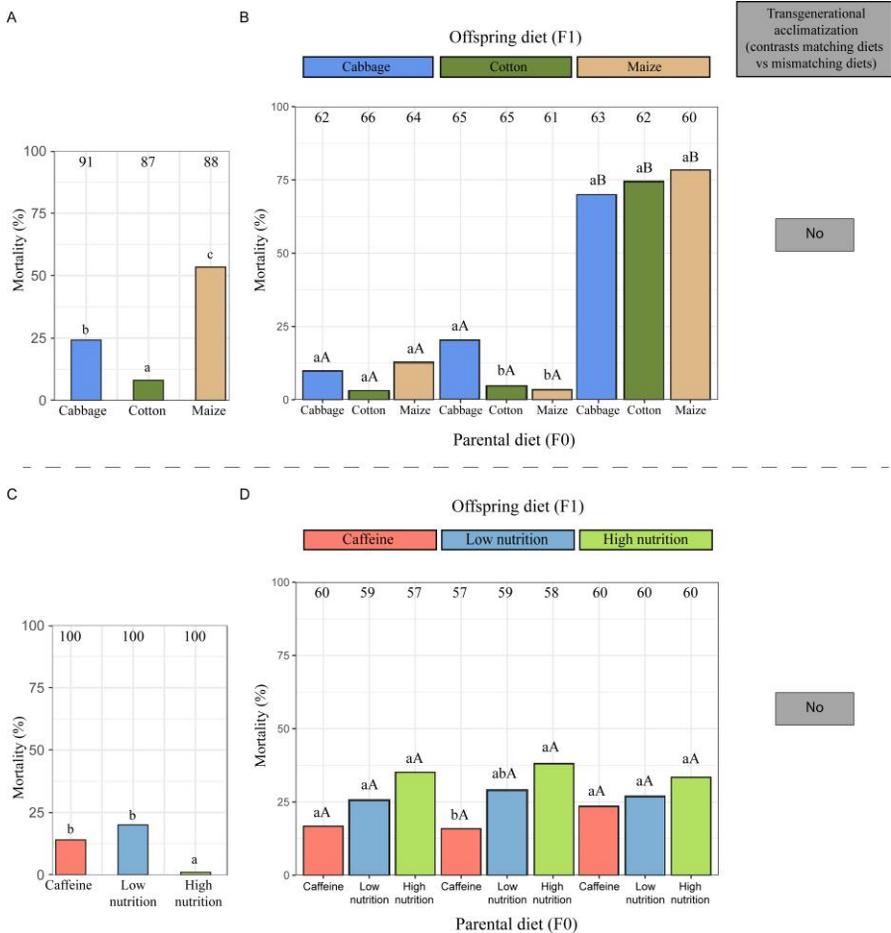
Figure 12. Mortality in *S. littoralis* from first instar until pupation in (A) first generation reared on plants, (B) second generation reared on plants, (C) first generation reared on artificial diet, (D) second generation reared on artificial diet. In A and C, different lowercase letters indicate significant differences among the diets (P<0.05). In B and D, different lowercase letters indicate significant differences among parent dietary backgrounds within each F1 diet while uppercase letters indicate significant differences within a specific parent's dietary background across each F1 diet (P<0.05). The numbers above the boxes represent the number of replicates. Numbers in boxes represent the number of replicates. Grey boxes to the right correspond to contrasts between matching and mismatching diets, where "no" represents no signs of transgenerational acclimatization. Modified from **paper III**.

We hypothesise that the transgenerational effects seen on reproductive output and mortality could be explained by carry-over effects or transgenerational preadaptation. Thus, the nutritional qualities and presence of secondary metabolites in the parental diets may influence offspring reproductive output and survival in the plant diets (figure 10a-c and 12b, **paper III**), which is supported by the artificial diets (figure 11a-c and 12d, **paper III**).

Interestingly, the high mortality on the maize diet and the ability to use previous experience when reared on maize to elevate the plant in the preference hierarchy (Thöming *et al.* 2013) indicate that certain plant species could act as ecological traps where offspring are put in a suboptimal environment (Dwernychuk & Boag 1972). Other aspects, however, that influence survival in a natural system should not be overlooked, e.g. natural enemies (Bernays & Graham 1988; Khallaf *et al.* 2023) and cultivar of the plant (Chiriboga Morales *et al.* 2021). This shows that the use of previous experience in the parental generation could be a mechanism that facilitates female host-plant choice at the expense of the offspring, causing a parent-offspring conflict on host-plant suitability in terms of development (Garcia-Robledo & Horvitz 2012).

6. Concluding remarks and future perspectives

Plant-insect interactions in heterogeneous environments show a complex interplay that causes physiological shifts in both organisms involved. The Egyptian cotton leaf worm, *Spodoptera littoralis*, with its wide host-plant range, has been used as a model organism during the last decades to investigate generalist behaviour in plant-insect interactions. In this thesis, the aim was to disentangle how variation in host-plant odour cues influences the behavioural output of *S. littoralis* in a heterogeneous setting and the consequences the behavioural decisions have on behaviour and life-history traits in a transgenerational context.

In the first part of this thesis (part I; **paper I**), I studied how herbivory affected odour emission and host-plant choice in male and female *S. littoralis* in three of its host plants. I demonstrated, in a within-species context, that males were attracted to two out of the three host plants in their damaged state compared to undamaged plants, namely cotton and cowpea. Contrarily, females showed deterrence to one of these plants, damaged cotton. I then investigated how changes in behaviour due to herbivory influenced *S. littoralis* innate preference hierarchy in a between-species context. The male preference hierarchy was unaffected, while females had a decreased preference for one of their preferred host plants in its damaged state. To elaborate on changes in chemosensory cues as a potential mechanism for the observed behaviour, I analysed the odour profiles of the plants in their damaged and undamaged state. This showed that in plants in which we saw a behavioural shift, we also found alterations in their odour profiles between their damaged and undamaged states. From the odour profiles of damaged and undamaged plants, a subset of compounds were identified as unique for a particular plant state. I used the cotton system to verify that the subset of compounds identified for the damaged state was sufficient to mimic the behavioural shifts seen in males and females between the two plant states. Further investigation of the compounds eliciting the different behavioural

responses between the sexes is warranted in field conditions, where their potential as semiochemicals should be investigated, with a focus on repelling females while attracting males.

In the following part (part II; **paper II**), I investigated whether previous experience in the parental generation influenced the host-plant choice of both larval and adult females. I found no evidence of transgenerational effects influencing the behavioural output of *S. littoralis*, but rather that their innate preference hierarchy and previous experience within the generation facilitate host-plant location.

Finally, in the last part of the thesis (part III; **papers II and III**), I hypothesized that females might adapt their offspring to the plant which they had experienced through transgenerational acclimatization, increasing offspring fitness on that plant. I found that larval pupal weight was increased in individuals who were put on a matching plant of that of the parents on a good host plant (cotton) but not on a suboptimal host plant (maize). Interestingly, I found no evidence of transgenerational acclimatization influencing reproductive output or survival but rather the opposite. In the plant diets, when comparing matching diets against mismatching diets, offspring that had a matching environment to that of the parents had a lower fecundity compared to those where parent-offspring diets were mismatched. The transgenerational effects observed, although not acclimatory in nature, could potentially be explained through carry-over effects. Yet, studies on transgenerational effects and their impact on insect life-history traits remain limited. Given the contrasting results of its influence on larval development and adult reproductive output found in this thesis, further exploration is needed, where *S. littoralis* may serve as a promising model.

References

Agnihotri, A.R., Roy, A.A. & Joshi, R.S. (2016). Gustatory receptors in Lepidoptera: chemosensation and beyond. *Insect. Mol. Biol.,* 25(5), 519-29. https://doi.org/10.1111/imb.12246

Agrawal, A.A., Laforsch, C. & Tollrian, R. (1999). Transgenerational induction of defences in animals and plants. *Nature,* 401(6748), 60-63. https://doi.org/10.1038/43425

Allmann, S., Späthe, A., Bisch-Knaden, S., Kallenbach, M., Reinecke, A., Sachse, S., Baldwin, I.T. & Hansson, B.S. (2013). Feeding-induced rearrangement of green leaf volatiles reduces moth oviposition. *Elife,* 2, e00421. https://doi.org/10.7554/eLife.00421

Anderson, P. & Anton, S. (2014). Experience-based modulation of behavioural responses to plant volatiles and other sensory cues in insect herbivores. *Plant Cell Environ.,* 37(8), 1826-35. https://doi.org/10.1111/pce.12342

Anderson, P., Hansson, B.S. & Löfqvist, J. (1995). Plant-odour-specific receptor neurones on the antennae of female and male *Spodoptera littoralis*. *Physiol. Entomol.,* 20(3), 189-198. https://doi.org/10.1111/j.1365-3032.1995.tb00001.x

Anderson, P., Hilker, M., Hansson, B.S., Bombosch, S., Klein, B. & Schildknecht, H. (1993). Oviposition deterring components in larval frass of *Spodoptera littoralis* (Boisd.)(Lepidoptera: Noctuidae): a behavioural and electrophysiological evaluation. *J. Insect Physiol.,* 39(2), 129-137. https://doi.org/10.1016/0022-1910(93)90104-Y

Anderson, P., Sadek, M.M., Larsson, M., Hansson, B.S. & Thöming, G. (2013). Larval host plant experience modulates both mate finding and oviposition choice in a moth. *Anim. Behav.,* 85(6), 1169-1175. https://doi.org/10.1016/j.anbehav.2013.03.002

Andersson, M.N., Löfstedt, C. & Newcomb, R.D. (2015). Insect olfaction and the evolution of receptor tuning. *Front. Ecol. Evol.,* 3, 53. https://doi.org/10.3389/fevo.2015.00053

Badra, Z., Larsson Herrera, S., Cappellin, L., Biasioli, F., Dekker, T., Angeli, S. & Tasin, M. (2021). Species-specific induction of plant volatiles by two aphid species in apple: real time measurement of plant emission and attraction of lacewings in the wind tunnel. *J. Chem. Ecol.,* 47(7), 653-663. https://doi.org/10.1007/s10886-021-01288-5

Baldwin, I.T., Halitschke, R., Paschold, A., Von Dahl, C.C. & Preston, C.A. (2006). Volatile signaling in plant-plant interactions:"talking trees" in the genomics era. *Science,* 311(5762), 812-815. https://doi.org/10.1126/science.1118446

Ballhorn, D.J., Kautz, S. & Heil, M. (2013). Distance and sex determine host plant choice by herbivorous beetles. *PLoS One,* 8(2), e55602. https://doi.org/10.1371/journal.pone.0055602

Barron, A.B. (2001). The life and death of Hopkins' host-selection principle. *J. Insect. Behav.,* 14(6), 725-737. https://doi.org/10.1023/a:1013033332535

Behmer, S.T. (2009). Insect herbivore nutrient regulation. *Annu. Rev. Entomol.,* 54(1), 165-187. https://doi.org/10.1146/annurev.ento.54.110807.090537

Bell, J.R., Bohan, D.A., Shaw, E.M. & Weyman, G.S. (2005). Ballooning dispersal using silk: world fauna, phylogenies, genetics and models. *Bull. Entomol. Res.,* 95(2), 69-114. https://doi.org/10.1079/BER2004350

Benton, R. (2015). Multigene family evolution: perspectives from insect chemoreceptors. *Trends Ecol. Evol.,* 30(10), 590-600. https://doi.org/10.1016/j.tree.2015.07.009

Bernays, E. & Graham, M. (1988). On the evolution of host specificity in phytophagous arthropods. *Ecology,* 69(4), 886-892. https://doi.org/10.2307/1941237

Bernays, E.A. (1999). Plasticity and the problem of choice in food selection. *Ann. Entomol. Soc. Am.,* 92(6), 944-951. https://doi.org/10.1093/aesa/92.6.944

Bernays, E.A. (2001). Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annu. Rev. Entomol.,* 46(1), 703-727. https://doi.org/10.1146/annurev.ento.46.1.703

Bernays, E.A. & Funk, D.J. (1999). Specialists make faster decisions than generalists: experiments with aphids. *Proc. R. Soc. B,* 266(1415), 151-156. https://doi.org/10.1098/rspb.1999.0615

Beukeboom, L.W. (2018). Size matters in insects–an introduction. *Entomol. Exp. Appl.,* 166(1), 2-3. https://doi.org/10.1111/eea.12646

Beyaert, I. & Hilker, M. (2014). Plant odour plumes as mediators of plant–insect interactions. *Biol. Rev.,* 89(1), 68-81. https://doi.org/10.1111/brv.12043

Binyameen, M., Anderson, P., Ignell, R., Birgersson, G., Razaq, M., Shad, S.A., Hansson, B.S. & Schlyter, F. (2014). Identification of plant semiochemicals and characterization of new olfactory sensory neuron types in a polyphagous pest moth, *Spodoptera littoralis*. *Chem. Senses,* 39(8), 719-733. https://doi.org/10.1093/chemse/bju046

Binyameen, M., Anderson, P., Ignell, R., Seada, M.A., Hansson, B.S. & Schlyter, F. (2012). Spatial organization of antennal olfactory sensory neurons in the female *Spodoptera littoralis* moth: differences in sensitivity and temporal characteristics. *Chem. Senses,* 37(7), 613-629. https://doi.org/10.1093/chemse/bjs043

Binyameen, M., Hussain, A., Yousefi, F., Birgersson, G. & Schlyter, F. (2013). Modulation of Reproductive Behaviors by Non-Host Volatiles in the Polyphagous Egyptian Cotton Leafworm, *Spodoptera littoralis*. *J. Chem. Ecol.,* 39, 1273-1283. https://doi.org/10.1007/s10886-013-0354-4

Blight, M.M., Pickett, J.A., Wadhams, L.J. & Woodcock, C.M. (1995). Antennal perception of oilseed rape, *Brassica napus* (Brassicaceae), volatiles by the

cabbage seed weevil *Ceutorhynchus assimilis* (Coleoptera, Curculionidae). *J. Chem. Ecol.,* 21(11), 1649-1664. https://doi.org/10.1007/bf02033667

Bonduriansky, R. & Crean, A.J. (2018). What are parental condition-transfer effects and how can they be detected? *Methods Ecol. Evol.,* 9(3), 450-456. https://doi.org/10.1111/2041-210X.12848

Borges, R.M., Bessière, J.M. & Hossaert-McKey, M. (2008). The chemical ecology of seed dispersal in monoecious and dioecious figs. *Funct. Ecol.,* 22(3), 484-493. https://doi.org/10.1111/j.1365-2435.2008.01383.x

Borrero-Echeverry, F., Becher, P.G., Birgersson, G., Bengtsson, M., Witzgall, P. & Saveer, A.M. (2015). Flight attraction of *Spodoptera littoralis* (Lepidoptera, Noctuidae) to cotton headspace and synthetic volatile blends. *Front. Ecol. Evol.,* 3(56). https://doi.org/10.3389/fevo.2015.00056

Borrero-Echeverry, F., Bengtsson, M., Nakamuta, K. & Witzgall, P. (2018). Plant odor and sex pheromone are integral elements of specific mate recognition in an insect herbivore. *Evolution,* 72(10), 2225-2233. https://doi.org/10.1111/evo.13571

Bracho-Nunez, A., Welter, S., Staudt, M. & Kesselmeier, J. (2011). Plant-specific volatile organic compound emission rates from young and mature leaves of Mediterranean vegetation. *J. Geophys. Res. Atmos.,* 116(D16). https://doi.org/10.1029/2010JD015521

Bruce, T.J.A. & Pickett, J.A. (2011). Perception of plant volatile blends by herbivorous insects – Finding the right mix. *Phytochemistry,* 72(13), 1605-1611. https://doi.org/10.1016/j.phytochem.2011.04.011

Bruce, T.J.A., Wadhams, L.J. & Woodcock, C.M. (2005). Insect host location: a volatile situation. *Trends in Plant Sci.,* 10(6), 269-274. https://doi.org/10.1016/j.tplants.2005.04.003

Busto, G.U., Cervantes-Sandoval, I. & Davis, R.L. (2010). Olfactory Learning in *Drosophila. Physiology,* 25(6), 338-346. https://doi.org/10.1152/physiol.00026.2010

CABI (2022). *Spodoptera littoralis (cotton leafworm).* https://doi.org/10.1079/cabicompendium.51070 [Accessed 28 September 2023]

Cahenzli, F. & Erhardt, A. (2013). Transgenerational acclimatization in an herbivore–host plant relationship. *Proc. R. Soc. B,* 280(1756). https://doi.org/10.1098/rspb.2012.2856

Cahenzli, F., Wenk, B.A. & Erhardt, A. (2015). Female butterflies adapt and allocate their progeny to the host-plant quality of their own larval experience. *Ecology,* 96(7), 1966-1973. https://doi.org/10.1890/14-1275.1

Carrasco, D., Larsson, M.C. & Anderson, P. (2015). Insect host plant selection in complex environments. *Curr. Opin. Insect Sci.,* 8, 1-7. https://doi.org/10.1016/j.cois.2015.01.014

Cheng, A.X., Lou, Y.G., Mao, Y.B., Lu, S., Wang, L.J. & Chen, X.Y. (2007). Plant terpenoids: biosynthesis and ecological functions. *J. Integr. Plant Biol.,* 49(2), 179-186. https://doi.org/10.1111/j.1672-9072.2006.00395.x

Chiriboga Morales, X., Tamiru, A., Sobhy, I.S., Bruce, T.J.A., Midega, C.A.O. & Khan, Z. (2021). Evaluation of African maize cultivars for resistance to fall armyworm *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae) larvae. *Plants,* 10(2), 392. https://doi.org/10.3390/plants10020392

Coley, P.D. & Bateman, M.L.K., T. A. (2006). The effects of plant quality on caterpillar growth and defense against natural enemies. *Oikos,* 115(2), 219-228. https://doi.org/10.1111/j.2006.0030-1299.14928.x

Conchou, L., Anderson, P. & Birgersson, G. (2017). Host plant species differentiation in a polyphagous moth: olfaction is enough. *J. Chem. Ecol.,* 43(8), 794–805. https://doi.org/10.1007/s10886-017-0876-2

Conchou, L., Lucas, P., Meslin, C., Proffit, M., Staudt, M. & Renou, M. (2019). Insect odorscapes: from plant volatiles to natural olfactory scenes. *Front. Physiol.,* 10, 972. https://doi.org/10.3389/fphys.2019.00972

Crozier, A., Clifford, M.N. & Ashihara, H. (2006). *Plant secondary metabolites.* (Occurrence, Structure and Role in the Human Diet). New Jersey: Blackwell-Publishing.

Cunningham, J.P. (2012). Can mechanism help explain insect host choice? *J. Evol. Biol.,* 25(2), 244-251. https://doi.org/10.1111/j.1420-9101.2011.02435.x

de Fouchier, A., Sun, X., Caballero-Vidal, G., Travaillard, S., Jacquin-Joly, E. & Montagné, N. (2018). Behavioral effect of plant volatiles binding to *Spodoptera littoralis* larval odorant receptors. *Front. Behav. Neurosci.,* 12(264). https://doi.org/10.3389/fnbeh.2018.00264

De Moraes, C.M., Mescher, M.C. & Tumlinson, J.H. (2001). Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature,* 410(6828), 577-580. https://doi.org/10.1038/35069058

del Campo, M.L., Miles, C.I., Schroeder, F.C., Mueller, C., Booker, R. & Renwick, J.A. (2001). Host recognition by the tobacco hornworm is mediated by a host plant compound. *Nature,* 411(6834), 186-189. https://doi.org/10.1038/35075559

Diamond, S.E. & Martin, R.A. (2021). Buying time: plasticity and population persistence. In: Pfennig, D.W. (ed.) *Phenotypic Plasticity & Evolution.* Colorado, USA: CRC Press. 185-209.

Dicke, M. (2009). Behavioural and community ecology of plants that cry for help. *Plant Cell Environ.,* 32(6), 654-665. https://doi.org/10.1111/j.1365-3040.2008.01913.x

Dicke, M. & Loreto, F. (2010). Induced plant volatiles: from genes to climate change. *Trends Plant Sci.,* 15(3), 115-117. https://doi.org/10.1016/j.tplants.2010.01.007

Dicke, M. & Lucas-Barbosa, D. (2020). Herbivore-induced plant volatiles as a source of information in plant–insect networks. In: Pichersky, E. & Dudareva, N. (eds) *Biology of plant volatiles*. Boca Raton, FL, USA: CRC Press. 327-346. https://doi.org/10.1201/9780429455612-20

Dicke, M. & Sabelis, M.W. (1988). Infochemical terminology: based on cost-benefit analysis rather than origin of compounds? *Funct. Ecol.*, 131-139. https://doi.org/10.2307/2389687

Dicke, M. & Van Loon, J.J.A. (2000). Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomol. Exp. Appl.,* 97(3), 237-249. https://doi.org/10.1046/j.1570-7458.2000.00736.x

Donelson, J.M., Salinas, S., Munday, P. L., & Shama, L. N. (2018). Transgenerational plasticity and climate change experiments: Where do we go from here? *Global Change Biology,,* 24(1), 13-34. https://doi.org/10.1111/gcb.13903

Dudareva, N., Klempien, A., Muhlemann, J.K. & Kaplan, I. (2013). Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytol.,* 198(1), 16-32. https://doi.org/10.1111/nph.12145

Dudareva, N., Negre, F., Nagegowda, D.A. & Orlova, I. (2006). Plant volatiles: recent advances and future perspectives. *Crit. Rev. Plant Sci.,* 25(5), 417-440. https://doi.org/10.1080/07352680600899973

Dukas, R. (2008). Evolutionary biology of insect learning. *Annu. Rev. Entomol.,* 53. https://doi.org/10.1146/annurev.ento.53.103106.093343

Dwernychuk, L.W. & Boag, D.A. (1972). Ducks nesting in association with gulls—an ecological trap? *Can. J. Zool.,* 50(5), 559-563. https://doi.org/10.1139/z72-076

Ehrlich, P.R. & Raven, P.H. (1964). Butterflies and plants: a study in coevolution. *Evolution*, 586-608. https://doi.org/10.2307/2406212

El-Sayed, A. (2023). *The Pherobase: database of pheromones and semiochemicals.* http://www.pherobase.com [Accessed 5 October 2023]

Engqvist, L. & Reinhold, K. (2016). Adaptive trans-generational phenotypic plasticity and the lack of an experimental control in reciprocal match/mismatch experiments. *Methods Ecol. Evol.*, 1482-1488. https://doi.org/10.1111/2041-210x.12618

EPPO (2015). PM 7/124 (1) *Spodoptera littoralis, Spodoptera litura, Spodoptera frugiperda, Spodoptera eridania*. *EPPO Bul.,* 45, 410-444. https://doi.org/10.1111/epp.12258

EPPO (2023). *Spodoptera littoralis. EPPO datasheets on pests recommended for regulation.* [Accessed 18 October 2023]

Farré-Armengol, G., Filella, I., Llusia, J. & Peñuelas, J. (2016). Bidirectional interaction between phyllospheric microbiotas and plant volatile emissions. *Trends Plant Sci.,* 21(10), 854-860. https://doi.org/10.1016/j.tplants.2016.06.005

Feeny, P. (1976). Plant apparency and chemical defense. In: Wallace, J.W. & Mansell, R.L. (eds) *Biochemical interaction between plants and insects*. Springer. 1-40.

Fox, C.W. & Czesak, M.E. (2000). Evolutionary ecology of progeny size in arthropods. *Annu. Rev. Entomol.,* 45(1), 341-369. https://doi.org/10.1146/annurev.ento.45.1.341

Franzke, A. & Reinhold, K. (2013). Transgenerational effects of diet environment on life-history and acoustic signals of a grasshopper. *Behav. Ecol.,* 24(3), 734-739. https://doi.org/10.1093/beheco/ars205

Galizia, C.G., Sachse, S., Rappert, A. & Menzel, R. (1999). The glomerular code for odor representation is species specific in the honeybee *Apis mellifera*. *Nat. Neurosci.,* 2(5), 473-478. https://doi.org/10.1038/8144

García-Robledo, C. & Horvitz, C.C. (2012). Parent–offspring conflicts,"optimal bad motherhood" and the "mother knows best" principles in insect herbivores colonizing novel host plants. *Ecol. Evol.,* 2(7), 1446-1457. https://doi.org/10.1002/ece3.267

Gowri, V., Dion, E., Viswanath, A., Piel, F. M., & Monteiro, A. (2019). Transgenerational inheritance of learned preferences for novel host plant odors in Bicyclus anynana butterflies. *Evolution*, 2401-2414. https://doi.org/10.1111/evo.13861

Gowri, V. & Monteiro, A. (2021). Inheritance of acquired Traits in insects and other animals and the epigenetic mechanisms that break the weismann barrier. *J. Dev. Biol.,* 9(4), 41. https://doi.org/10.3390/jdb9040041

Grimaldi, D. & Engel, M.S. (2005). *Evolution of the Insects.* Cambridge University Press.

Gripenberg, S., Mayhew, P.J., Parnell, M. & Roslin, T. (2010). A meta-analysis of preference–performance relationships in phytophagous insects. *Ecol. Lett.,* 13(3), 383-393. https://doi.org/10.1111/j.1461-0248.2009.01433.x

Hansson, B.S. (1999). *Insect Olfaction.* Springer Science & Business Media.

Hardy, N.B., Kaczvinsky, C., Bird, G. & Normark, B.B. (2020). What we don't know about diet-breadth evolution in herbivorous insects. *Annu. Rev. Ecol. Evol. Syst.,* 51, 103-122. https://doi.org/10.1146/annurev-ecolsys-011720-023322

Heil, M. & Karban, R. (2010). Explaining evolution of plant communication by airborne signals. *Trends Ecol. Evol.,* 25(3), 137-144. https://doi.org/10.1016/j.tree.2009.09.010

Hern, A. & Dorn, S. (1999). Sexual dimorphism in the olfactory orientation of adult *Cydia pomonella* in response to α-farnesene. *Entomol. Exp. Appl.,* 92(1), 63-72. https://doi.org/10.1046/j.1570-7458.1999.00525.x

Herrera, C.M. & Bazaga, P. (2010). Epigenetic differentiation and relationship to adaptive genetic divergence in discrete populations of the violet *Viola cazorlensis*. *New Phytol.,* 187(3), 867-876. https://doi.org/10.1111/j.1469-8137.2010.03298.x

Hilker, M. & Fatouros, N.E. (2015). Plant responses to insect egg deposition. *Annu. Rev. Entomol.,* 60, 493-515. https://doi.org/10.1146/annurev-ento-010814-020620

Hinks, C.F. & Byers, J.R. (1976). Biosystematics of the genus *Euxoa* (Lepidoptera: Noctuidae): V. Rearing procedures, and life cycles of 36 species. *Can. Entomol.,* 108(12), 1345-1357. https://doi.org/10.4039/Ent1081345-12

Hodgkison, R., Ayasse, M., Kalko, E.K.V., Häberlein, C., Schulz, S., Mustapha, W.A.W., Zubaid, A. & Kunz, T.H. (2007). Chemical ecology of fruit bat foraging behavior in relation to the fruit odors of two species of paleotropical bat-dispersed figs (*Ficus hispida* and *Ficus scortechinii*). *J. Chem. Ecol.,* 33, 2097-2110. https://doi.org/10.1007/s10886-007-9367-1

Holopainen, J.K. & Blande, J.D. (2013). Where do herbivore-induced plant volatiles go? *Front. Plant Sci.,* 4, 185. https://doi.org/10.3389/fpls.2013.00185

Hopkins, A.D. (1917). A discussion of CG Hewitt's paper on "Insect Behaviour". *J. Econ. Entomol.,* 10, 92-93.

Jablonka, E., Oborny, B., Molnar, I., Kisdi, E., Hofbauer, J. & Czaran, T. (1995). The adaptive advantage of phenotypic memory in changing environments. *Philos. Trans. R. Soc.,* 350(1332), 133-41. https://doi.org/10.1098/rstb.1995.0147

Jaenike, J. (1978). On optimal oviposition behavior in phytophagous insects. *Theor. Popul. Biol.,* 14(3), 350-356. https://doi.org/10.1016/0040-5809(78)90012-6

Jansen, R.M.C., Wildt, J., Kappers, I.F., Bouwmeester, H.J., Hofstee, J.W. & Van Henten, E.J. (2011). Detection of diseased plants by analysis of volatile organic compound emission. *Annu. Rev. Phytopathol.,* 49, 157-174. https://doi.org/10.1146/annurev-phyto-072910-095227

Janz, N. (2003). The cost of polyphagy: oviposition decision time vs error rate in a butterfly. *Oikos,* 100(3), 493-496. https://doi.org/10.1034/j.1600-0706.2003.12290.x

Jiménez-Pérez, A. & Wang, Q. (2004). Effect of body weight on reproductive performance in *Cnephasia jactatana* (Lepidoptera: Tortricidae). *J. Insect Behav.,* 17, 511-522. https://doi.org/10.1023/B:JOIR.0000042538.19559.09

Jones, L.C. (2022). Insects allocate eggs adaptively according to plant age, stress, disease or damage. *Proc. R. Soc. B.,* 289(1978), 20220831. https://doi.org/10.1098/rspb.2022.0831

Jun-Wen, C. & Cao, K.-F. (2005). Plant VOCs emission: a new strategy of thermotolerance. *J. For. Res.,* 16, 323-326. https://doi.org/10.1007/BF02858200

Jönsson, M. (2005). *Responses to oilseed rape and cotton volatiles in insect herbivores and parasitoids.* Dept. of Crop Science. Alnarp, Sweden: Swedish University of Agricultural Sciences.

Jönsson, M. & Anderson, P. (1999). Electrophysiological response to herbivore-induced host plant volatiles in the moth *Spodoptera littoralis. Physiol. Entomol.,* 24(4), 377-385. https://doi.org/10.1046/j.1365-3032.1999.00154.x

Kandasamy, D., Gershenzon, J., Andersson, M.N. & Hammerbacher, A. (2019). Volatile organic compounds influence the interaction of the Eurasian spruce bark beetle (*Ips typographus*) with its fungal symbionts. *ISME J.,* 13(7), 1788-1800. https://doi.org/10.1038/s41396-019-0390-3

Karlsson, M.F., Proffit, M. & Birgersson, G. (2017). Host-plant location by the Guatemalan potato moth *Tecia solanivora* is assisted by floral volatiles. *Chemoecology,* 27, 187-198. https://doi.org/10.1007/s00049-017-0244-2

Kemp, D.J. (2019). Manipulation of natal host modifies adult reproductive behaviour in the butterfly *Heliconius charithonia*. *Proc. R. Soc. B.,* 286(1910), 20191225. https://doi.org/10.1098/rspb.2019.1225

Khallaf, M.A., Sadek, M.M. & Anderson, P. (2023). Predator efficacy and attraction to herbivore-induced volatiles determine insect pest selection of inferior host plant. *iScience*. https://doi.org/10.1016/j.isci.2023.106077

Khan, Z., Bohman, B., Ignell, R. & Hill, S.R. (2023). Odour-mediated oviposition site selection in *Aedes aegypti* depends on aquatic stage and density. *Parasites Vectors,* 16(1), 1-12. https://doi.org/10.1186/s13071-023-05867-1

Kigathi, R.N., Weisser, W.W., Reichelt, M., Gershenzon, J. & Unsicker, S.B. (2019). Plant volatile emission depends on the species composition of the neighboring plant community. *BMC plant biol.,* 19, 1-17. https://doi.org/10.1186/s12870-018-1541-9

Knudsen, J.T., Tollsten, L. & Bergström, L.G. (1993). Floral scents–a checklist of volatile compounds isolated by head-space techniques. *Phytochemistry,* 33(2), 253-280. https://doi.org/10.1016/0031-9422(93)85502-i

Krist, M. (2011). Egg size and offspring quality: a meta-analysis in birds. *Biol. Rev.,* 86(3), 692-716. https://doi.org/10.1111/j.1469-185X.2010.00166.x

Kromann, S.H., Saveer, A.M., Binyameen, M., Bengtsson, M., Birgersson, G., Hansson, B.S., Schlyter, F., Witzgall, P., Ignell, R. & Becher, P.G. (2015). Concurrent modulation of neuronal and behavioural olfactory responses to sex and host plant cues in a male moth. *Proc. R. Soc. B,* 282(1799), 20141884. https://doi.org/10.1098/rspb.2014.1884

Kuijper, B. & Hoyle, R.B. (2015). When to rely on maternal effects and when on phenotypic plasticity? *Evolution,* 69(4), 950-968. https://doi.org/10.1111/evo.12635

Ledón-Rettig, C.C., Richards, C.L. & Martin, L.B. (2013). Epigenetics for behavioral ecologists. *Behav. Ecol.,* 24(2), 311-324. https://doi.org/10.1093/beheco/ars145

Leimar, O. & McNamara, J.M. (2015). The evolution of transgenerational integration of information in heterogeneous environments. *Am. Nat.,* 185(3), E55-E69. https://doi.org/10.1086/679575

Lejfalk, C. & Birgersson, G. (1997). Wick-baits–a novel delivery system for testing potential semiochemicals. In: *Proceedings of the 14th Annual Meeting of the International Society of Chemical Ecology*, Vancouver, Canada.

Lelito, J.P., Böröczky, K., Jones, T.H., Fraser, I., Mastro, V.C., Tumlinson, J.H. & Baker, T.C. (2009). Behavioral evidence for a contact sex pheromone component of the emerald ash borer, *Agrilus planipennis* Fairmaire. *J. Chem. Ecol.,* 35, 104-110. https://doi.org/10.1007/s10886-008-9583-3

Levin, E., McCue, M.D. & Davidowitz, G. (2017). More than just sugar: allocation of nectar amino acids and fatty acids in a Lepidopteran. *Proc. R. Soc. B,* 284(1848), 20162126. https://doi.org/10.1098/rspb.2016.2126

Levins, R. (1968). *Evolution in Changing Environments: Some Theoretical Explorations.* Princeton University Press.

Levis, N.A. & Pfennig, D.W. (2016). Evaluating 'plasticity-first' evolution in nature: key criteria and empirical approaches. *Trends Ecol. Evol.,* 31(7), 563-574. https://doi.org/10.1016/j.tree.2016.03.012

Lhomme, P., Carrasco, D., Larsson, M., Hansson, B. & Anderson, P. (2018a). A context-dependent induction of natal habitat preference in a generalist herbivorous insect-. *Behav. Ecol.,* 29(2), 360-367. https://doi.org/10.1093/beheco/arx173

Lhomme, P., Carrasco, D., Larsson, M., Hansson, B. & Anderson, P. (2018b). A context-dependent induction of natal habitat preference in a generalist herbivorous insect. *Behavioral Ecology,* 29(2), 360-367. https://doi.org/10.1093/beheco/arx173

Lhomme, P., Khallaf, M., Larsson, M. & Anderson, P. (2020). A sensitive period for the induction of host plant preference in a generalist herbivorous insect. *Anim. Behav.,* 169, 1-8. https://doi.org/10.1016/j.anbehav.2020.08.020

Li, Z., Li, D., Xie, B., Ji, R. & Cui, J. (2005). Effect of body size and larval experience on mate preference in *Helicoverpa armigera* (Hübner)(Lep., Noctuidae). *J. Appl. Entomol.,* 129(9-10), 574-579. https://doi.org/10.1111/j.1439-0418.2005.01012.x

Lindström, J. (1999). Early development and fitness in birds and mammals. *Trends Ecol. Evol.,* 14(9), 343-348. https://doi.org/10.1016/s0169-5347(99)01639-0

Liu, H., Xiu, C., Zhang, T. & Lu, Y. (2022). Odor perception in the cotton bollworm, *Helicoverpa armigera*, exposed to *Juglans regia*, a marginal host plant. *J. Chem. Ecol.,* 48(7-8), 618-627. https://doi.org/10.1007/s10886-022-01374-2

Ljunggren, J., Borrero-Echeverry, F., Chakraborty, A., Lindblom, T.U.T., Hedenström, E., Karlsson, M., Witzgall, P. & Bengtsson, M. (2019). Yeast volatomes differentially affect larval feeding in an insect herbivore. *Appl. Environ. Microbiol.,* 85(21), e01761-19. https://doi.org/10.1128/AEM.01761-19

Loughrin, J.H., Manukian, A.R.A., Heath, R.R., Turlings, T.C. & Tumlinson, J.H. (1994). Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plant. *Proc. Natl. Acad. Sci. U.S.A.,* 91(25), 11836-11840. https://doi.org/10.1073/pnas.91.25.11836

Maeda, H. & Dudareva, N. (2012). The shikimate pathway and aromatic amino acid biosynthesis in plants. *Annu. Rev. Plant Biol.,* 63, 73-105. https://doi.org/10.1146/annurev-arplant-042811-105439

Mann, L., Laplanche, D., Turlings, T.C.J. & Desurmont, G.A. (2021). A comparative study of plant volatiles induced by insect and gastropod herbivory. *Sci. Rep.,* 11(1), 23698.

Marshall, D.J. & Uller, T. (2007). When is a maternal effect adaptive? *Oikos,* 116(12), 1957-1963. https://doi.org/10.1111/j.2007.0030-1299.16203.x,

Mayhew, P.J. (2001). Herbivore host choice and optimal bad motherhood. *Trends Ecol. Evol.,* 16(4), 165-167. https://doi.org/10.1016/S0169-5347(00)02099-1

Mayhew, P.J. (2007). Why are there so many insect species? Perspectives from fossils and phylogenies. *Biol. Rev.,* 82(3), 425-454. https://doi.org/10.1111/j.1469-185X.2007.00018.x

McCall, P.J., Turlings, T.C.J., Loughrin, J., Proveaux, A.T. & Tumlinson, J.H. (1994). Herbivore-induced volatile emissions from cotton (*Gossypium hirsutum* L.) seedlings. *J. Chem. Ecol.,* 20(12), 3039-3050. https://doi.org/10.1007/BF02033709

Mechaber, W.L. & Hildebrand, J.G. (2000). Novel, non-solanaceous hostplant record for *Manduca sexta* (Lepidoptera: Sphingidae) in the southwestern United States. *Ann. Entomol. Soc. Am.,* 93(3), 447-451. https://doi.org/10.1603/0013-8746(2000)093[0447:NNSHRF]2.0.CO;2

Mendesil, E., Tefera, T., Blanco, C.A., Paula-Moraes, S.V., Huang, F., Viteri, D.M. & Hutchison, W.D. (2023). The invasive fall armyworm, *Spodoptera frugiperda*, in Africa and Asia: responding to the food security challenge, with priorities for integrated pest management research. *J. Plant Dis. Prot.*, 1-32. https://doi.org/10.1007/s41348-023-00777-x

Menzel, R. & Müller, U. (1996). Learning and memory in honeybees: from behavior to neural substrates. *Annu. Rev. Neurosci.,* 19(1), 379-404. https://doi.org/10.1146/annurev.ne.19.030196.002115

Mikulski, A. & Pijanowska, J. (2010). When and how can *Daphnia* prepare their offspring for the threat of predation? *Hydrobiologia,* 643(1), 21-26. https://doi.org/10.1007/s10750-010-0131-0

Mitra, S., Firake, D.M., Umesh, K.P., Pandey, P.P. & Pandit, S. (2021). Polyphagous caterpillars of *Spodoptera litura* switch from a trap crop to the main crop, improve fitness, and shorten generation time. *J. Pest Sci.,* 94(4), 1091-1103. https://doi.org/10.1007/s10340-021-01351-w

Mousseau, T.A. & Fox, C.W. (1998a). *Maternal Effects as Adaptations.* New York: Oxford University Press.

Mousseau, T.A. & Fox, C.W. (1998b). The adaptive significance of maternal effects. *Trends Ecol. Evol.,* 13(10), 403-7. https://doi.org/10.1016/s0169-5347(98)01472-4

Muhlemann, J.K., Klempien, A. & Dudareva, N. (2014). Floral volatiles: from biosynthesis to function. *Plant Cell Environ.,* 37(8), 1936-1949. https://doi.org/10.1111/pce.12314

Mumm, R. & Dicke, M. (2010). Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. *Can. J. Zool.,* 88(7), 628-667. https://doi.org/10.1139/Z10-032

Munday, P.L. (2014). Transgenerational acclimation of fishes to climate change and ocean acidification. *F1000Prime Rep.,* 6. https://doi.org/10.12703/P6-99

Ninkovic, V., Markovic, D. & Rensing, M. (2021). Plant volatiles as cues and signals in plant communication. *Plant Cell Environ.,* 44(4), 1030-1043. https://doi.org/10.1111/pce.13910

Nordlund, D.A. & Lewis, W.J. (1976). Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. *J. Chem. Ecol.,* 2, 211-220. https://doi.org/10.1007/BF00987744

Nottingham, S.F., Hardie, J., Dawson, G.W., Hick, A.J., Pickett, J.A., Wadhams, L.J. & Woodcock, C.M. (1991). Behavioral and electrophysiological responses of Aphids to host and nonhost plant volatiles. *J. Chem. Ecol.,* 17(6), 1231-1242. https://doi.org/10.1007/bf01402946

Olsson, P.O.C., Anderbrant, O. & Löfstedt, C. (2006). Experience influences oviposition behaviour in two pyralid moths, *Ephestia cautella* and *Plodia interpunctella*. *Anim. Behav.,* 72(3), 545-551. https://doi.org/10.1016/j.anbehav.2005.10.023

Paniagua Voirol, L.R., Frago, E., Kaltenpoth, M., Hilker, M. & Fatouros, N.E. (2018). Bacterial symbionts in Lepidoptera: their diversity, transmission, and impact on the host. *Front. Microbiol.,* 9(556), 556. https://doi.org/10.3389/fmicb.2018.00556

Pare, P.W. & Tumlinson, J.H. (1997). De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiol.,* 114(4), 1161-1167. https://doi.org/10.1104/pp.114.4.1161

Penuelas, J. & Llusià, J. (2001). The complexity of factors driving volatile organic compound emissions by plants. *Biol. Plant.,* 44, 481-487. https://doi.org/10.1023/A:1013797129428

Petrén, H., Gloder, G., Posledovich, D., Wiklund, C. & Friberg, M. (2021). Innate preference hierarchies coupled with adult experience, rather than larval imprinting or transgenerational acclimation, determine host plant use in *Pieris rapae*. *Ecol. Evol.,* 11(1), 242-251. https://doi.org/10.1002/ece3.7018

Picazo-Aragonés, J., Terrab, A. & Balao, F. (2020). Plant volatile organic compounds evolution: Transcriptional regulation, epigenetics and polyploidy. *Int. J. Mol. Sci.,* 21(23), 8956. https://doi.org/10.3390/ijms21238956

Pichersky, E., Noel, J.P. & Dudareva, N. (2006). Biosynthesis of plant volatiles: nature's diversity and ingenuity. *Science,* 311(5762), 808-811. https://doi.org/10.1126/science.1118510

Pierce, N.E. & Berry, A. (2011). The herbivore's dilemma: never enough nitrogen. In: Loso, J.B. (ed.) *In the Light of Evolution: Essays From the Laboratory*

and Field Roberts and Company. Colorado, USA: W. H. Freeman. 121-133.

Pigliucci, M., Murren, C.J. & Schlichting, C.D. (2006). Phenotypic plasticity and evolution by genetic assimilation. *J. Exp. Biol.,* 209(12), 2362-2367. https://doi.org/10.1242/jeb.02070

Proffit, M., Khallaf, M.A., Carrasco, D., Larsson, M.C. & Anderson, P. (2015). 'Do you remember the first time?' Host plant preference in a moth is modulated by experiences during larval feeding and adult mating. *Ecol. Lett.,* 18(4), 365-374. https://doi.org/10.1111/ele.12419

Qvarnström, A. & Price, T.D. (2001). Maternal effects, paternal effects and sexual selection. *Trends Ecol. Evol.,* 16(2), 95-100. https://doi.org/10.1016/S0169-5347(00)02063-2

Reddy, G.V.P. & Guerrero, A. (2004). Interactions of insect pheromones and plant semiochemicals. *Trends Plant Sci.,* 9(5), 253-261. https://doi.org/10.1016/j.tplants.2004.03.009

Rhainds, M. (2015). Size-dependent realized fecundity in two lepidopteran capital breeders. *Environ. Entomol.,* 44(4), 1193-1200. https://doi.org/10.1093/ee/nvv075

Rieger, J.F., Binckley, C.A. & Resetarits Jr, W.J. (2004). Larval performance and oviposition site preference along a predation gradient. *Ecology,* 85(8), 2094-2099. https://doi.org/10.1890/04-0156

Riffell, J.A., Lei, H., Christensen, T.A. & Hildebrand, J.G. (2009). Characterization and coding of behaviorally significant odor mixtures. *Curr. Biol.,* 19(4), 335-340. https://doi.org/10.1016/j.cub.2009.01.041

Rotem, K., Agrawal, A.A. & Kott, L. (2003). Parental effects in *Pieris rapae* in response to variation in food quality: adaptive plasticity across generations? *Ecol. Entomol.,* 28(2), 211-218. https://doi.org/10.1046/j.1365-2311.2003.00507.x

Röse, U.S.R. & Tumlinson, J.H. (2005). Systemic induction of volatile release in cotton: how specific is the signal to herbivory? *Planta,* 222, 327-335. https://doi.org/10.1007/s00425-005-1528-2

Rösvik, A., Lhomme, P., Khallaf, M.A. & Anderson, P. (2020). Plant-induced transgenerational plasticity affecting performance but not preference in a polyphagous moth. *Front. Ecol. Evol.,* 8(254). https://doi.org/10.3389/fevo.2020.00254

Sachse, S. & Krieger, J. (2011). Olfaction in insects. *E-Neuroforum,* 17(3), 49-60. https://doi.org/10.1007/s13295-011-0020-7

Sadek, M.M., Hansson, B.S. & Anderson, P. (2010). Does risk of egg parasitism affect choice of oviposition sites by a moth? A field and laboratory study. *Basic Appl. Ecol.,* 11(2), 135-143. https://doi.org/10.1016/j.baae.2009.09.003

Salinas, S., Brown, S.C., Mangel, M. & Munch, S.B. (2013). Non-genetic inheritance and changing environments. *Non-Genet. Inherit.,* 1. https://doi.org/10.2478/ngi-2013-0005

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N. & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nat. Ecol. Evol.,* 3(3), 430-439. https://doi.org/10.1038/s41559-018-0793-y

Saveer, A.M., Becher, P.G., Birgersson, G., Hansson, B.S., Witzgall, P. & Bengtsson, M. (2014). Mate recognition and reproductive isolation in the sibling species *Spodoptera littoralis* and *Spodoptera litura*. *Front. Ecol. Evol.,* 2, 18. https://doi.org/10.3389/fevo.2014.00018

Saveer, A.M., Kromann, S.H., Birgersson, G., Bengtsson, M., Lindblom, T., Balkenius, A., Hansson, B.S., Witzgall, P., Becher, P.G. & Ignell, R. (2012). Floral to green: mating switches moth olfactory coding and preference. *Proc. R. Soc. B,* 279(1737), 2314-2322. https://doi.org/10.1098/rspb.2011.2710

Scheiner, S.M. (1993). Genetics and evolution of phenotypic plasticity.*,* 24(1), 35-68. https://doi.org/10.1146/annurev.es.24.110193.000343

Schiestl, F.P. (2015). Ecology and evolution of floral volatile-mediated information transfer in plants. *New Phytol.,* 206(2), 571-577. https://doi.org/10.1111/nph.13243

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P. & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nat. Methods,* 9(7), 676-82. https://doi.org/10.1038/nmeth.2019

Schoonhoven, L.M., Van Loon, J.J.A. & Dicke, M. (2005). *Insect-Plant Biology.* Oxford: Oxford university press.

Schulz-Bohm, K., Gerards, S., Hundscheid, M., Melenhorst, J., de Boer, W. & Garbeva, P. (2018). Calling from distance: attraction of soil bacteria by plant root volatiles. *ISME J.,* 12(5), 1252-1262. https://doi.org/10.1038/s41396-017-0035-3

Segers, F.H.I.D. & Taborsky, B. (2011). Egg size and food abundance interactively affect juvenile growth and behaviour. *Funct. Ecol.,* 25(1), 166-176. https://doi.org/10.1111/j.1365-2435.2010.01790.x

Sheehy, K.A. & Laskowski, K.L. (2023). Correlated behavioural plasticities: insights from plasticity evolution, the integrated phenotype and behavioural syndromes. *Anim. Behav.* https://doi.org/10.1016/j.anbehav.2023.04.007

Silva, R. & Clarke, A.R. (2020). The "sequential cues hypothesis": a conceptual model to explain host location and ranking by polyphagous herbivores. *Insect Sci.,* 27(6), 1136-1147. https://doi.org/10.1111/1744-7917.12719

Sultan, S.E., Barton, K. & Wilczek, A.M. (2009). Contrasting patterns of transgenerational plasticity in ecologically distinct congeners. *Ecology,* 90(7), 1831-1839. https://doi.org/10.1890/08-1064.1

Taborsky, B. (2006). The influence of juvenile and adult environments on life-history trajectories. *Proc. Biol. Sci.,* 273(1587), 741-50. https://doi.org/10.1098/rspb.2005.3347

Tang, Q.-B., Zhan, H., Cao, H., Berg, B.G., Yan, F.-M. & Zhao, X.-C. (2014). Central projections of gustatory receptor neurons in the medial and the lateral sensilla styloconica of *Helicoverpa armigera* larvae. *PLoS One,* 9(4), e95401. https://doi.org/10.1371/journal.pone.0095401

Tholl, D., Boland, W., Hansel, A., Loreto, F., Röse, U.S.R. & Schnitzler, J.P. (2006). Practical approaches to plant volatile analysis. *Plant J.,* 45(4), 540-560. https://doi.org/10.1111/j.1365-313X.2005.02612.x

Thompson, J.N. & Pellmyr, O. (1991). Evolution of oviposition behavior and host preference in Lepidoptera. *Annu. Rev. Entomol.,* 36(1), 65-89. https://doi.org/10.1146/annurev.en.36.010191.000433

Thöming, G. (2021). Behavior matters - future need for insect studies on odor-mediated host plant recognition with the aim of making use of allelochemicals for plant protection. *J. Agric. Food Chem.,* 69(36), 10469-10479. https://doi.org/10.1021/acs.jafc.1c03593

Thöming, 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(8), 1744-1752. https://doi.org/10.1890/12-0907.1

Turlings, T.C. & Tumlinson, J.H. (1992). Systemic release of chemical signals by herbivore-injured corn. *Proc. Natl. Acad. Sci. U.S.A.,* 89(17), 8399-8402. https://doi.org/10.1073/pnas.89.17.8399

Turlings, T.C.L., Wäckers, F.L., Vet, L.E.M., Lewis, W.J. & Tumlinson, J.H. (1993). Learning of host-finding cues by hymenopterous parasitoids. In: Papaj, D.R. (ed.) *Insect Learning : Ecological and Evolutionary Perspectives*. (3). Boston, MA.: Springer US. 51-78. https://doi.org/10.1007/978-1-4615-2814-2_3

Uller, T. (2008). Developmental plasticity and the evolution of parental effects. *Trends Ecol. Evol.,* 23(8), 432-438. https://doi.org/10.1016/j.tree.2008.04.005

Uller, T., Nakagawa, S. & English, S. (2013). Weak evidence for anticipatory parental effects in plants and animals. *J. Evol. Biol.,* 26(10), 2161-70. https://doi.org/10.1111/jeb.12212

Vallat, A. & Dorn, S. (2005). Changes in volatile emissions from apple trees and associated response of adult female codling moths over the fruit-growing season. *J. Agric. Food Chem.,* 53(10), 4083-4090. https://doi.org/10.1021/jf048499u

Valtonen, T.M., Kangassalo, K., Polkki, M. & Rantala, M.J. (2012). Transgenerational effects of parental larval diet on offspring development time, adult body size and pathogen resistance in *Drosophila melanogaster*. *PLoS One,* 7(2), e31611. https://doi.org/10.1371/journal.pone.0031611

van de Pol, M., Bruinzeel, L.W., Heg, D.I.K., van der Jeugd, H.P. & Verhulst, S. (2006). A silver spoon for a golden future: long-term effects of natal origin on fitness prospects of oystercatchers (*Haematopus ostralegus*). *J. Anim. Ecol.,* 75(2), 616-626. https://doi.org/10.1111/j.1365-2656.2006.01079.x

van der Meijden, E. (2014). Herbivorous insects–a threat for crop production. In: Lugtenberg, B. (ed.) *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture.* Cham: Springer. 103-114. https://doi.org/10.1007/978-3-319-08575-3_12

Vivaldo, G., Masi, E., Taiti, C., Caldarelli, G. & Mancuso, S. (2017). The network of plants volatile organic compounds. *Sci. Rep.,* 7(1), 11050. https://doi.org/10.1038/s41598-017-10975-x

Wang, J.W., Wong, A.M., Flores, J., Vosshall, L.B. & Axel, R. (2003). Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain. *Cell,* 112(2), 271-282. https://doi.org/10.1016/S0092-8674(03)00004-7

Wang, W., He, P., Zhang, Y., Liu, T., Jing, X. & Zhang, S. (2020). The population growth of *Spodoptera frugiperda* on six cash crop species and implications for its occurrence and damage potential in China. *Insects,* 11(9), 639. https://doi.org/10.3390/insects11090639

Webster, B., Bruce, T., Pickett, J. & Hardie, J. (2010). Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid. *Anim. Behav.,* 79(2), 451-457. https://doi.org/10.1016/j.anbehav.2009.11.028

West-Eberhard, M.J. (1989). Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Evol. Syst.,* 20(1), 249-278. https://doi.org/10.1146/annurev.es.20.110189.001341

Whitman, D.W. & Agrawal, A.A. (2009). What is phenotypic plasticity and why is it important. In: Whitman, D. (ed.) *Phenotypic Plasticity of Insects: Mechanisms and Consequences.* 1st edition. Boca Raton, USA: CRC Press. 1-63. https://doi.org/10.1201/b10201

Wiegmann, B.M., Kim, J.-W. & Trautwein, M.D. (2009). Holometabolous insects (holometabola). In: Hedges, B. & Kumar, S. (eds) *The Timetree of Life.* (31). Oxford, UK: Oxford University Press. 260-263.

Williams, I.S. (1999). Slow-growth, high-mortality–a general hypothesis, or is it? *Ecol. Entomol.,* 24(4), 490-495. https://doi.org/10.1046/j.1365-2311.1999.00217.x

Woestmann, L. & Saastamoinen, M. (2016). The importance of trans-generational effects in Lepidoptera. *Curr. Zool.,* 62(5), 489-499. https://doi.org/10.1093/cz/zow029

Wright, G.A., Lillvis, J.L., Bray, H.J. & Mustard, J.A. (2012). Physiological state influences the social interactions of two honeybee nest mates. *PLoS One,* 7(3), e32677. https://doi.org/10.1371/journal.pone.0032677

Wright, G.A. & Schiestl, F.P. (2009). The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. *Funct. Ecol.,* 23(5), 841-851. https://doi.org/10.1111/j.1365-2435.2009.01627.x

Yin, J., Zhou, M., Lin, Z., Li, Q.Q. & Zhang, Y.Y. (2019). Transgenerational effects benefit offspring across diverse environments: a meta-analysis in plants and animals. *Ecol. Lett.,* 22(11), 1976-1986. https://doi.org/10.1111/ele.13373

Youngson, N.A. & Whitelaw, E. (2008). Transgenerational epigenetic effects. *Annu. Rev. Genomics Hum. Genet.,* 9, 233-257. https://doi.org/10.1146/annurev.genom.9.081307.164445

Youngsteadt, E., Nojima, S., Häberlein, C., Schulz, S. & Schal, C. (2008). Seed odor mediates an obligate ant–plant mutualism in Amazonian rainforests. *Proc. Natl. Acad. Sci. U.S.A.,* 105(12), 4571-4575. https://doi.org/10.1073/pnas.0708643105

Zakir, A., Bengtsson, M., Sadek, M.M., Hansson, B.S., Witzgall, P. & Anderson, P. (2013a). Specific response to herbivore-induced de novo synthesized plant volatiles provides reliable information for host plant selection in a moth. *J. Exp. Biol.,* 216(17), 3257-63. https://doi.org/10.1242/jeb.083188

Zakir, A., Sadek, M.M., Bengtsson, M., Hansson, B.S., Witzgall, P. & Anderson, P. (2013b). Herbivore-induced plant volatiles provide associational resistance against an ovipositing herbivore. *J. Ecol.,* 101(2), 410-417. https://doi.org/10.1111/1365-2745.12041

Zalucki, M.P., Clarke, A.R. & Malcolm, S.B. (2002). Ecology and behavior of first instar larval Lepidoptera. *Annu. Rev. Entomol.,* 47(1), 361-393. https://doi.org/10.1146/annurev.ento.47.091201.145220

Zeender, V., Sbilordo, S.H., Roy, J. & Lupold, S. (2023). Paternal condition affects offspring reproduction and life history in a sex-specific manner in *Drosophila melanogaster*. *Evolution,* 77(2), 467-481. https://doi.org/10.1093/evolut/qpac051

Zizzari, Z.V., van Straalen, N.M. & Ellers, J. (2016). Transgenerational effects of nutrition are different for sons and daughters. *J. Evol. Biol.,* 29(7), 1317-27. https://doi.org/10.1111/jeb.12872

Popular science summary

Herbivorous insects pose a serious threat towards food security by reducing the yield and quality in cropping systems. The Egyptian cotton leafworm, with its scientific name *Spodoptera littoralis*, is one such herbivorous insect that is considered a problem. From its native range in Africa, this moth has spread to Europe and is now considered a quarantine pest. Its generalist nature makes it able to develop on a wide range of unrelated plants from over 40 plant families with at least 87 plants of economic importance. The ability to find and feed on such a large variety of plant species makes *S. littoralis* interesting not only from an agricultural perspective but also from an evolutionary and ecological perspective.

Like most herbivorous insects, the adult *S. littoralis* utilizes odour cues released from plants to locate suitable egg-laying or mating sites. The location of a suitable plant is of great importance, specifically for egg-laying females, since offspring are heavily dependent on their mother to allocate them to a plant on which they can develop and thrive. Due to its ability to sustain itself on many different, but far from all, plants, *S. littoralis* is up for a daunting task when it comes to locating its potential hosts based on the widely different odour cues released from plants in its surroundings. As if this was not a hard task on its own, the odour cues released from the plants can change with e.g. the time of the day, temperature, and damage from insect herbivores chewing on them, further complicating host-plant location. In part I of this thesis, we investigated how changes in the plant odour, due to herbivory, influenced host-plant choice in *S. littoralis*. We found that herbivory changed the odours of plants in some but not all plant species tested. In the plant species where the odours changed, we found an influence on both male and female behaviour where damaged plants attracted males while females were deterred.

Interestingly, in *S. littoralis*, previous experience from its larval stage is maintained throughout metamorphosis as larvae undergo their transformation into winged adults. The experience can then be used by the adult stage to facilitate host-plant location, increasing the preference for the plant experienced during their larval stage. Lately, there has been an increasing interest from researchers in whether previous experience in the parental generation could influence offspring behaviour. In part II, we therefore investigated whether previous experience in the parental generation influences offspring host-plant choice. We found no evidence of parental experience influencing offspring behaviour, either in larva or adult individuals. Instead, offspring behaviour was shown to depend on an innate preference that could be influenced by their own previous host-plant experience.

Due to the ability to use previous experience from the larval stage of the insect to facilitate host-plant location in the adult, the chances of the offspring ending up on a similar plant as their parents increase. It is believed that parents could prepare their offspring for the environment which they have experienced, increasing the offspring's fitness in that type of environment. In part III of this thesis, we investigated whether parents could acclimatize their offspring to the host plant that they experienced, increasing the offspring's fitness on that type of host plant. We found that parents were able to acclimatize their offspring, producing offspring that were heavier when the offspring was reared on the same plant species as their parent. However, we found no evidence of such effects influencing offspring reproductive output but rather the opposite. Offspring that were put on a different plant than their parent showed an increased reproductive output. To disentangle the complex relationship between generalist insects and their surrounding plant community, future work with an emphasis on both intra- and transgenerational aspects is warranted.

Populärvetenskaplig sammanfattning

Växtätande insekter utgör ett allvarligt hot mot livsmedelsförsörjningen genom att reducera avkastning och kvalitet i våra odlingssystem. Det egyptiska bomullsflyet, vars vetenskapliga namn är *Spodoptera littoralis*, är just en sådan herbivor som anses vara ett problem. Från sin ursprungsplats i Afrika har denna nattfjäril spridit sig till Europa där den anses vara en karantänskadegörare. Dess generalistiska natur gör att den kan fullfölja sin livscykel på ett brett utbud av värdväxter från över 40 växtfamiljer med minst 87 växter av ekonomisk betydelse. Förmågan att hitta och utnyttja ett så brett spektrum av värdväxter gör *S. littoralis* intressant, inte bara ur ett agrikulturellt perspektiv, utan även ur ett evolutionärt och ekologiskt perspektiv.

Likt de flesta växtätande insekter använder *S. littoralis* sig av växtdofter för att hitta lämpliga äggläggnings- eller parningsplatser. Att hitta en lämplig värdväxt är av yttersta vikt, speciellt för honor, vars avkomma är i allra högsta grad beroende av att deras moder placerar dem på en växt där de kan utvecklas och frodas. Eftersom *S. littoralis* kan livnära sig på många olika, men långt ifrån alla, växter står den inför en svår uppgift när det gäller att hitta sina potentiella värdväxter baserat på de vitt skilda doftsignaler som växterna i dess omgivning avger. Som om detta inte vore en svår uppgift i sig, kan de doftämnen som frigörs från växterna förändras med t.ex. tiden på dygnet, temperaturen och skador som uppstår av bladtuggande herbivorer, vilket ytterligare försvårar lokaliseringen av värdväxten. I del I av denna avhandling undersökte vi hur förändringar i växtens doft, på grund av herbivori, påverkade valet av värdväxt. Vi fann att herbivori förändrade växternas doft hos vissa, men inte alla, testade växtarter. Hos de växtarter där dofterna förändrades fann vi även en påverkan på både hanars och honors

beteende där växter som blivit skadade av herbivorer attraherade hanar medan de avskräckte honor.

Intressant är att hos *S. littoralis* kan tidigare erfarenhet från larvstadiet bibehållas under metamorfosen där larverna omvandlas till bevingade vuxna. Denna erfarenhet kan därefter användas för att underlätta lokalisering av värdväxter hos den vuxna individen där preferensen ökar för den växten individen har erfarenhet av från larvstadiet. På senare tid har det funnits ett ökat intresse från forskare om huruvida tidigare erfarenheter i föräldragenerationen kan påverka avkommans beteende. Av denna anledning undersökte vi om värdväxterfarenhet i föräldragenerationen påverkade avkommans värdväxtval. Vi fann inga bevis för att föräldragenerationens erfarenhet påverkar avkommans beteende, varken hos larver eller vuxna individer. Istället visade det sig att avkommans beteende bygger på medfödda preferenser som kan påverkas av tidigare värdväxterfarenhet.

Genom att använda erfarenheter från insektens larvstadium för att underlätta lokalisering av värdväxter hos den vuxna individen, ökar chansen att avkomman hamnar på samma växtart som sina föräldrar. Föräldrar antas ibland kunna förbereda sin avkomma för den miljö som de själva har upplevt, vilket resulterar i att avkommans framgång ökar i den typen av miljö. I del III av denna avhandling undersökte vi om föräldrar kunde anpassa sin avkomma till ett liv på den värdväxt som de själva växte upp på, och på så sätt öka avkommans förmåga att klara sig på den typen av värdväxt. Vi fann att föräldrar kunde anpassa sin avkomma på så sätt att deras vikt ökade när de föddes upp på samma växtart som föräldern. Vi fann däremot inga bevis för att sådana effekter skulle påverka avkommans reproduktionsförmåga utan snarare tvärtom, där avkomma som placerades på en annan värdväxt än föräldern hade en högre reproduktiv förmåga. För att ta reda ut det komplexa förhållandet mellan generalistiska insektsarter och deras omgivande växtsamhällen är det motiverat att framtida forskning betonar aspekter både inom och över generationsgränsen.

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I'm out.

Appendix

Table A1. Reference list of plant-derived compounds electrophysiologically active (electroantennography or single sensillum recording) on *S. littoralis* antennae found in the odour samples. Table from supplementary material **paper I**.

Compound	References
α-pinene	e, g
myrcene	a, c, f, h, i, j, k
eucalyptol	a
(*Z*)-β-ocimene	a, c, h, k
(*E*)-β-ocimene	a, c, h, i, j, k
linalool	a, c, d, e, h, i, k
(*E*)-DMNT	a, c, d, i, j
(*Z*)-3-hexenyl acetate	a, c, d, h, i, j, k
(*Z*)-3-hexen-1-ol	a, c, d, i
methyl salicylate	a
benzaldehyde	b, d, h, j, k
(*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene	c, d, i
(*E,E*)-α-farnesene	c, i
indole	c, d, i
β-caryophyllene	c, d, e, i, j
α-humulene.	c, d, e, i, j
(*E*)-2-hexenal	c, d, e, i
β-pinene	c
nonanal	e, h, j, k
sabinene	f
3-carene	f
p-cymene	f, k
α-terpinolene	f
3-methylbutyl ethanoate	g
camphene	g
β-phellandrene	g

(*E*)-β-farnesene	g
phenylmethanol	a, h
limonene	j, k
hexan-1-ol	d, e, g
hexanal	a
α-phellandrene	i
6-methyl-5-hepten-2-one	a, g

Electrophysiologically-active compounds include compounds that have been shown to give an electrophysiological response reported in either single sensillum recording or electroantennography from *S. littoralis* antennae. References:

[a] Conchou, L., Anderson, P., & Birgersson, G. (2017). Host plant species differentiation in a polyphagous moth: olfaction is enough. J. Chem. Ecol., 43, 794-805.

[b] Anderson, P., Hilker, M., Hansson, B. S., Bombosch, S., Klein, B., & Schildknecht, H. (1993). Oviposition deterring components in larval frass of *Spodoptera littoralis* (Boisd.)(Lepidoptera: Noctuidae): a behavioural and electrophysiological evaluation. J. Insect Physiol., 39(2), 129-137.

[c] Jönsson, M., & Anderson, P. (1999). Electrophysiological response to herbivore-induced host plant volatiles in the moth *Spodoptera littoralis*. Physiol. Entomol., 24(4), 377-385.

[d] Binyameen, M., Anderson, P., Ignell, R., Seada, M. A., Hansson, B. S., & Schlyter, F. (2012). Spatial organization of antennal olfactory sensory neurons in the female *Spodoptera littoralis* moth: differences in sensitivity and temporal characteristics. Chem. Senses, 37(7), 613-629.

[e] Anderson, P., Hansson, B. S., & Löfqvist, J. (1995). Plant-odour-specific receptor neurones on the antennae of female and male *Spodoptera littoralis*. Physiol. Entomol., 20(3), 189-198.

[f] Binyameen, M., Hussain, A., Yousefi, F., Birgersson, G., & Schlyter, F. (2013). Modulation of reproductive behaviors by non-host volatiles in the polyphagous Egyptian cotton leafworm, *Spodoptera littoralis*. J. Chem. Ecol., 39, 1273-1283.

[g] Binyameen, M., Anderson, P., Ignell, R., Birgersson, G., Razaq, M., Shad, S. A., ... & Schlyter, F. (2014). Identification of plant semiochemicals and characterization of new olfactory sensory neuron types in a polyphagous pest moth, *Spodoptera littoralis*. Chem. Senses, 39(8), 719-733.

[h] Saveer, A. M., Kromann, S. H., Birgersson, G., Bengtsson, M., Lindblom, T., Balkenius, A., ... & Ignell, R. (2012). Floral to green: mating switches moth olfactory coding and preference. Proc. Royal Soc. B, 279(1737), 2314-2322.

[i] 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. J. Exp. Biol., 216(17), 3257-3263.

[j] Borrero-Echeverry, F., Becher, P. G., Birgersson, G., Bengtsson, M., Witzgall, P., & Saveer, A. M. (2015). Flight attraction of *Spodoptera littoralis* (Lepidoptera, Noctuidae) to cotton headspace and synthetic volatile blends. Front. Ecol. Evol., 3, 56.

[k] Kromann, S. H., Saveer, A. M., Binyameen, M., Bengtsson, M., Birgersson, G., Hansson, B. S., ... & Becher, P. G. (2015). Concurrent modulation of neuronal and behavioural olfactory responses to sex and host plant cues in a male moth. Proc. Royal Soc. B, 282(1799), 20141884.

II

Plant-Induced Transgenerational Plasticity Affecting Performance but Not Preference in a Polyphagous Moth

Axel Rösvik[1]*[†][‡], Patrick Lhomme[1,2][†][‡], Mohammed A. Khallaf[1,3][‡] and Peter Anderson[1][‡]

[1] Division of Chemical Ecology, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden, [2] Biodiversity and Crop Improvement Program, International Center of Agricultural Research in the Dry Areas, Rabat, Morocco, [3] Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Jena, Germany

Environmental variation experienced by a single genotype can induce phenotypic plasticity in various traits, such as behavioural, physiological and developmental characteristics. It can occur within the lifetime of an individual through within-generation phenotypic plasticity (WGP) or vertically across generations through transgenerational phenotypic plasticity (TGP). However, knowledge about TGP and the co-occurrence of WGP and TGP is still limited. In insect host-plant selection, the ability to alter phenotypic traits through WGP is well documented while the importance of TGP and the possible co-occurrence between the two is largely unknown. Host-plant selection of both larvae and adults of the polyphagous moth *Spodoptera littoralis* can be modified by previous experience through WGP. Thus, the aim of this study was to investigate if parental host-plant experience can influence host-plant choice behaviour and performance of *S. littoralis* offspring through TGP. For this, we tested effects of rearing parents on different host plants on the offspring's first instar larval migration and host plant choice, larval development and adult oviposition. A transgenerational effect on larval development was found, with increased pupal weight on a matching host-plant diet to that of the parent, when larvae were reared on cotton (good larval host plant) while no such effect was found on maize (poor larval host plant). These findings indicate that TGP of *S. littoralis* progeny development traits may only occur under favourable conditions. Parental diet did not affect larval host plant choice or migration. Furthermore, no effect of parental diet was found on offspring oviposition behaviour, indicating that adult female host-plant selection is governed by innate preference hierarchy and WGP, rather than TGP. Thus, parental diet may influence offspring performance but not behaviour, indicating that WGP is most important for host-plant selection behaviours in *S. littoralis*, but TGP can affect progeny development. If so, the importance of different types of plasticity may vary among traits of *S. littoralis* associated with host plant utilisation.

Keywords: within-generation phenotypic plasticity, transgenerational phenotypic plasticity, anticipatory plasticity, larval performance, insect behaviour, Lepidoptera, *Spodoptera littoralis*

INTRODUCTION

Phenotypic plasticity is the ability of individual genotypes to modify traits, such as physiological, morphological and behavioural characteristics, quickly in response to biotic and abiotic environmental variation (West-Eberhard, 1989; Agrawal, 2001a; Whitman and Agrawal, 2009). It can increase the fitness of individuals in their experienced environments (Lande, 2009), and may include changes in both behaviour and development within the lifetime of an organism. Such within-generation plasticity (WGP) has been observed in diverse taxa, and theoretical models of the phenomenon have been widely supported with empirical data (West-Eberhard, 1989; Lande, 2009). Furthermore, phenotypic traits can be transferred vertically across generations through transgenerational plasticity (TGP), a non-genetic process that has been described in various ways, e.g., as parental effects, maternal effects, paternal effects, non-genetic inheritance, epigenetic inheritance, and prenatal learning (Mousseau and Fox, 1998; Uller, 2008; Bonduriansky et al., 2012; Peralta Quesada and Schausberger, 2012). TGP has been documented in plants (Herman and Sultan, 2011), vertebrates (Salinas and Munch, 2012), and invertebrates (Mousseau and Fox, 1998). However, there is still much less evidence of TGP than WGP, and theoretical models of TGP are not well supported by empirical data. Thus, there are substantial gaps in knowledge of TGP's roles and importance (Bonduriansky et al., 2012). Some empirical support for TGP in morphological and physiological traits has been reported (Herman and Sultan, 2011; Donelson et al., 2018; Yin et al., 2019). For example, herbivory of *Raphanus raphanistrum* plants may increase their offspring's leaf trichome density (Agrawal et al., 1999; Agrawal, 2001b) and changes in defensive features, earlier maturation and increased reproductive output have been observed in progeny of *Daphnia* parents exposed to predator cues (Agrawal et al., 1999; Walsh et al., 2015). Effects of parental experience on offspring behaviour have also been found, for example, fear conditioning in mice (Dias and Ressler, 2014) and feeding preferences of predatory mites (Peralta Quesada and Schausberger, 2012). However, although behaviour is considered to show high phenotypic plasticity, and organisms' behavioural traits are often the first to change in response to environmental changes (West-Eberhard, 1989), effects of TGP on behaviour have received less attention.

Predictive models have indicated that both WGP and TGP are favoured by spatial and temporal environmental heterogeneity, environmental cues that are reliable predictors of upcoming environmental conditions, and low costs of plasticity (Uller, 2008; Bonduriansky et al., 2012; Dury and Wade, 2019). However, there are differences in the theoretical frameworks regarding the kinds of conditions that favour WGP, TGP and their possible co-occurrence. Studies on *Daphnia spp.* have supported the decoupling of WGP and TGP, indicating that selective pressures tend to favour either one or the other (Walsh et al., 2015, 2016). It has also been argued that if either WGP or TGP can optimise a trait there is no need for a combination of the two (Donelson et al., 2018). Contrarily, other models have suggested that WGP and TGP can co-exist and that information from environmental cues can be integrated for a specific phenotypic

trait (Leimar and McNamara, 2015). Empirical support for this theory has been provided, e.g., by indications of their co-occurrence in *Daphnia* defence mechanisms and development (Agrawal et al., 1999; Mikulski and Pijanowska, 2010) and drought adaptations of *Polygonium persicaria* (Sultan et al., 2009). Furthermore, it has been suggested that species with high ability to express WGP should also have high ability to express TGP (Woestmann and Saastamoinen, 2016), thus potentially favouring their co-occurrence.

Behavioural changes in foraging and host-finding induced by WGP have been well documented in various insects, including parasitoids (Turlings et al., 1993), honeybees (Menzel and Müller, 1996) and herbivores (Anderson and Anton, 2014). There is also evidence of insects' morphological and physiological traits being altered by TGP (Woestmann and Saastamoinen, 2016; Donelson et al., 2018). For example, parental exposure to UV light has been shown to affect wing coloration of offspring of the butterfly *Papilio polytes* (Katoh et al., 2018). Moreover, feeding on host plants of similar quality to plants their parents fed on has been found to promote development of offspring of both *Coenonympha pamphilus* and *Pieris rapae* (Cahenzli and Erhardt, 2013; Cahenzli et al., 2015). In addition, a study on the moth *Bicyclus anynana* showed that offspring preference for a synthetic odour was increased if the parents were reared on plant material coated with high doses of the same odour (Gowri et al., 2019). However, studies on effects of TGP on behaviour, particularly host-plant choice, using natural plant material are still lacking.

The Egyptian cotton leaf worm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), is a generalist phytophagous insect (Pogue, 2002), with an innate preference hierarchy for host plants that can be modified by WGP in both larval and adult stages. Larval host plant feeding experience has been found to induce a preference for the experienced host plant in later larval stages (Carlsson et al., 1999), and subsequent adult moth oviposition and mating behaviour (Anderson et al., 2013; Thöming et al., 2013; Proffit et al., 2015). Moreover, mating experience affects subsequent reproductive behaviour of both male and female adults (Proffit et al., 2015). As the importance of WGP for host plant choice in *S. littoralis* is well established it provides a good model to investigate the occurrence of TGP and possible interactions between, and co-occurrence of, WGP and TGP. Thus, the objective of this study was to investigate whether TGP, induced by parental experience to host plants, can affect preferences and performance of the species' offspring. First, we investigated whether first instar larval host-plant choice and migratory behaviour are influenced by parental diet. We then followed the performance of larvae reared on the parental host plant or a different plant in a cross-comparison experiment. Finally, we tested whether the oviposition preference of the offspring was influenced by the parental diet.

MATERIALS AND METHODS

Plants and Insects

Plants of three species – cotton (*Gossypium hirsutum*, cv. Delta Pineland 90, Malvaceae), cowpea (*Vigna unguiculata* subsp.

Unguiculata, Fabaceae) and maize (*Zea mays*, cv. Sweet Nugget, Poaceae) – were used in this study. They were cultivated until use (before flowering) in experiments in a commercial substrate (Kronmull, Weibull Trädgård AB, Hammenhög, Sweden) in 1.5 L pots for 5–6 weeks at $25 \pm 2°C$, $70 \pm 5\%$ RH in a greenhouse with artificial light provided by Osram Powerstar HQI-T, 400 W/D lamps in 16:8 h L:D cycles.

The rearing strain of *S. littoralis* was founded from moths collected in Alexandria, Egypt, in 2008 and has been regularly refreshed with new wild-collected specimens from Egypt. Larvae were fed a potato-based artificial diet (Hinks and Byers, 1976) and kept at $25 \pm 2°C$, RH $65 \pm 2\%$ with 17:7 h L:D cycles. Adults were kept at $25 \pm 2°C$, RH $50 \pm 2\%$ with 16:8 h L:D cycles. Adult males and females were separated at the pupal stage and kept separate until mating.

Adult Oviposition Preference Rearing Procedure

The hypothesis that parental experience of this moth may affect oviposition preference of the offspring was tested in a four-generation rearing experiment, as follows. First-generation (F0) insects were reared on the artificial diet. Resulting pupae were sexed and kept separate. Sugar solution was provided as an energy source for the merging adults. Two to three days after hatching, single couples were mated in the absence of plants (F0). Mating of all replicates was observed and directly after mating the females were introduced to a cage with cotton plants to lay eggs. The offspring was then fed on cotton and adult females were introduced to a cage with cotton plants to mate and lay eggs. This was repeated for three generations (F1–F3). Females of the fourth generation were introduced to either cages with cotton plants or in the absence of plants for mating and oviposition, creating two separate rearing lines. One, consisting of larvae from eggs laid on cotton, was kept on cotton for the fourth generation (designated F4 cotton) while larvae of the other line, from eggs laid in cages with no plants, were kept on artificial diet (F4 artificial diet).

Adult Oviposition Preference

In this experiment, the females were allowed to choose to lay eggs on either cotton or cowpea plants. Larval experience of feeding on cotton has been previously shown to induce a preference for cotton over the innately preferred cowpea (Thöming et al., 2013). Females that had not been exposed to plant odours as adults of F0, F1, F2, F3, F4 generations fed on cotton and F4 fed on artificial diet were mated with unexposed males from the same feeding background and put in mating cages (length and width 28 cm; height 29 cm, $N = 25$ per treatment) containing detached cotton and cowpea leaves in water-filled vials (diameter 2 cm, height 9 cm). Detached leaves were used because they have previously given comparable results to intact plants in preference experiments (Thöming et al., 2013). Females were left in the cages and were able to oviposit on the leaves for 3 days. Cages were checked for eggs on a daily basis and eggs oviposited on the plants were removed and weighed.

Rearing Procedure for Larval Behaviour and Performance Assays

Eggs produced by the parental generation (F0) reared on artificial diet were collected and placed in plastic boxes (width 24 cm, height 7 cm, depth 18 cm) until hatching. Hatched larvae were randomly divided into two groups, one of which was fed on detached maize leaves and the other on detached cotton leaves. The plants were chosen partly because third and fourth instar larvae have different innate preferences for them, and partly because they differ in suitability as hosts, with cotton being considered a good host and maize a poor host (Anderson et al. unpublished data). Larvae were reared in groups of 60 individuals. Males and females were separated at the pupal stage, then after emergence males and females were transferred to a mating cage where mating occurred in the presence of the larval host plant. Eggs deposited on the plants were then removed and left to hatch in plastic boxes with no plant material.

Larval Host-Plant Choice Assay

Naïve first instar larvae (F1), from parents reared on either cotton ($N = 318$) or maize ($N = 326$), were placed in Petri dishes (diameter 8.5 cm, height 1.5 cm), each containing a maize leaf disc and a cotton leaf disc (both 0.5 cm diameter). Leaf discs were placed 5 cm apart in diametric opposition. To avoid positional effects, alternate replicates were rotated at 180° with respect to the others, so the leaf discs had different orientations. Since *S. littoralis* is known to use olfactory cues for host-plant identification (Salloum et al., 2011), the first choice was noted when a larva had oriented towards one of the leaf discs and touched it. Twenty-four batches of thirty randomly selected larvae were used in the tests, to ensure that the behaviour could be successfully observed. Larvae that did not make a choice within 4 h were excluded from further analysis.

Migration Assay

Naïve first instar larvae (F1) from parents reared on either cotton or maize were put in boxes (length 24 cm, width 18 cm, height 7 cm) containing cotton and maize leaves placed in water-filled vials (diameter 2 cm, height 9 cm). The larvae were put on either the plant that their parent was reared on or the other one. This resulted in four possible combinations, designated cotton × cotton, maize × maize, maize × cotton and cotton × maize, where the first and second plants are those that the parents and offspring were reared on, respectively. There were 20 replicates of each combination except cotton-maize (19) and 20 larvae in each replicate. To avoid positional effects, alternate replicates were rotated at 180° with respect to the others, so the leaves had different orientations. After 72 h, the number of larvae present on each plant was counted. Larvae that were not present on any of the plants were excluded from the experiment.

Performance Assay

Individual first instar larvae (F1), from parents fed on cotton or maize, were transferred to individual plastic cups (30 ml) containing either cotton or maize, creating four possible parent-offspring combinations (maize × maize, maize × cotton, cotton × maize, cotton × cotton: $N = 80$ in each case). Food

was provided *ad libitum* during their entire development, and their mortality, larval development time (period from hatching to pupation) and pupal weight were recorded. The larvae were checked daily during the later larval instars to see if pupation had occurred, and pupae were weighed 24 h after pupation.

Statistics

A preference index, based on the total egg weight oviposited on the two plants by each female in the adult oviposition preference assay, was defined as follows:

$$Adult\ oviposition\ preference\ index$$
$$= \frac{(egg\ weight\ on\ cotton\ -\ egg\ weight\ on\ cowpea)}{(total\ egg\ weight)}$$

The index ranges from 1 (absolute preference for cotton) to −1 (absolute preference for cowpea), with 0 indicating no preference. Larval migration was calculated as the percentage of larvae that migrated from one plant to the other:

$$Larval\ migration = \frac{(migrated\ larvae)}{(total\ number\ of\ larvae)}$$

This variable ranges from 0 (no migration) to 1 (migration of all larvae).

The Wilcoxon signed rank test was used to test the significance of between-treatment differences in non-parametric datasets, such as the female oviposition preference, larval migration and development time values (which did not satisfy the normal distribution null hypothesis of the Shapiro-Wilk test, at $P = 0.009$, $P < 0.001$ and $P = 0.003$, respectively). *Post hoc* pairwise comparisons were conducted using Dunn's test as implemented in the R package dunn.test (Dinno, 2017), with Bonferroni correction for the larval migration and development time. In the female oviposition bioassay, the response of the first generation (F0) was compared to the response of every other generation (F1–F4). To compensate for mass-significance, the *P*-values were multiplied by the number of relevant comparisons. A chi-square test was performed to assess the significance of preference differences in the larval host plant choice bioassay.

As pupal weight data obtained in the performance bioassay were not normally distributed (according to the Shapiro-Wilk test; $P < 0.001$) they were subjected to square-root transformation, then fitted using a linear model. Differences in pupal weight were analysed using ANOVA, with *post hoc* (Tukey's HSD) pairwise comparisons implemented using the *glht* function in the multicomp R package (Hothorn et al., 2008).

A chi-square test was applied to assess the significance of between-treatment differences in mortality rates in the performance bioassay. In all tests, $P < 0.05$ were considered statistically significant. All statistical analyses were performed using R statistical software version 3.6.1 (R Core Team, 2019), and figures were created with the software packages ggplot2 version 3.2.1 (Wickham, 2016) and ggpubr version 0.3.0 (Kassambara, 2020).
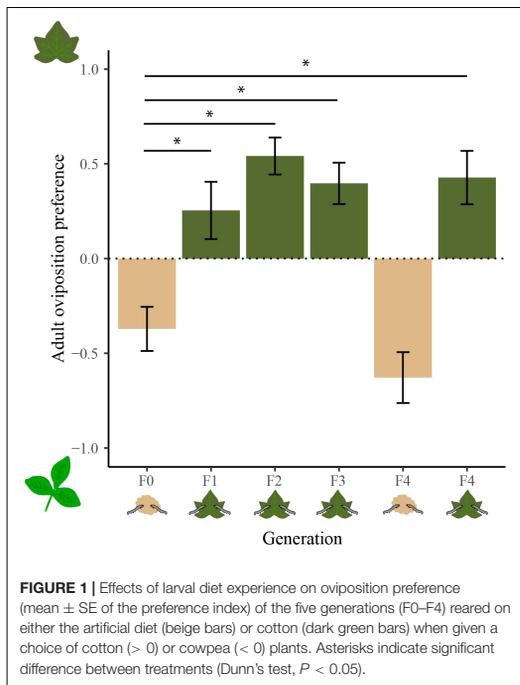

FIGURE 1 | Effects of larval diet experience on oviposition preference (mean ± SE of the preference index) of the five generations (F0–F4) reared on either the artificial diet (beige bars) or cotton (dark green bars) when given a choice of cotton (> 0) or cowpea (< 0) plants. Asterisks indicate significant difference between treatments (Dunn's test, $P < 0.05$).
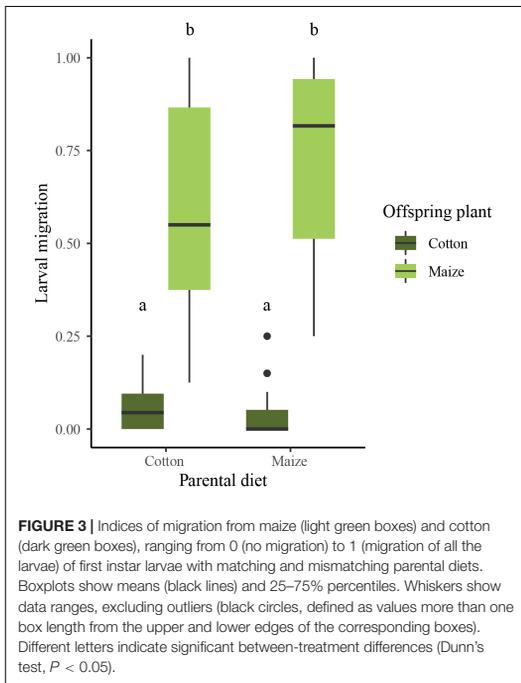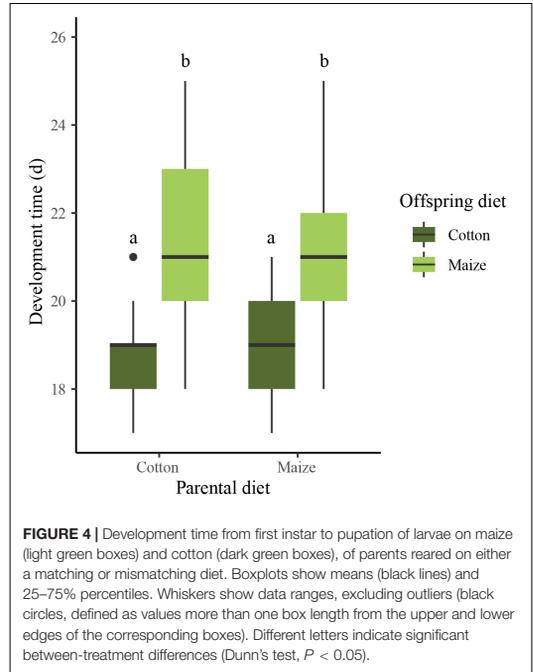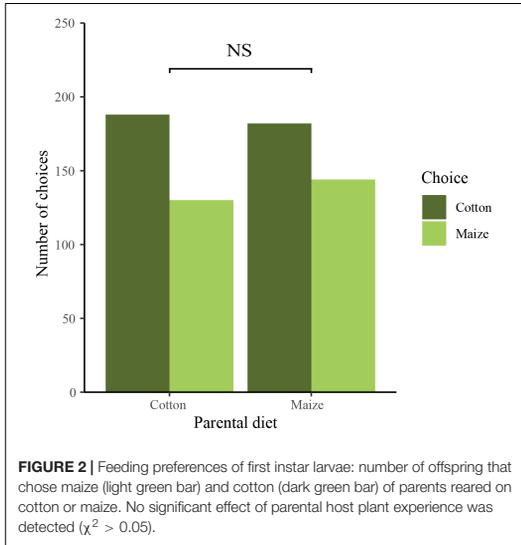
RESULTS

Adult Oviposition Preference

We detected significant between-treatment differences in adults' host plant oviposition preferences (Wilcoxon signed rank test, $Z = -14.456$, $P < 0.001$; **Figure 1**). These included differences between the generation (F0) reared on the artificial diet and subsequent generations (F1–F4) reared on cotton ($N = 25$ in each case, Dunn's test: $Z = -2.884$, $P = 0.019$; $Z = -3.749$, $P < 0.001$; $Z = -2.950$, $P = 0.016$; and $Z = -3.470$, $P = 0.003$). In contrast, no difference in oviposition preference was found between the F0 and F4 generations reared on artificial diet ($N = 25$; $Z = 1.240$, $P = 1$).

Larval Host Plant Choice

Parental host plant experience had no significant effect on offspring host plant choice [$\chi^2(1) = 0.585$, $P = 0.444$; **Figure 2**]. Offspring from parents reared on cotton ($N = 318$) choose maize 41% and cotton 59% of the time while offspring from parents reared on maize ($N = 326$) choose maize 44% and cotton 56% of the time.

Larval Migration

We detected between-treatment differences in migratory behaviour of first instar larvae (Wilcoxon rank sum test, $Z = -6.782$, $P < 0.001$; **Figure 3**). Larvae placed on maize migrated more frequently [on average 73% migration in the
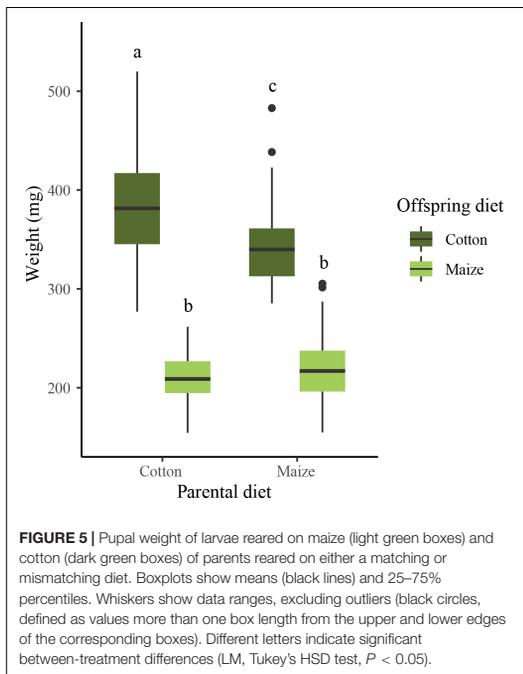
FIGURE 2 | Feeding preferences of first instar larvae: number of offspring that chose maize (light green bar) and cotton (dark green bar) of parents reared on cotton or maize. No significant effect of parental host plant experience was detected ($\chi^2 > 0.05$).

FIGURE 3 | Indices of migration from maize (light green boxes) and cotton (dark green boxes), ranging from 0 (no migration) to 1 (migration of all the larvae) of first instar larvae with matching and mismatching parental diets. Boxplots show means (black lines) and 25–75% percentiles. Whiskers show data ranges, excluding outliers (black circles, defined as values more than one box length from the upper and lower edges of the corresponding boxes). Different letters indicate significant between-treatment differences (Dunn's test, $P < 0.05$).

FIGURE 4 | Development time from first instar to pupation of larvae on maize (light green boxes) and cotton (dark green boxes), of parents reared on either a matching or mismatching diet. Boxplots show means (black lines) and 25–75% percentiles. Whiskers show data ranges, excluding outliers (black circles, defined as values more than one box length from the upper and lower edges of the corresponding boxes). Different letters indicate significant between-treatment differences (Dunn's test, $P < 0.05$).

($N = 20$)]. This applied to offspring of parents reared on both maize (maize × cotton versus maize × maize, Dunn's test, $Z = -5.783$, $P < 0.001$) and cotton (cotton × cotton versus cotton × maize, Dunn's test, $Z = -4.896$, $P < 0.001$). However, the parental diet had no effect on the migratory behaviour of offspring larvae (cotton × cotton versus maize × cotton, Dunn's test, $Z = 0.069$, $P = 1$; maize × maize versus cotton × maize, Dunn's test, $Z = -0.744$, $P = 1$).

Development Time

Development time from first larval instar to pupation differed between the treatments (Wilcoxon rank sum test, $Z = -21.6$, $P < 0.001$; **Figure 4**). Offspring on cotton developed faster than offspring on maize independently of parental diet [maize × cotton ($N = 79$) versus maize × maize ($N = 78$), Dunn's test, $Z = -7.154$, $P < 0.001$; cotton × cotton ($N = 77$) versus cotton × maize ($N = 76$), Dunn's test, $Z = -9.678$, $P < 0.001$]. The parental diet did not affect the development time when offspring were reared on cotton (cotton × cotton versus maize × cotton, Dunn's test, $Z = -1.884$, $P = 0.179$) or maize (maize × maize, cotton × maize, Dunn's test, $Z = 0.752$, $P = 1$).

Pupal Weight

Results of the larval performance bioassay showed that pupal weight differed between the treatments (LM, $F = 389$, $df = 3/306$, $P < 0.001$; **Figure 5**). Larvae reared on cotton had a higher pupal weight than larvae reared on maize, independently of

maize × maize treatment ($N = 20$) and 59% migration in the cotton × maize treatment ($N = 20$)] than those placed on cotton [on average 4.5% migration in the maize × cotton treatment ($N = 20$) and 5% migration in the cotton × cotton treatment

FIGURE 5 | Pupal weight of larvae reared on maize (light green boxes) and cotton (dark green boxes) of parents reared on either a matching or mismatching diet. Boxplots show means (black lines) and 25–75% percentiles. Whiskers show data ranges, excluding outliers (black circles, defined as values more than one box length from the upper and lower edges of the corresponding boxes). Different letters indicate significant between-treatment differences (LM, Tukey's HSD test, $P < 0.05$).

parental diet (maize × cotton versus maize × maize, $t = -20.925$, $P < 0.001$; cotton × cotton versus cotton × maize, $t = -26.889$, $P < 0.001$). Offspring reared on cotton with a matching parental diet had a higher pupal weight than offspring reared on cotton with mismatching parental diet (cotton × cotton versus maize × cotton, $t = -4.919$, $P < 0.001$). In contrast, no effect of parental diet on pupal weight was detected in offspring reared on maize (cotton × maize versus maize × maize, $t = 1.365$, $P = 0.523$).

Mortality

No difference in mortality rate was detected between the treatments: $\chi^2(3) = 2.06$, $P = 0.560$. Offspring reared on cotton with matching (cotton × cotton) and mismatching (maize × cotton) parental diets had mortality rates of 3.75 and 1.25%, respectively. Offspring reared on maize with matching (maize × maize) and mismatching (cotton × maize) parental diets had mortality rates of 2.5 and 5%, respectively.

DISCUSSION

In this study we found transgenerational effects of parental diet on larval development, but not the behaviour of progeny larvae or adults of *S. littoralis*. Larvae reared on cotton from parents reared on the same diet reached a higher pupal weight than larvae from parents reared on maize, but there was no difference in their development time. The difference observed between offspring reared on cotton with matching and

mismatching parental diets could be explained by anticipatory transgenerational effects affecting offspring weight, e.g., through epigenetic modulations (Glastad et al., 2019) or vertically transferred symbionts (Paniagua Voirol et al., 2018). Increases in the fitness of offspring relative to parental fitness under matching conditions has been predicted in theoretical studies (Uller et al., 2013; Engqvist and Reinhold, 2016) and detected in both plants (Herman and Sultan, 2011) and vertebrates (Salinas and Munch, 2012). Anticipatory transgenerational effects have also been observed in invertebrates, including findings that offspring of the lepidopterans *Pieris rapae* and *Coenonympha pamphilus* developed best on food with the same nitrogen content as food that the parental generation had received (Rotem et al., 2003; Cahenzli and Erhardt, 2013). However, in our study a positive effect on offspring development was only found when offspring and parent diet was matched on the good host plant cotton, as no increase on offspring larval weight was found for offspring reared on maize with parents on a matching diet compared to the mismatching diet. When reared on a suboptimal host plant, the stressful environment limits the resources available for the parental generation and could reduce means of cue transfer through TGP to subsequent generations, thereby limiting the adaptive adjustment of the offspring (Uller et al., 2013). Effects of host plant quality have been observed in maritime pine, as Vivas et al. (2013) found that offspring of parents reared in benign conditions had higher pathogen resistance and growth rates than individuals grown in less favourable conditions. Furthermore, we have in *S. littoralis* found that on high quality food, larval olfactory experience is transferred to the adult through WGP and affect host plant choice while this does not occur on low quality food (Lhomme et al., 2018). Another possible explanation to the results could be silver spoon effects that allow parents from benign environments to give their offspring a heads start in life through transmission of abundant resources that would increase fitness independent of the environment of the offspring (Bonduriansky et al., 2012; Uller et al., 2013; Engqvist and Reinhold, 2016). In our study, we would expect that a silver spoon effect should increase the weight of offspring from parents reared on cotton irrespective of the larval diet, but we only found this effect on progeny that were fed cotton and not on those fed maize. Negative carry-over effects could potentially also be involved, we would then expect detrimental effects on the development of larvae with parents fed on maize. However, we detected no difference in pupal weight of offspring reared on maize related to the parental diet. Thus, we found no clear evidence for either silver spoon or carry-over effects.

The oviposition experiments revealed no transgenerational effects, as cotton versus cowpea preferences did not differ between females reared on the artificial diet after three generations on cotton and females of the first generation with no experience of plants during the larval stage. If transgenerational effects had influenced the oviposition preference, the females transferred to the artificial diet after three generations on cotton should have had a stronger preference for cotton than the first generation reared on the artificial diet. The oviposition results corroborate findings from our earlier studies that host plant selection of *S. littoralis* relies on an innate preference hierarchy

between host plants that is modified through WGP, where larval feeding experience induces a preference for the experienced plant (Thöming et al., 2013).

The migration assay clearly showed that there was a difference in the behaviour of the larvae on the two host plants. First instar larvae that were placed on maize migrated towards cotton at a much higher extent compared to the number of larvae migrating from cotton to maize. This is most likely due to that cotton is a more suitable host plant for larval development than maize (Anderson et al. unpublished data). The lower food quality of maize could induce larval movement and increase their search for an alternative host plant. A difference in migration behaviour has also been shown for larger larvae of *S. littoralis*, where more larvae were found to leave damaged cotton plants with induced defence than undamaged plants (Anderson et al., 2011). However, although *S. littoralis* larvae may actively migrate from less suitable plants, and WGP can strongly influence the species' feeding preference (Carlsson et al., 1999; Salloum et al., 2011), we detected no effect of parental diet on migration behaviour of first instar larvae, or larval host-plant choice.

Recent genetic and mathematical models of WGP, TGP and their possible coexistence predict that the two types of plasticity can operate either separately or additively, depending on the environmental conditions (Lande, 2009; Ezard et al., 2014; Leimar and McNamara, 2015; Dury and Wade, 2019). Empirical support for this has been found in both plants and animals (Agrawal et al., 1999; Sultan et al., 2009; Mikulski and Pijanowska, 2010; Walsh et al., 2015, 2016; Katoh et al., 2018). The experiments reported here provided no evidence of TGP affecting the behaviour of *S. littoralis*, supporting the hypothesis that the two types of plasticity operate separately for a specific trait, as postulated by Walsh et al. (2015, 2016). Theoretical models of WGP, TGP and their possible co-occurrence indicate that WGP is the dominating type of plasticity (Kuijper and Hoyle, 2015; Leimar and McNamara, 2015). However, the developmental data obtained in this study show that transgenerational effects may be present in *S. littoralis* when environmental conditions are favourable, and there may be interactions between WGP and TGP under these conditions. Thus, WGP and TGP could potentially influence specific traits connected to host plant utilisation either separately or additively.

WGP may influence behavioural choices of *S. littoralis* more strongly than TGP because the associated cues are temporally closer to them than parental cues, and more accurate predictors of current availabilities and qualities of host plants (Kuijper and Hoyle, 2015). We have previously identified a sensitive period in the late larval instar of *S. littoralis* in which larval host plant experience modifies subsequent adult behavioural decisions, while early larval experience is not retained (Lhomme et al., in press). Thus, the information is gathered close to the adult stage, and the decision mechanism takes into account factors that affect larval development. Such WGP increases the salience of the previously experienced plant, reduces risks of mismatching conditions in the ovipositing female's environment, and could make the transfer of parental experience through TGP redundant (Donelson et al., 2018). Furthermore, the host plant choice is under maternal control and she will likely lay her eggs

on the same plant (through WGP), thereby reducing selective pressures favouring the evolution of TGP-mediated effects on the behaviour of offspring larvae. However, under such conditions, a transgenerational transfer of cues that increases the ability of the progeny to develop on that specific plant species could be very valuable and promote TGP of such traits.

In conclusion, this study suggests that TGP may modify the physiological state of *S. littoralis* offspring in a manner that enhances their performance on the parental host plant. However, such enhancement may only occur when conditions are favourable. The results also indicate that host plant-mediated behaviours of both adult females and first instar larvae are strongly influenced by innate preferences and WGP, but not by the parental diet. However, mechanisms underlying the higher pupal weight of offspring reared on cotton with a matching parental diet are still unknown, and further studies on anticipatory TGP, WGP and their possible co-occurrence in *S. littoralis* focused on these mechanisms in both favourable and unfavourable conditions are warranted.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

AR, PA, PL, and MK designed the study. AR, PL, and MK collected and analysed the data. AR wrote the manuscript together with PL and PA. PA obtained the funding. All authors contributed to revisions.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Agrawal, A. A. (2001a). Phenotypic plasticity in the interactions and evolution of species. *Science* 294, 321–326. doi: 10.1126/science.1060701

Agrawal, A. A. (2001b). Transgenerational consequences of plant responses to herbivory: an adaptive maternal effect? *Am. Natural.* 157, 555–569. doi: 10.1086/319932

Agrawal, A. A., Laforsch, C., and Tollrian, R. (1999). Transgenerational induction of defences in animals and plants. *Nature* 401, 60–63. doi: 10.1038/43425

Anderson, P., and Anton, S. (2014). Experience-based modulation of behavioural responses to plant volatiles and other sensory cues in insect herbivores. *Plant Cell Environ.* 37, 1826–1835. doi: 10.1111/pce.12342

Anderson, P., Sadek, M. M., Larsson, M., Hansson, B. S., and Thöming, G. (2013). Larval host plant experience modulates both mate finding and oviposition choice in a moth. *Anim. Behav.* 85, 1169–1175. doi: 10.1016/j.anbehav.2013.03.002

Anderson, P., Sadek, M. M., and Wackers, F. L. (2011). Root herbivory affects oviposition and feeding behavior of a foliar herbivore. *Behav. Ecol.* 22, 1272–1277. doi: 10.1093/beheco/arr124

Bonduriansky, R., Crean, A. J., and Day, T. (2012). The implications of nongenetic inheritance for evolution in changing environments. *Evol. Appl.* 5, 192–201. doi: 10.1111/j.1752-4571.2011.00213.x

Cahenzli, F., and Erhardt, A. (2013). Transgenerational acclimatization in an herbivore-host plant relationship. *Proc. R. Soc. Lond. B Biol. Sci.* 280:2856. doi: 10.1098/rspb.2012.2856

Cahenzli, F., Wenk, B. A., and Erhardt, A. (2015). Female butterflies adapt and allocate their progeny to the host-plant quality of their own larval experience. *Ecology* 96, 1966–1973. doi: 10.1890/14-1275.1

Carlsson, M. A., Anderson, P., Hartlieb, E., and Hansson, B. S. (1999). Experience-dependent modification of orientational response to olfactory cues in larvae of *Spodoptera littoralis*. *J. Chem. Ecol.* 25, 2445–2454. doi: 10.1023/A:1020865922827

Dias, B. G., and Ressler, K. J. (2014). Parental olfactory experience influences behavior and neural structure in subsequent generations. *Nat. Neurosci.* 17, 89–96. doi: 10.1038/nn.3594

Dinno, A. (2017). *Dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums*. Available online at: https://CRAN.R-project.org/package=dunn.test (accessed June 1, 2020).

Donelson, J. M., Salinas, S., Munday, P. L., and Shama, L. N. (2018). Transgenerational plasticity and climate change experiments: where do we go from here? *Glob. Chang. Biol.* 24, 13–34. doi: 10.1111/gcb.13903

Dury, G. J., and Wade, M. J. (2019). When mother knows best: a population genetic model of transgenerational versus intragenerational plasticity. *J. Evol. Biol.* 33, 127–137. doi: 10.1111/jeb.13545

Engqvist, L., and Reinhold, K. (2016). Adaptive trans-generational phenotypic plasticity and the lack of an experimental control in reciprocal match/mismatch experiments. *Methods Ecol. Evol.* 7, 1482–1488. doi: 10.1111/2041-210x.12618

Ezard, T. H. G., Prizak, R., Hoyle, R. B., and Marshall, D. (2014). The fitness costs of adaptation via phenotypic plasticity and maternal effects. *Funct. Ecol.* 28, 693–701. doi: 10.1111/1365-2435.12207

Glastad, K. M., Hunt, B. G., and Goodisman, M. A. D. (2019). Epigenetics in insects: genome regulation and the generation of phenotypic diversity. *Annu. Rev. Entomol.* 64, 185–203. doi: 10.1146/annurev-ento-011118-111914

Gowri, V., Dion, E., Viswanath, A., Piel, F. M., and Monteiro, A. (2019). Transgenerational inheritance of learned preferences for novel host plant odors in *Bicyclus anynana* butterflies. *Evolution* 73, 2401–2414. doi: 10.1111/evo.13861

Herman, J. J., and Sultan, S. E. (2011). Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. *Front. Plant Sci.* 2:102. doi: 10.3389/fpls.2011.00102

Hinks, C., and Byers, J. (1976). Biosystematics of the genus Euxoa (Lepidoptera: Noctuidae): V. rearing procedures, and life cycles of 36 species. *Can. Entomol.* 108, 1345–1357. doi: 10.4039/Ent1081345-12

Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometr. J.* 50, 346–363. doi: 10.1002/bimj.200810425

Kassambara, A. (2020). *ggpubr: 'ggplot2' Based Publication Ready Plots*. Available online at: https://rpkgs.datanovia.com/ggpubr/ (accessed June 1, 2020).

Katoh, M., Tatsuta, H., and Tsuji, K. (2018). Ultraviolet exposure has an epigenetic effect on a Batesian mimetic trait in the butterfly *Papilio polytes*. *Sci. Rep.* 8:31738. doi: 10.1038/s41598-018-31732-31738

Kuijper, B., and Hoyle, R. B. (2015). When to rely on maternal effects and when on phenotypic plasticity? *Evolution* 69, 950–968. doi: 10.1111/evo.12635

Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *J. Evolut. Biol.* 22, 1435–1446. doi: 10.1111/j.1420-9101.2009.01754.x

Leimar, O., and McNamara, J. M. (2015). The evolution of transgenerational integration of information in heterogeneous environments. *Am. Natural.* 185, E55–E69. doi: 10.1086/679575

Lhomme, P., Carrasco, D., Larsson, M., Hansson, B., and Anderson, P. (2018). A context-dependent induction of natal habitat preference in a generalist herbivorous insect. *Behav. Ecol.* 29, 360–367. doi: 10.1093/beheco/arx173

Lhomme, P., Khallaf, M., Larsson, M., and Anderson P. (in press). Sensitive period for the induction of host plant preference in a generalist herbivorous insect. *Anim. Behav.*

Menzel, R., and Müller, U. (1996). Learning and memory in honeybees: from behavior to neural substrates. *Annu. Rev. Neurosci.* 19, 379–404. doi: 10.1146/annurev.ne.19.030196.002115

Mikulski, A., and Pijanowska, J. (2010). When and how can Daphnia prepare their offspring for the threat of predation? *Hydrobiologia* 643, 21–26. doi: 10.1007/s10750-010-0131-130

Mousseau, T. A., and Fox, C. W. (1998). *Maternal Effects AS Adaptations*. New York, NY: Oxford University Press.

Paniagua Voirol, L. R., Frago, E., Kaltenpoth, M., Hilker, M., and Fatouros, N. E. (2018). Bacterial symbionts in lepidoptera: their diversity, transmission, and impact on the host. *Front. Microbiol.* 9:556. doi: 10.3389/fmicb.2018.00556

Peralta Quesada, P. C., and Schausberger, P. (2012). Prenatal chemosensory learning by the predatory mite *Neoseiulus californicus*. *PLoS One* 7:e53229. doi: 10.1371/journal.pone.0053229

Pogue, M. G. (2002). A world revision of the genus Spodoptera Guenée:(Lepidoptera: Noctuidae). *Memoirs Am. Entomol. Soc.* 43, 1–202.

Proffit, M., Khallaf, M. A., Carrasco, D., Larsson, M. C., and Anderson, P. (2015). 'Do you remember the first time?' Host plant preference in a moth is modulated by experiences during larval feeding and adult mating. *Ecol. Lett.* 18, 365–374. doi: 10.1111/ele.12419

R Core Team (2019). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Rotem, K., Agrawal, A. A., and Kott, L. (2003). Parental effects in *Pieris rapae* in response to variation in food quality: adaptive plasticity across generations? *Ecol. Entomol.* 28, 211–218. doi: 10.1046/j.1365-2311.2003.00507.x

Salinas, S., and Munch, S. B. (2012). Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. *Ecol. Lett.* 15, 159–163. doi: 10.1111/j.1461-0248.2011.01721.x

Salloum, A., Colson, V., and Marion-Poll, F. (2011). Appetitive and Aversive Learning in Spodoptera littoralis Larvae. *Chem. Senses* 36, 725–731. doi: 10.1093/chemse/bjr041

Sultan, S. E., Barton, K., and Wilczek, A. M. (2009). Contrasting patterns of transgenerational plasticity in ecologically distinct congeners. *Ecology* 90, 1831–1839. doi: 10.1890/08-1064.1

Thöming, G., Larsson, M. C., Hansson, B. S., and Anderson, P. (2013). Comparison of plant preference hierarchies of male and female moths and the impact of larval rearing hosts. *Ecology* 94, 1744–1752. doi: 10.1890/12-0907.1

Turlings, T. C. L., Wäckers, F. L., Vet, L. E. M., Lewis, W. J., and Tumlinson, J. H. (1993). "Learning of host-finding cues by hymenopterous parasitoids," in *Insect Learning: Ecological and Evolutionary Perspectives*, eds D. R. Papaj and A. C. Lewis (Boston, MA: Springer), 51–78. doi: 10.1007/978-1-4615-2814-2_3

Uller, T. (2008). Developmental plasticity and the evolution of parental effects. *Trends Ecol. Evol.* 23, 432–438. doi: 10.1016/j.tree.2008.04.005

Uller, T., Nakagawa, S., and English, S. (2013). Weak evidence for anticipatory parental effects in plants and animals. *J. Evolut. Biol.* 26, 2161–2170. doi: 10.1111/jeb.12212

Vivas, M., Zas, R., Sampedro, L., and Solla, A. (2013). Environmental maternal effects mediate the resistance of maritime pine to biotic stress. *PLoS One* 8:e70148. doi: 10.1371/journal.pone.0070148

Walsh, M. R., Castoe, T., Holmes, J., Packer, M., Biles, K., Walsh, M., et al. (2016). Local adaptation in transgenerational responses to predators. *Proc. R. Soc. B Biol. Sci.* 283:2271. doi: 10.1098/rspb.2015.2271

Walsh, M. R., Cooley, F., Biles, K., and Munch, S. B. (2015). Predator-induced phenotypic plasticity within-and across-generations: a challenge for theory? *Proc. R. Soc. B Biol. Sci.* 282:2205. doi: 10.1098/rspb.2014.2205

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* 20, 249–278. doi: 10.1146/annurev.es.20.110189.001341

Whitman, D. W., and Agrawal, A. A. (2009). "What is phenotypic plasticity and why is it important," in *Phenotypic Plasticity Of Insects: Mechanisms And Consequences*, 1st Edn, ed. D. Whitman (Boca Raton: CRC Press), 1–63.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer-Verlag.

Woestmann, L., and Saastamoinen, M. (2016). The importance of trans-generational effects in *Lepidoptera*. *Curr. Zool.* 62, 489–499. doi: 10.1093/cz/zow029

Yin, J., Zhou, M., Lin, Z., Li, Q. Q., and Zhang, Y. Y. (2019). Transgenerational effects benefit offspring across diverse environments: a meta-analysis in plants and animals. *Ecol. Lett.* 22, 1976–1986. doi: 10.1111/ele.13373

Insect herbivores and plants have an intricate relationship where the herbivore must be able to locate their host plants in complex environments. This thesis investigates host-plant choice and its influence on behaviour and life-history traits in a transgenerational context in the generalist herbivore *Spodoptera littoralis*.

Axel Rösvik completed his graduate education at the Department of Plant Protection Biology, SLU, Alnarp. He received his Master of Science in Biology from SLU, Alnarp.

Acta Universitatis agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

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