Plant priming of anti-herbivore defence by insect oviposition: Dynamics and fitness consequences

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Affidavit

I hereby declare that my thesis, entitled *Plant priming of anti-herbivore defence by insect oviposition: Dynamics and fitness consequences*, is the result of my own work. I did not receive any help or support from commercial consultants. All sources and/or materials applied in this thesis are listed and specified. Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

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Part I

Main Part

1 Abstract

1.1 Summary

Plants evolved elaborate strategies to cope with the ubiquitous threat posed by herbivorous insects. Beside constitutive or induced defence mechanisms, priming of plant defences describes a strategy by which the plants improve their defence response upon perceiving a previous stimulus indicative of the stress. For instance, insect egg deposition can serve as stimuli for the plant which indicate future herbivory, as numerous herbivorous insects deposit their eggs directly on the future host plant of their larvae. Indeed, various plant species improve their defence against herbivorous larvae when they previously perceived insect egg deposition as a priming signal. However, knowledge of the signalling and mechanisms which facilitate such an enhanced anti-herbivore defence in oviposited plants is scarce. Therefore, this dissertation aims to further investigate the temporal dynamics of the activation of different signalling pathways as well as the fitness consequences of oviposition-mediated priming of anti-herbivore defences in two solanaceous plant species in interaction with generalist and specialist lepidopteran herbivores.
Within the first part, responses of the bittersweet nightshade (*Solanum dulcamara*) to oviposition and / or larval feeding by the beet armyworm *Spodoptera exigua* and the leaf mining specialist *Acrolepia autumnitella* were examined to investigate the largely unknown temporal dynamics of the primed state.
Firstly, the accumulation of phytohormones and associated transcripts of defence-related genes in oviposited (primed) leaves were compared to those of untreated control leaves at different time points within and after the natural egg incubation time, as well as a time point matching the time of larval hatching. The so far undescribed phytohormonal and transcriptional responses of *S. dulcamara* to oviposition by *A. autumnitella* were largely similar to those of *S. exigua* oviposition. The induction of salicylic acid (SA) by oviposition was restricted to the period of egg exposure, while a differentially transcriptional induction in oviposited leaves was detectable for at least ten days after oviposition, i.e. six days after egg removal. Interestingly, jasmonic acid (JA) and jasmonic acid-isoleucine (JA-Ile), highly important phytohormones for the induction of defence mechanisms against chewing herbivores, were induced in small quantities within and consistently after period of egg exposure, which could indicate for a preparation of the defence response against the hatching larvae.

Consequently, the next aim was to investigate if oviposition-mediated defence priming alters responses to the feeding larvae during the beginning of the larval attack. Therefore, full-factorial experiments with oviposition by *S. exigua* (priming stimulus) and a short phase of herbivory or simulated herbivory (triggering stimulus) were conducted, while both stimuli were applied on different but vascular fully-connected leaves. During the onset of the response, oviposition and natural/simulated herbivory caused an additive effect on the accumulation of abscisic acid (ABA). Moreover, the primed jasmonate induction in oviposited plants in response to natural or simulated herbivory suggests an earlier or faster induction which could entail a more effective defence against the larvae. Furthermore, transcriptional results suggest an involvement of cytokinins in oviposition-mediated priming, which could further point to an important role of the phytohormonal interplay for a primed defence induction.

Defence priming is postulated as adaptive strategy, however, actual knowledge regarding the effect of oviposition priming on the plant fitness is largely missing. Consequently, the aim of the second part of this thesis was to examine the fitness consequences of oviposition priming for the annual plant *Nicotiana attenuata* in interaction with the generalist herbivore *S. exigua* and the tobacco specialist *Manduca sexta*.

Therefore, full factorial priming experiments with both herbivores were conducted which assessed the growth (stalk length) and fitness (flowering, number of capsules and seed weight) of oviposited and non-oviposited plants, induced and non-induced by natural or simulated herbivory. Larval feeding by both herbivores and associated induced defence caused a clearly diminished growth and fitness of *N. attenuata*. Oviposition by both herbivores without subsequent larval feeding had no effect on growth or plant fitness, indicating that fitness incurs as a consequence of the onset and maintenance of the primed state are minimal in occasions when herbivory does not occur. Consistent with the diminished larval performance of *S. exigua* on prior oviposited plants, the fitness loss due to larval feeding by *S. exigua* was slightly smaller for oviposited plants in terms of capsule and seed production. Benefits of oviposition priming likely lie in the decline of fitness losses due to herbivory. In contrast, the fitness loss due to herbivory by *M. sexta* was not affected by prior oviposition, but in this interaction also the larval performance is not impaired. The induced defence, triggered by simulated herbivory without leaf tissue loss, had only an effect on flowering but not on the reproductive output. Oviposition in combination with simulated herbivory, i.e. a higher primed defence induction, had no further negative effect on fitness of *N. attenuata*. Probably fit-

ness consequences of oviposition priming are mainly influenced by the effect of the primed defence induction on the herbivore and associated lower leaf tissue loss than due to physiological costs caused by a higher defence induction.
Interestingly, *M. sexta* herbivory on *N. attenuata* is also known to induce tolerance responses, such as transient carbon allocation to the roots that could enhance the ability to regrow after the herbivore threat is gone. To assess if such induced tolerance mechanisms are enhanced by oviposition-mediated priming, experiments including oviposition and / or larval feeding by *M. sexta* followed by a removal of all aboveground plant parts were conducted to observe the fitness of regrown plants. Interestingly, the fitness of regrown plants was enhanced if plants were exposed to oviposition in combination with subsequent larval feeding before defoliation, which suggests that oviposition priming affects tolerance responses. As the physiological state of a plant changes during plant development, which is assumed to affect the inducibility of defence and tolerance responses such as carbon reallocation, further experiments with plants in different developmental stages were conducted. Young rosette and matured flowering plants were exposed to the similar experimental setup as early elongating plants in the previous experiments. Varying effects of larval feeding and prior oviposition on the plant fitness of regrown plants in the distinctive developmental stages, suggest that the ability to regrow and the enhancing effect of prior oviposition follow a developmental pattern.
Overall, this doctoral thesis highlights the involvement of different phytohormones in the context of oviposition-mediated defence priming. Within an elaborate phytohormonal interplay involving salicylic acid, absicic acid, jasmonates facilitate an earlier or faster response to larval feeding, which may enable the oviposited plant to mount a more effective defence. Furthermore, this thesis indicates that oviposition-mediated defence priming may not just be beneficial for plant fitness if the defence is effective against the herbivore but additionally by increasing tolerance responses to larval feeding.

1.2 Zusammenfassung

Pflanzen haben im Laufe der Evolution ausgefeilte Strategien entwickelt, um unter der allgegenwärtigen Bedrohung durch pflanzenfressende Insekten zu bestehen. Neben konstitutiven oder induzierten pflanzlichen Abwehrmechanismen versteht man unter „*Priming*“ der Pflanzenabwehr eine Strategie, die besagt das Pflanzen ihre Abwehrreaktion gegen Herbivore verbessern, wenn sie einen früheren Stimulus wahrnehmen der auf den folgenden Stress hinweist. Beispielsweise kann die Eiablage von Insekten als ein Stimulus für die Pflanze dienen, der auf zukünftige Herbivorie hinweist, da zahlreiche pflanzenfressende Insekten ihre Eier direkt auf der zukünftigen Wirtspflanze ihrer Larven ablegen. In der Tat zeigen verschiedene Pflanzenarten eine verbesserte Abwehr gegen pflanzenfressende Larven, wenn sie zuvor eine Eiablage erlebt haben (im Folgenden bezeichnet als „*Eiablage-Priming*“). Das Wissen über die involvierte Signalübermittlung und Mechanismen, die eine solche verbesserte Verteidigung gegen Herbivore in eierbelegten Pflanzen ermöglichen, ist jedoch spärlich. Ziel dieser Dissertation ist es daher, die zeitliche Dynamik der Aktivierung verschiedener Signalwege sowie die Konsequenzen für die pflanzliche Fitness in Folge des Eiablage-Primings in zwei Nachtschattengewächsen (Solanaceae) in Interaktion mit generalistischen und spezialisierten Herbivoren (Lepidopteren) zu untersuchen.
Im ersten Teil der Dissertation wurden die Reaktionen des Bittersüßen Nachtschattens (*Solanum dulcamara*) auf Eiablage und / oder Larvenfraß durch die Zuckerrübeneule (*Spodoptera exigua*) und des spezialisierten Blattminierer *Acrolepia autumnitella* untersucht, um die weitgehend unbekannte zeitliche Dynamik des vorbereiteten Zustands nach der Eiablage zu untersuchen.
Zunächst wurde die Akkumulation von Phytohormonen und damit assoziierten Transkripten abwehrrelevanter Gene in eierbelegten Blättern mit denen von unbehandelten Kontrollblättern zu verschiedenen Zeitpunkten verglichen. Diese umfassten Zeitpunkte innerhalb und nach der natürlichen Inkubationszeit der Eier, sowie einen Zeitpunkt, der dem Zeitpunkt des Larvenschlupfes entsprach. Die bisher unbeschriebenen phytohormonalen und transkriptionellen Reaktionen von *S. dulcamara* auf die Eiablage durch *A. autumnitella* waren denen der Eiablage von *S. exigua* weitgehend ähnlich. Die Induktion von Salicylsäure (SA) durch die Eiablage war auf den Zeitraum der Ei-Exposition beschränkt, während eine differentielle Transkriptionsinduktion in eibelegten Blättern mindestens zehn Tage nach der Eiablage, d.h. sechs Tage nach der Entfernung der Eier, nachweisbar war. Interessanterweise wurden Jasmonsäure (JA) und Jasmonsäure-Isoleucin (JA-Ile),

Phytohormone mit zentralen Funktionen in der Induktion von Abwehrmechanismen gegen kauende Herbivore, in geringen Mengen innerhalb und durchgehend nach der Phase der Ei-Exposition induziert, was auf eine Vorbereitung der Abwehrreaktion gegen die schlüpfenden Larven hinweisen könnte.
Das nächste Ziel war daher, zu untersuchen ob sich durch eine vorherige Eiablage und die damit verbundene gesteigerte Abwehrreaktion die Reaktionen auf die fressenden Larven zu Beginn des Angriffes verändert. Daher wurden vollfaktorielle Experimente mit Eiablage durch *S. exigua* (Priming-Stimulus) und einer kurzen Phase von Larvenfraß oder simulierten Fraß (Auslösungs-Stimulus) durchgeführt, wobei beide Stimuli auf verschiedene, aber vaskulär voll verbundene Blätter appliziert wurden. Zu Beginn der Reaktion verursachten Eiablage und natürlicher / simulierter Larvenfraß einen additiven Effekt auf die Akkumulation von Abscisinsäure (ABA). Darüber hinaus deutet eine höhere Induktion von Jasmonaten in eierbelegten Pflanzen, in Reaktion auf natürlichen oder simulierten Larvenfraß, auf eine frühere oder schnellere Induktion hin, die eine wirksamere Abwehr gegen die Larven zur Folge haben könnte. Des weiteren deuten Ergebnisse auf der transkriptionellen Ebene auf eine Beteiligung von Cytokininen an der Ei-vermittelten gesteigerten Abwehrreaktion nach Fraß hin, was die Bedeutung des phytohormonalen Zusammenspiels für den Mechanismus des Eiablage-Primings unterstreicht.
Die gesteigerte pflanzliche Abwehrreaktion nach einer Eiablage im Rahmen des Eiablage-Primings wird als adaptive Strategie postuliert, jedoch fehlen weitgehend Nachweise über die Auswirkung dieser Strategie auf die pflanzliche Fitness. Daher war es Ziel des zweiten Teils dieser Dissertation, die Konsequenzen der gesteigerten pflanzlichen Abwehrreaktion nach einer Eiablage für die Fitness der einjährigen Pflanze *Nicotiana attenuata* in Interaktion mit dem Generalisten *S. exigua* und dem spezialisierten Tabakschwärmer (*Manduca sexta*) zu untersuchen.
Dafür wurden vollfaktorielle Experimente mit beiden Herbivorenarten durchgeführt, in denen das Wachstum (Stiellänge) und die pflanzliche Fitness (Blüte, Anzahl der Kapseln und Samengewicht) gemessen wurde. In diesen Experimenten wurden eierbelegten und eifreien Pflanzen miteinander verglichen, die zudem durch natürlichen / simulierten Larvenfraß induzierten wurden oder uninduziert blieben. Larvenfraß beider Arten und die damit verbundene induzierte Abwehr verursachten ein deutlich vermindertes Wachstum und eine deutlich verringerte pflanzliche Fitness von *N. attenuata*. Die alleinige Eiablage durch Herbivoren beider Arten ohne anschließenden Larvenfraß, hatte keinen Einfluss auf das Wachstum oder die pflanzliche Fitness. Dies weist darauf hin, dass die Fitness durch die

gesteigerte pflanzliche Abwehrreaktion infolge der Eiablage in Fällen, in denen keine Pflanzenfresser auftreten, minimal ist. In Übereinstimmung mit der verminderten und verzögerten Entwicklung von *S. exigua* Larven auf zuvor eierbelegten Pflanzen (Effekt des Eiablage-Primings auf *S. exigua*), war der Fitnessverlust aufgrund des Larvenfraßes durch *S. exigua* bei eibelegten Pflanzen in Bezug auf die Kapsel- und Samenproduktion leicht geringer. Die Vorteile des Eiablage-Primings liegen wahrscheinlich in der Reduzierung der Fitnessverluste, die durch die Herbivorie hervorgerufen werden. Im Gegensatz dazu sind die Fitnessverluste die durch Herbivorie von *M. sexta* hervorgerufen wurden nicht durch eine vorherige Eiablage beeinflusst, während in dieser Interaktion auch die Entwicklung der Larven durch eine erhöhte Abwehrreaktion auf zuvor eierbelegten Pflanzen nicht beeinträchtigt wird. Die induzierte Abwehr, ausgelöst durch simulierte Herbivorie ohne Verlust des Blattgewebes, hatte nur einen Einfluss auf die Anzahl der Blüten, nicht jedoch auf die Fortpflanzungsparameter der Pflanze. Eine vorherige Eiablage in Kombination mit simulierter Herbivorie, d.h. eine erhöhte Abwehrinduktion aufgrund der vorherigen Eiablage, hatte keinen zusätzlichen Effekt auf die Fitness von *N. attenuata*. Wahrscheinlich werden die Fitness-Konsequenzen des Eiablage-Primings hauptsächlich durch die Wirkung der gesteigerten Abwehrreaktion auf den Herbivor, d.h. dem damit verbundenen geringeren Verlust an Blattgewebe, beeinflusst als durch physiologische Kosten die durch eine höhere Abwehrinduktion verursacht werden.

Interessanterweise werden in *N. attenuata* durch Larvenfraß von *M. sexta* neben Abwehrmechanismen auch Toleranzreaktionen induziert, wie z.B. eine vorübergehende Umverteilung von Kohlenstoff-Assimilaten zu den Wurzeln. Eine solche Umverteilung könnte die Fähigkeit zum Nachwachsen der Pflanze verbessern, nachdem die Bedrohung durch den Herbivor verschwunden ist. Um zu untersuchen, ob solche induzierten Toleranzmechanismen durch Eiablage-Priming verstärkt werden, wurden weitere Experimente durchgeführt. In diesen wurden die Pflanzen einer Eiablage und / oder Larvenfraß durch *M. sexta* ausgesetzt, gefolgt von einer Entfernung aller oberirdischen Pflanzenteile, um die Fitness der Nachwachsen Pflanzen zu begutachten. Interessanterweise war die Fitness der nachgewachsenen Pflanzen verbessert, wenn die Pflanzen vor dem Rückschnitt einer Eiablage in Kombination mit anschließenden Larvenfraß ausgesetzt waren, was darauf hindeutet, dass das Eiablage-Priming die Toleranzreaktionen von *N. attenuata* beeinflusst. Da sich der physiologische Zustand einer Pflanze im Laufe der Entwicklung ändert, was wahrscheinlich ebenfalls die induzierten pflanzlichen Abwehr- und Toleranzreaktionen wie die Assimilatumverteilung beeinflusst, wurden weitere

Experimente mit einem Rückschnitt der oberirdischen Pflanzenteile mit Pflanzen in verschiedenen Entwicklungsstadien durchgeführt. Junge Rosetten-Pflanzen und ausgewachsene Blütenpflanzen wurden einem ähnlichen Versuchsaufbau ausgesetzt wie in den vorhergehenden Versuchen. Unterschiedliche Auswirkungen des Larvenfraßes und der vorherigen Eiablage auf die Fitness der nachgewachsener Pflanzen in den unterschiedlichen Entwicklungsstadien legen nahe, dass die Fähigkeit zum Nachwachsen und verbessernde Wirkung einer vorherigen Eiablage einem Entwicklungsmuster folgen.
Insgesamt unterstreicht diese Dissertation die Beteiligung verschiedener Phytohormone in der Signalübermittlung einer gesteigerten pflanzliche Abwehrreaktion durch vorherige Eiablage. Mittels eines ausgeklügelten phytohormonalen Zusammenspiels ermöglichen Salicylsäure, Absicinsäure und Jasmonaten eine frühere oder schnellere Reaktion auf Herbivorie, wodurch die eierbelegte Pflanze wahrscheinlich eine effektivere Abwehr aufbauen kann. Darüber hinaus zeigt diese Dissertation, dass Eiablage-Priming nicht nur für die pflanzliche Fitness von Vorteil sein kann, wenn diese Ei-vermittelte gesteigerte Abwehrreaktion gegen den Herbivor wirksam ist, sondern zusätzlich durch die Erhöhung von Toleranzreaktionen in Reaktion auf Larvenfraß.

2 Introduction

2.1 Insect herbivory

Like all organisms, plants are in constant interaction with their physical environment and various other organisms. These interactions can be favourable or unfavourable for the plant, whereby factors which lead to unfavourable conditions constitute stress factors for the plant (Jones et al., 2012). Particularly due to their sessile lifestyle, plants are challenged to either take advantage of favourable conditions or to cope with unfavourable ones. Especially biotic interactions are challenging, as they comprise two or more dynamically interacting organisms which may react to each other in diverse ways resulting in a unilateral or bilateral favourable respectively unfavourable interaction (although this classification is not always straightforward) (van Dam, 2009). Well-known examples for a bilateral beneficial interaction are plant-pollinator interactions (Faegri and Van Der Pijl, 2013). However, other biotic interactions are detrimental for the plant. As plants represent by far the biggest biomass on earth (Bar-On et al., 2018), it is not surprising that the herbivorous lifestyle, i.e. exploiting plant material as source of nutrients, is common among the other kingdoms of life.

Herbivory by insects represents a major biotic stress factor for plants, as almost every plant species is getting fed upon by at least one of over 400 000 herbivorous insect species (Fürstenberg-Hägg et al., 2013; Schoonhoven et al., 2005). Herbivorous insects use various feeding strategies to obtain nutrients from all above- and belowground plant parts (Howe and Jander, 2008).

The often close relationship between host plant and insect herbivores has given rise to the co-evolutionary theory, which proposes that herbivory on plants has been a determining factor in increasing species diversity in both herbivores and hosts (Ehrlich and Raven, 1964). During plant-insect coevolution, insects developed different feeding modes, which can be classified into chewing-biting (mandibulate insects; e.g. folivore herbivores like caterpillars, beetles and grasshoppers) or piercing-sucking (haustellate insects; e.g. cell-sucking or sap-feeding herbivores like trips or spider mites) (Schoonhoven et al., 2005). Many chewing-biting insects ingest relatively large chunks of leaf material while piercing-sucking herbivores suck the liquid content from lateral cells (Fürstenberg-Hägg et al., 2013; Schoonhoven et al., 2005). Furthermore, herbivorous insects can life on the outside or inside their host plant. For example, leaf miners live and feed during their

larval stage between the upper and lower epidermis of a leaf blade and feed from parenchymal tissues (Fürstenberg-Hägg et al., 2013). Another categorization of herbivorous insects can be made based on their degree of dietary specialization. Species can be considered as monophagous (limited to feed from one or a few closely related plant taxa), oligophagous (feed on various plant species usually within one botanical family), or polyphagous (feed on plants of different families) (Ali and Agrawal, 2012; Fürstenberg-Hägg et al., 2013; Schoonhoven et al., 2005). In addition, it is often more convenient to distinguish between specialist (monophagous and oligophagous species) and generalist (polyphagous species) herbivores, albeit specialist herbivores are more common than generalist herbivores (Ali and Agrawal, 2012; Fürstenberg-Hägg et al., 2013; Schoonhoven et al., 2005; Howe and Jander, 2008). However, it has to be considered that in reality the distribution of insects from mono- to polyphagous feeding is a continuum (Ali and Agrawal, 2012).

2.2 Plant responses to insect herbivory

Plants attacked by herbivores are anything but vulnerable and passive victims at the bottom of the food chain. To withstand herbivory and to ensure their survival, plants possess an effective resistance system which can imply a combination of physical, chemical and developmental features (Schoonhoven et al., 2005; Howe and Jander, 2008). On the one hand, countermeasures against herbivores can comprise mechanisms or traits which allow the infested plant to reduce the degree of how much the plant is affected by the herbivore damage or even compensate the herbivore damage, here referred as plant tolerance to herbivory (see 2.2.1). On the other hand, plants can target the herbivore by mechanisms or traits which aim to repel, harm, or poison the herbivorous insect to prevent or reduce the inflicted damage, here defined as defence against herbivory (see 2.2.2). Albeit tolerance to and defence against herbivory are not mutually exclusive, most plant-insect interactions likely combine both strategies (Núñez-Farfán et al., 2007; Carmona and Fornoni, 2013). In addition to mounting defence or tolerance responses upon encounter the herbivore, plants may already prepare their defence upon stimuli indicating a high probability of herbivore attack, referred as priming of plant defence (see 2.2.4). Nevertheless, herbivory and also mounded plant responses to deal with herbivory cause distinct effects on plant fitness (see 2.2.3).

2.2.1 Plant tolerance to herbivory

One countermeasure of the plant to withstand herbivory could be to induce or activate mechanisms or traits which enable the plant to tolerate herbivory. Plant tolerance to herbivory is referred as the ability of plants to minimise or buffer fitness losses of damage without directly affecting the herbivore (Strauss and Agrawal, 1999; Heil, 2010). A term closely related to tolerance is compensation, which refers to the degree of tolerance observed: If (otherwise phenotypic similar) damaged and undamaged plants have the same fitness, then the damaged plant compensates fully for herbivory (Strauss and Agrawal, 1999). If damaged plants have a greater fitness than their undamaged relatives, these plants have overcompensated and if they have a lower fitness, they have undercompensated for herbivory (Strauss and Agrawal, 1999). In this context, a central difficulty becomes obvious as tolerance can only be quantified in a comparative manner by examining the fitness of plants affected by herbivory in contrast to the fitness of plants in the undamaged state (Strauss and Agrawal, 1999; Heil, 2010; Stowe et al., 2000). Whereas overcompensation is obvious as soon as herbivory increases rather than decreases plant fitness, it is difficult to determine effects attributed to tolerance if the fitness of invested plants is lower than the fitness of uninfested plants, as one has to quantify what fitness would be expected without the tolerance mechanism (Heil, 2010; Stowe et al., 2000). Even when growth and/or reproduction of damaged individuals appear equivalent to that of undamaged plants, tolerance may be overestimated because e.g. the quality of reproduction (i.e. seed viability, seedling survivorship and/or seed output) could differ (Stowe et al., 2000).
But how is tolerance to herbivory achieved? The ability to tolerate herbivory has a heritable basis and consequently tolerance can evolve in natural plant populations (Strauss and Agrawal, 1999). At the physiological level, different mechanisms can be involved in causing an increased tolerance to herbivory: increased photosynthetic activity, increased (compensatory) growth (e.g. branching, or tilling after release of apical dominance), storage in belowground tissues (allocation of photoassimilates toward roots or higher root:shoot-ratio), mobilisation of stored reserves, activation of dormant meristems and related changes in allocation patterns, altered flowering phenology (Strauss and Agrawal, 1999; Tiffin, 2000). Through an induced or enhanced allocation and storage of resources like photoassimilates in belowground tissues upon herbivory, these resources would be diverted away from the attacked part of the plant and stored for regrowth processes when the threat has passed (Schwachtje et al., 2006). Such an allocation toward roots upon induction has been confirmed in several plant species from various families, including

tomato (*Solanum lycopersicum*; Gómez et al. (2010, 2012)), *A. thaliana* (Ferrieri et al., 2013), maize (*Zea mays*; Holland et al. (1996); Robert et al. (2012, 2014)), barley (*Hordeum vulgare*; Henkes et al. (2008)), poplar (*Populus* spp.; Babst et al. (2005, 2008)), *Nicotiana attenuata* (Schwachtje et al., 2006) and *Nicotiana tabacum* (Kaplan et al., 2008). However, the molecular basis of plant tolerance to herbivory remains poorly understood (Erb and Reymond, 2019).

2.2.2 Plant defence strategies

Other countermeasures of plants to withstand herbivory can involve plant traits or mechanisms which aim to reduce encounters with the herbivore to prevent or reduce the inflicted damage and thereby minimize the negative impact of herbivory, referred to as plant defence against herbivores (Fürstenberg-Hägg et al., 2013; Howe and Jander, 2008; Erb, 2018b). Within over 300 million years of co-evolution with insect herbivores, plants evolved an elaborate spectrum of effective defence strategies to avert insect herbivory (Fürstenberg-Hägg et al., 2013). These defensive mechanisms or traits either aim to affect the behaviour of the herbivore (e.g. host plant selection, oviposition, feeding behaviour) or to decrease their performance (e.g. growth rate, development, reproductive success) (Schaller, 2008). Plants can defend themselves by mechanisms that directly and negatively affect herbivore growth, reproduction, or fecundity (direct defence), for example through the formation of physical barriers (mechanical defence) like thorns, trichomes and cuticles (Howe and Jander, 2008; Wu and Baldwin, 2010). Moreover, plants rely on chemical defences based on a tremendous number of compounds and metabolites that exert repellent, antinutritive or toxic effects on herbivores (Mithöfer and Boland, 2012; Fürstenberg-Hägg et al., 2013). These compounds comprise among others terpenoids, alkaloids, glucosinolates, phenolics or polypeptides (Mithöfer and Boland, 2012; Fürstenberg-Hägg et al., 2013). In addition to these direct defences, plants can also attract, nourish or house members of the third trophic level (i.e. predators or parasitoids of herbivores) to reduce enemy pressure, a mechanism referred to as indirect defence (Fürstenberg-Hägg et al., 2013; Heil, 2008). This attraction is achieved for example by emission of volatile organic compounds (VOCs) or by provision of extrafloral nectar, food bodies, nesting or refuge sites (Fürstenberg-Hägg et al., 2013; Heil, 2008). Another crucial aspect of defence is the timing: Some defence mechanisms are expressed constitutively, irrespective of the herbivore threat level, while inducible defences are mounted only after plants are attacked by herbivores (Fürstenberg-Hägg et al., 2013; Howe and Jander, 2008;

Wu and Baldwin, 2010). A major disadvantage of induced defences compared to constitutive defences is that attacked plants experience a window of vulnerability during the time that it takes until defences are activated and effective against the herbivore (Karban, 2011; Cipollini et al., 2003). However, the different defence strategies are not mutually exclusive, as different strategies can dynamically occur within the same plant at the same time (Núñez-Farfán et al., 2007; Carmona and Fornoni, 2013).

2.2.3 Fitness consequences of herbivory

Plant fitness is defined as the contribution of a plant to the gene pool of the next generation, which is not only represented by the number of offspring produced by an individual, but also by the survival and fecundity of the offspring (Stowe et al., 2000; Erb, 2018a). On the one hand, the fitness of a plant depends on external factors, i.e. environmental conditions like for example nutrient, light, and water availability (Steppuhn and Baldwin, 2008). On the other hand, fitness is affected by internal factors, like different physiological capacities (e.g. resource uptake rates, photosynthetic rates, and metabolic efficiency) and the ability to maintain these under stress conditions (Steppuhn and Baldwin, 2008).
Herbivory and plant responses to herbivory can have positive (i.e. benefits) as well as negative effects (i.e. costs) on plant fitness and ultimately, in order to cope with herbivory effectively, plants need to balance these costs and benefits. Fitness costs associated with plant responses to herbivory are assumed to be a driving force behind the evolution of inducible defences (Simms and Rausher, 1987). The negative impact of herbivory is obvious as herbivory reduces the leaf area and associated production of assimilates, disrupt tissue connectivity or even cause death. Contrary, responses to herbivory like defence or tolerance help the plant to survive in the presence of herbivores and reduce the impact of herbivory which increases the plant fitness (Vos et al., 2013a). But production or activation of defences or tolerance mechanisms comes at a price. In first place defensive traits or tolerance mechanism demand metabolic costs, i.e. energy and resources for the production and maintenance of the traits as well as for the machinery involved in synthesis, modification, transport and maintenance or storage (Gershenzon, 1994; Züst and Agrawal, 2017). Furthermore, associated costs can form fitness penalties like for example trade-offs with other plant functions (Vos et al., 2013a; Züst and Agrawal, 2017). Such allocation costs occur when fitness-limiting resources are tied in defences and consequently not available for growth or reproduction (Heil

and Baldwin, 2002; Strauss and Agrawal, 1999; Züst and Agrawal, 2017). Mainly focusing on these allocation costs, the commonly invoked cost-benefit theory of plant resistance assumes that resistance traits are costly for the plant (Simms and Rausher, 1987; Harvell, 1990). However, diverse linkages among various plant responses to herbivory (e.g. crosstalk between signalling cascades which fine-tune the metabolism) suggests that a more dynamic view of the costs and benefits of anti-herbivore defence mechanisms would be more appropriate (Steppuhn and Baldwin, 2008; Vos et al., 2013a). Furthermore, an emerging consensus suggests that negative associations between for example growth and defence not as the direct result of allocation costs, but rather as prioritization of one process over another (Stanton et al., 2013; Huot et al., 2014; Campos et al., 2016; Kliebenstein, 2016). Trade-offs may more often reflect the range of trait combinations that achieve optimal fitness rather than representing strict physiological limits (Züst and Agrawal, 2017).
Further costs of anti-herbivore defence mechanisms on the physiological level can comprise for example autotoxicity costs, when induced compounds are toxic to the plant itself (Heil and Baldwin, 2002; Strauss et al., 2002). Costs can also be observed more indirectly on the ecological level, as defences may negatively affect interactions of plants with their environment (Steppuhn and Baldwin, 2008). Additional, more indirect, costs occur when considering evolutionary aspects, as frequently or consistently deployed defences may provide a stronger selection pressure for herbivores to evolve counter resistance (Steppuhn and Baldwin, 2008). However, ecological and evolutionary costs are undetectable if plants are examined outside of their natural environment as these costs manifest themselves only through interactions with the biotic and abiotic environment of a plant (Simms et al., 1992; Züst and Agrawal, 2017). Due to these different costs associated with defence or tolerance traits, inducibility of these traits in an environment with unpredictable and variable herbivore pressure is assumed to allow plants to forgo these costs when countermeasures are unnecessary, i.e. when herbivores are not present (Baldwin, 1998; Zavala et al., 2004a; Schoonhoven et al., 2005).

2.2.4 Priming of plant defence

In addition to defence or tolerance responses to herbivory, plants have evolved adaptive strategies to optimize such stress responses to herbivory. For example, plants can perceive environmental stimuli that reliably indicate a probable stress and prime their stress response in advance in order to positively affect

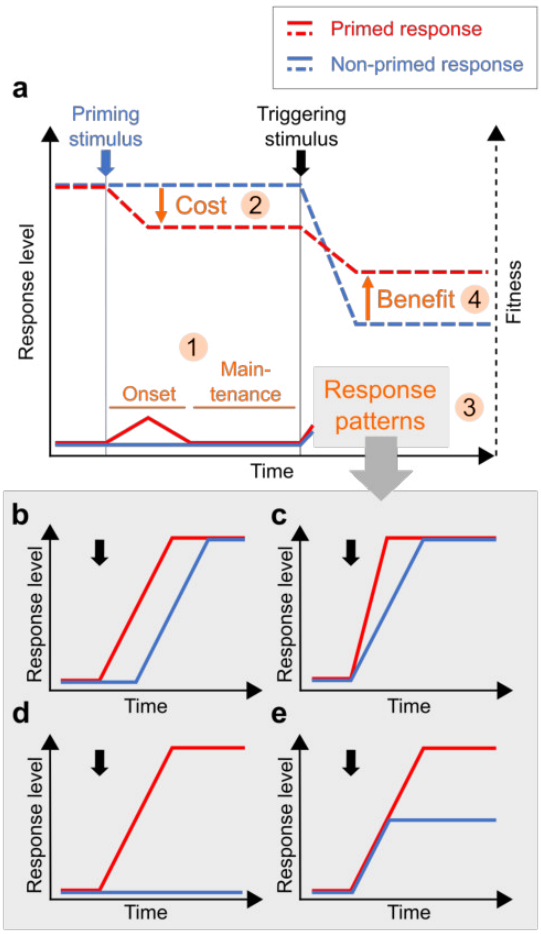

Figure 1: Characteristics of defence priming in a scheme of the relation between defence responses (solid lines) and fitness (dashed lines) of primed (red lines) and non-primed (blue lines) plants. (1) Memory: In response to the priming stimulus (blue arrow) the plant is transferred in the primed state and the information about the priming experience is stored during the lag phase. (2) Low costs: Establishing and maintaining the primed state is expected to cause modest costs. (3) Altered stress response: In response to the stress (triggering stimulus, black arrow) the plant that experienced a priming stimulus exhibits a primed stress response. The response of the primed plant could be earlier (B), faster (C), more sensitive (D) or stronger (E) compared to the response of a non-primed plant. (4) Fitness benefit: Due to the more efficient defence, priming is expected to enhance fitness of primed and triggered plants (adapted from Hilker et al. (2016) and Martinez-Medina et al. (2016))

their future performance with preferably minimal investment, a phenomenon referred as priming (Conrath et al., 2015; Hilker et al., 2016; Hilker and Schmülling, 2019; Martinez-Medina et al., 2016). Priming of organismic responses to stress is defined as an altered stress response (Fig. 1 b – e), whereby perception of a temporally limited environmental stimulus (i.e. priming stimulus) prepares and modifies/improves the response to a future stress incident (i.e. triggering stimulus/stress) (Hilker et al., 2016). Both stimuli (priming and triggering) could be of the same nature (cis-priming) or of different nature (trans-priming), whereby the priming stimulus may be a stress itself, an indicative of an imminent stress, a compound or a beneficial organism (Hilker et al., 2016; Martinez-Medina et al., 2016). Beside cues indicating future herbivory, numerous other biotic or abiotic cues have been shown to prime a plant for improved stress response against various threats (reviewed by Conrath et al. (2015); Hilker et al. (2016); Martinez-Medina et al. (2016)). For example, exposure of a plant to a mild abiotic stress can prepare its resistance to subsequent occurring abiotic stress (heat, cold, drought or osmotic stress; reviewed by e.g. Baier et al. (2019); Avramova (2019)).

Especially in the context of anti-herbivore defence, priming is an intriguing phenomenon which comprises several characteristics (Fig. 1, characteristics highlighted as 1 to 4), described in the following. Exposure to the priming stimulus does not (or only in a slightly or transient way) induce or activate defence responses, it rather promotes the plant to a persistently primed state of enhanced defence readiness (Hilker et al., 2016; Martinez-Medina et al., 2016). In the time gap upon encounter with the triggering stress, the plant needs to store information to maintain the primed state (Hilker et al., 2016; Martinez-Medina et al., 2016) (Fig. 1 a 1). Responses to the priming stimulus and the onset of the primed state are expected to be associated with some costs (e.g. changes in the regulatory network) while maintaining the primed state is expected to have modest fitness costs (Fig. 1 a 2) (Martinez-Medina et al., 2016). In response to the triggering stress, the primed plant then mounts an improved stress response (Fig. 1 a 3). Compared to a non-primed/naïve plant which experience the same stress, such an altered stress response of a primed plant could be earlier (Fig. 1 b), faster (Fig. 1 c), more sensitive (i.e. organism already react to a lower dose of stress; Fig. 1 d) or stronger (Fig. 1 e) (Hilker et al., 2016). Certainly, these general response patterns could occur in combination or other patterns could occur, which might account for more complex signalling networks that may involve up- and downregulation of certain responses (Hilker et al., 2016). Due to an improved defence response to the triggering stress, primed plants are expected to perform better and have an improved fitness compared to non-primed plants after experiencing the stress (Fig. 1 a 4) (Hilker et al., 2016; Martinez-Medina et al., 2016). Reduced fitness costs in response to the triggering stimulus would benefit primed plants and outweigh potential costs that occur during onset and maintenance of the primed state (Hilker et al., 2016). The mechanisms which facilitate the memory and the altered primed stress response remain largely unknown, albeit they might include epigenetic, cellular, hormonal, and other phenotypic changes (Hilker et al., 2016).
In context of anti-herbivore defence priming, plants can be primed for example by oviposition of herbivorous insects on the plant (described later on, see 2.4.3 Priming of anti-herbivore defence by oviposition), by volatiles of attacked plants or volatiles emitted by herbivores. Plants can for example perceive VOCs of herbivore invested neighbouring plants as stimulus to prime their defence response (Frost et al., 2008). For instance, VOCs from *Spodoptera littoralis*-infested maize plants can prime neighbouring maize plants for an earlier and/or stronger defence induction upon subsequent triggering, which correlates with reduced caterpillar feeding and development (Ton et al., 2007). Furthermore, the primed plants ex-

hibit enhanced emissions of aromatic as well as terpenoid compounds and are significantly more attractive to parasitic wasps (Ton et al., 2007). Additionally, volatile cues from herbivores can prime plant defence responses. Tall goldenrod plants (*Solidago altissima*) exposed to E,S-conophthorin, part of the putative male sex attractant of the goldenrod gall fly (*Eurosta solidaginis*), exhibit a stronger defence induction and reduced herbivory relative to unexposed control plants (Helms et al., 2013, 2014, 2017; Yip et al., 2017).

2.3 Physiological plant responses to herbivory

Successful implementation of an induced response to herbivory requires that plants respond both rapidly and accurately. To do so, plants depend on fast and precise perception of the herbivore (see 2.3.1), a subsequent regulatory network to process the information (see 2.3.2) comprising phytohormonal signalling (see 2.3.3), which mediates the biosynthesis and activation of metabolites that function as defences (see 2.3.4) (Fürstenberg-Hägg et al., 2013; Howe and Jander, 2008; Wu and Baldwin, 2010).

2.3.1 Perception of herbivory

The feeding process of chewing herbivorous insects combines mechanical wounding with introduction of oral secretion (OS) and regurgitate from the herbivore into the wounded tissue. Thus, the attacked plant is challenged by a vast array of mechanical as well as chemical cues that may be perceived by the plant (Acevedo et al., 2015; Mithöfer and Boland, 2008). Certain compounds abundant in the OS or regurgitate belong to the broad group of elicitors, chemicals that upon recognition by the host plant activate a defensive response (Mithöfer and Boland, 2008; Wu and Baldwin, 2009; Howe and Jander, 2008). Such elicitors can be herbivore-derived or plant-derived compounds that are modified by the herbivorous insect, which are referred to as herbivore-associated molecular patterns (HAMPs; Mithöfer and Boland (2008); Howe and Jander (2008); Heil (2009)). Within some plant herbivore interactions elicitors have been identified (reviewed e.g. by Acevedo et al. (2015); Basu et al. (2018)). One of the best studied groups of elicitors are fatty acid-amino acid conjugates (FACs). This group of elicitors is examined since more than twenty years when the first fully characterized herbivore-derived elicitor, volicitin, a hydroxyl FAC (N-(17-hydroxylinolenoyl)-L-glutamine) was identified in OS of the beet armyworm (*Spodoptera exigua*; Alborn et al. (1997)).

Moreover, chewing herbivores inflict mechanical damage, which releases components that are normally inside the cell in the extracellular space. Such delocalized molecules referred to as damage associated molecular patterns (DAMPs) can also operate as elicitors (Heil, 2009; Heil et al., 2012; Heil and Land, 2014). DAMPs can comprise delocalized molecules like oligosaccharides, extracellular adenosine 5'-triphosphate (eATP) or DNA, fragmented cell walls or extracellular matrices and fragments of macromolecules that are released when pre-existing metabolites come into contact with enzymes from which they are separated in the intact cell (Heil et al., 2012; Heil and Land, 2014). In several studies eATP was shown to induce multiple defence responses in different plant species (reviewed e.g. by Tanaka et al. (2014)). Additionally, extracellular nicotinamide adenine dinucleotide (phosphate) (eNAD(P)) functions in plant signalling by inducing defence responses (Zhang and Mou, 2009).

Plants are assumed to perceive herbivory through the binding of HAMPs and/or DAMPs to pattern recognition receptors (PRRs), which are surface-localized receptor kinases or receptor-like proteins (Zipfel, 2014; Erb and Reymond, 2019). Some PRRs involved in DAMP perception have been identified (Erb and Reymond, 2019). For example, in *A. thaliana* a lectin receptor kinases were identified as receptor for eATP (LecRK-I.9; Choi et al. (2014)) and eNAD+ (LecRK-I.8; Wang et al. (2017)). In contrast, heretofore no receptor for HAMP-perception has been identified although there are indications that PRRs are important in this context. In wild tobacco (*N. attenuata*) a lectin receptor kinase contributes to resistance against the tobacco hornworm (*Manduca sexta*), but the corresponding ligand is unknown (Gilardoni et al., 2011). However, perception of HAMPs could also occur independently of PRRs (Erb and Reymond, 2019). For example, glucose oxidase is found in saliva of different caterpillar species (Acevedo et al., 2015). By oxidizing glucose, this enzyme produces the signalling molecule hydrogen peroxide (H_2O_2), which could diffuse through membranes or enter the plant cells via aquaporins (Erb and Reymond, 2019). Also lipase activity in the OS from the desert locust (*Schistocerca gregaria*) could be related to defence elicitation as it was found to directly release defence hormone precursors from membrane lipids in Arabidopsis (Schäfer et al., 2011).

Furthermore, feeding behaviours, i.e. mode, speed and frequency of tissue damage, may be recognized by plants and important for herbivore perception (Wu and Baldwin, 2009). Mechanical damage that strongly resembles the caterpillar feeding process in timing and amount of damaged leaf area ("MecWorm") is sufficient to elicit the same blend of volatiles in lima bean (*Phaseolus lunatus*) as those induced

by different herbivores, albeit in different quantities (Mithöfer et al., 2005). When this pattern of mechanical damage is further combined with continuous application of OS ("SpitWorm") a volatile bouquet is induced, mimicking insect herbivory qualitatively and quantitatively almost identically compared to real larvae feeding (Li et al., 2019). This indicates that mechanical wounding can trigger most of the defence reactions, while chemical factors in insect OS may have a 'fine-tune' function by enhancing or attenuating the induction of gene expression by mechanical wounding (Li et al., 2019).

2.3.2 Processing of the herbivory signal

After perceiving herbivory, elaborate signalling networks are activated in the plant. In order to transduce, process and amplify the signal, receptors often modify activities of other proteins or employ second messengers, small molecules or ions that are rapidly produced or metabolized at relatively high levels after signal perception (Taiz et al., 2015). Early signalling steps following upon perception of herbivory or wounding are detectable within seconds after the stimulus and comprise depolarization of the plasma transmembrane potential (V_m), increase of the cytosolic calcium concentration ($[Ca^{2+}]_{cyt}$), formation of reactive oxygen species (ROS) and activation of mitogen-activated protein kinase (MAPK) signalling cascades (Fig. 2; Maffei et al. (2007); Bricchi et al. (2010); Wu and Baldwin (2010)).
Among the earliest events after perception are ion fluxes (e.g. Ca^{2+}, Na^+, K^+ and Cl^-) at the plasma membrane, which usually result in temporary changes of V_m (Maffei et al., 2007; Wu and Baldwin, 2010; Zebelo and Maffei, 2015). Herbivory-induced V_m changes are followed by a fast electrical signal (action potential) that travels through the entire plant (Maffei and Bossi, 2006). Such plasma membrane depolarizations in response to environmental stimuli are common in plants (Fromm and Lautner, 2007) and correlated to elevated $[Ca^{2+}]_{cyt}$, ion channel activity and ROS bursts (Zebelo and Maffei, 2015). In general, Ca^{2+} is an important second messenger in all eukaryotes (Wu and Baldwin, 2010). When lepidopteran larvae feed on plants, the cell membrane depolarizes at the vicinity of the bite zone followed by a transient increase of $[Ca^{2+}]_{cyt}$ (Maffei et al., 2006; Howe and Jander, 2008). Wound- and insect triggered long-distance propagation of membrane depolarizations and $[Ca^{2+}]_{cyt}$ changes were shown to be dependent on glutamate receptor-like ion channels (Nguyen et al., 2018; Toyota et al., 2018). *S. littoralis* larvae growth is enhanced on plants lacking these receptors, indicating their role in progressing the signal (Nguyen et al., 2018). Several Ca^{2+} sensor

proteins like calmodulins or calmodulin-like proteins and calcium-dependent protein kinases (CPDKs), translate Ca^{2+} signals into downstream actions (Wu and Baldwin, 2010; Lecourieux et al., 2006). Furthermore, ROS such as hydrogen peroxide (H_2O_2) or nitric oxide (NO), which function at low concentrations as second messengers, are induced by herbivory and associated with plant defence regulation (Maffei et al., 2007; Wu and Baldwin, 2010). For example, in lima bean, H_2O_2 is released upon *S. littoralis* feeding and, to a lesser extent, upon mechanical damage (Maffei et al., 2006). ROS are primarily produced by plasma membrane NADPH oxidases (respiratory burst oxidase homologues), which have the ability to integrate calcium signalling and protein phosphorylation with ROS formation (Maffei et al., 2007; Zebelo and Maffei, 2015). Herbivory and wound-induced defence signalling also involve activation of several types of MAPK cascades, which represent well-conserved signalling pathways in the response of eukaryotes to many types of environmental stress (Maffei et al., 2007; Wu and Baldwin, 2010; Heil and Land, 2014).

As herbivores are highly mobile and potentially move from attacked to non-attacked tissues, plants require additional countermeasures to establish an efficient defence for the entire plant. To do so, plants either transport defensive metabolites within the plant or induce responses, like the formation of defensive metabolites, in distal (systemic) leaves that were not actually wounded or attacked by herbivores (Heil and Ton, 2008). To accomplish the latter, the information of infestation or injury needs to be transduced by a signal from the side of actual attack throughout the plant or parts of the plant. Although systemic responses were already observed almost fifty years ago (Green and Ryan, 1972), the signals involved in systemic responses are still not fully understood (Wasternack, 2015; Wu et al., 2007). Several studies indicated that the vascular system is involved in the transportation of systemic signals (Jones et al., 1993; Schittko et al., 2000; Orians et al., 2000) and others indicate that an electrical signal is involved (Wildon et al., 1992; Stanković and Davies, 1997; Zimmermann et al., 2009). Wounding or insect feeding triggers the production of plant peptides, which are considered as endogenous secondary danger signals, that are released into the apoplastic space (Erb and Reymond, 2019). For example, systemin, an 18-amino acid polypeptide that is cleaved from the precursor prosystemin, spreads systemically throughout tomato plants and induces defences that negatively impact chewing herbivores (Orozco-Cardenas et al., 1993). In addition to their function in indirect defence, VOCs can also be perceived by undamaged parts of the same plant and serve as signals and eliciting defence responses in distal systemic plant parts (Karban et al., 2014; Pierik et al.,

2014; Heil and Ton, 2008). This could be particularly important in whines and shrubs where vascular distances between adjacent plant parts limit the spread of internal signals (Erb and Reymond, 2019; Heil and Ton, 2008).

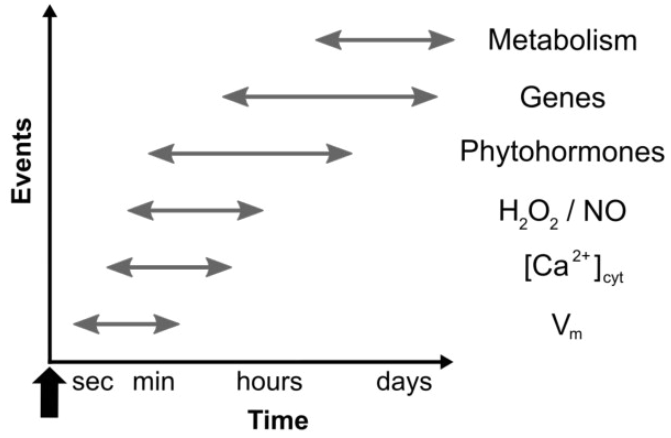

Figure 2: Timed hierarchy of consecutive events detectable following perception of insect feeding. V_m(plasma transmembrane potential) changes at the plasma membrane are the earliest events measurable, immediately followed by or associated with changes in $[Ca^{2+}]_{cyt}$ (cytosolic calcium concentration) and the generation of H_2O_2 and NO. Within minutes activity of kinases and induction of phytohormones are detectable. Gene activations and subsequent metabolic changes regularly occur after around 30 minutes to an hour (adapted from Maffei et al. (2007) and Bricchi et al. (2010)).

2.3.3 Phytohormonal signalling in response to herbivory

A later element of the signal transduction cascade is represented by the induced signalling of diverse phytohormones (Fig. 2). Phytohormones comprise a group of structurally unrelated small molecules derived from various essential metabolic pathways and function as endogenous chemical messengers (Santner et al., 2009; Santner and Estelle, 2009). Nine major groups of hormones in plants, auxins, gibberellins, cytokinins (CK), ethylene (ET), abscisic acid (ABA), brassinosteroids, jasmonic acid (JA), salicylic acid (SA) and strigolactones, collectively regulate every act of plant life (Santner et al., 2009). They mediate communication across cells, tissues and organs to coordinate growth, metabolism and responses to environmental conditions into the transcriptional and metabolomic response of plants (Santner et al., 2009; Santner and Estelle, 2009; Taiz et al., 2015). Transcription factors play a key role in regulating defences both up- and downstream of phyto-

hormone signalling and contribute to the complexity and specificity of signalling outputs (Howe et al., 2018; Erb and Reymond, 2019). Although the details of hormonal control are complex, all basic hormonal pathways share common features: Perception of an environmental signal often results in an increase or decrease of hormone biosynthesis, which causes transcriptional or post-transcriptional changes that ultimately result in a physiological or developmental response (Taiz et al., 2015; Santner et al., 2009). To return to base levels of the hormone and to reacquires the ability to respond to the next signal input, the response can be attenuated by catabolism or sequestration of the active hormone or by a negative feedback mechanism that repress hormone biosynthesis (Taiz et al., 2015; Santner et al., 2009). An important and complex aspect of phytohormonal signalling, is the interaction of the different phytohormones with each other, also denoted as phytohormonal crosstalk (Santner and Estelle, 2009; Pieterse et al., 2012). The different phytohormonal signalling pathways are interconnected at various points, forming a complex network of positive and negative feedback loops by means of synergistic and antagonistic interactions (Bari and Jones, 2009; Maffei et al., 2007). In the context of defence signalling, this phytohormonal network or more precisely the quality, composition, and timing of the hormonal blend tailors the appropriate response to the attacker and allow plants to integrate information from multiple external stimuli into transcriptional programs (Maffei et al., 2007; Santner and Estelle, 2009; Pieterse et al., 2012). Jasmonic acid (JA) with its derivatives (collectively referred to as jasmonates; see 2.3.3.1) are well established as the core hormone regulating defences against chewing herbivores (Pieterse et al., 2012; Wasternack, 2015; Howe et al., 2018). However, also other stress-related phytohormones such as ABA (see 2.3.3.2), SA (see 2.3.3.3) and ET as well as growth hormones such as gibberellins, auxin and CKs are induced upon herbivory and have modulating roles in the regulation of antiherbivore defences (van Loon et al., 2006; Erb and Reymond, 2019; Pieterse et al., 2012).

2.3.3.1 Jasmonate signalling

Among the most prominent plant hormones active in stress responses are jasmonic acid (JA) and its derivatives, collectively referred to as jasmonates (Wasternack and Hause, 2013; Wasternack, 2015). In addition to their crucial role in stress responses, jasmonates are involved in numerous developmental processes such as seed germination, growth, stamen development and senescence (Wasternack and Hause, 2013; Wasternack and Song, 2017).

The octadecanoid pathway

Jasmonates are derived from lipids in the octadecanoid pathway (Fig. 3; reviewed for example by Wasternack and Hause (2013); Wasternack and Song (2017); Wasternack and Feussner (2018)), a biosynthesis pathway investigated since more than forty years (history of JA research reviewed by Wasternack (2015)).

The first part of the pathway is localized in the plastid (Fig. 3, grey box) and is initiated by the lipase-mediated release of tri-unsaturated fatty acid -linolenic acid (18:3, α-linolenic acid (α-LeA)) from chloroplastic glycerolipids (Ishiguro et al., 2001; Kelly and Feussner, 2016). 13-lipoxygenase (13-LOX) then oxidises α-LeA to 13(S)-hydroperoxy-octadecatrienoic acid (13 (S)-HPOT), which can also be metabolized to other oxylipins (Feussner and Wasternack, 2002; Andreou and Feussner, 2009). Subsequently, 13 (S)-HPOT is converted by allene oxide synthase (AOS) to the highly unstable allene oxide 12,13(S)-epoxy-octadecatrienoic acid (Brash, 2009), which is then converted by allene oxide cyclase (AOC) to (9S, 13S)-12-oxo-phytodienoic acid (cis-(+)-OPDA), the first cyclic compound and the end-product of the plastid localized part of the pathway (Hofmann et al., 2006; Hofmann and Pollmann, 2008; Schaller and Stintzi, 2009). AOC enforces strong steric restrictions which results in the exclusive accumulation of cis-(+)-OPDA (Hofmann et al., 2006). The following export of cis-(+)-OPDA from the plastid to the cytosol is suggested to be mediated by a channel protein from the outer envelope of the plastid called JASSY (Guan et al., 2019). An ABC transporter of the peroxisomal membrane, COMATOSE (CTS), facilitates the subsequent import of cis-(+)-OPDA into peroxisomes (Theodoulou et al., 2005). There, OPDA reductase 3 (OPR3) catalyzes the reduction of cis-(+)-OPDA to OPC-8 (3-oxo-2- (2‘[Z]-pentenyl)-cyclopentan-1-octanoic acid) (Schaller and Weiler, 1997; Breithaupt et al., 2001, 2006). Ultimately, the carboxylic side chain is shortened by the fatty acid β-oxidation machinery catalysed by acyl-CoA oxidase (ACX; Li et al. (2005); Schilmiller et al. (2007)), L-3-ketoacyl CoA thiolase (KAT; Castillo et al. (2004)) and 4-coumarate:CoA ligase-like enzymes (4CL; Schneider et al. (2005); Koo et al. (2006)).

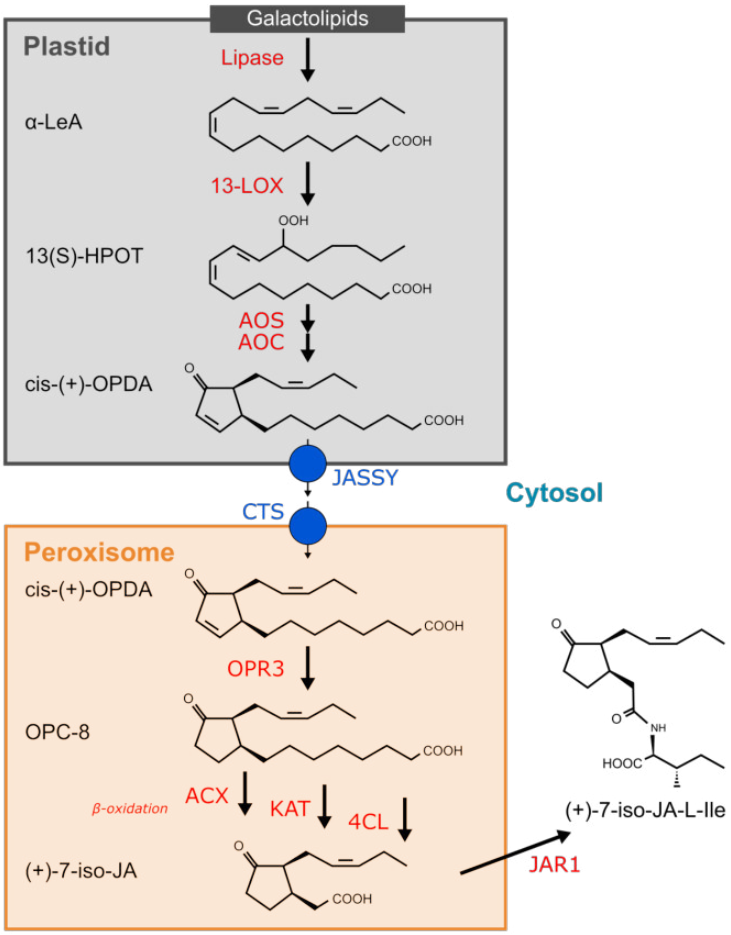

Figure 3: The octadecanoid pathway. Upon the generation of α-linolenic acid (α-LeA) from galactolipids by lipases in the plastid (grey box), (13S)-hydroperoxy octadecatrienoic acid (13(S)-HPOT) is formed by 13-lipoxygenase (13-LOX). The unstable allene oxide 12,13(S)-epoxy-octadecatrienoic acid is generated by allene oxide synthase (AOS) and further converted by allene oxide cyclase (AOC) to (9S, 13S)-12-oxo-phytodienoic acid (cis-(+)-OPDA). The membrane protein JASSY facilitates the export of OPDA in the cytosol, while the import of OPDA into the peroxisomes is mediated by the ABC transporter comatose (CTS). In the peroxisome (orange box) OPDA is reduced to 3-oxo-2- (2‘[Z]-pentenyl)-cyclopentan-1-octanoic acid (OPC-8) by OPDA reductase 3 (OPR3). Subsequently the carboxylic acid side chain is shortened in three rounds of fatty acid β-oxidation, mediated by acyl-CoA oxidase (ACX), L-3-ketoacyl-CoA thiolase (KAT) and 4-coumarate:CoA ligase-like enzymes (4CL). The end product, (+)-7-iso-JA is conjugated by JA-amino acid synthetase (JAR1) to (+)-7-iso-jasmonoyl-L-isoleucine ((+)-7-iso-JA-L-Ile) (adapted from Wasternack and Feussner (2018) and Wasternack and Hause (2019)).

Derivatives of JA

The initial product, (+)-7-iso-JA, is transported by an unknown mechanism into the cytosol (Wasternack and Feussner, 2018). In addition to epimerization to the more stable stereoisomer (−)-JA (trans-configuration), (+)-7-iso-JA (in the following denoted as JA) can be subjected to numerous metabolic reactions (e.g. conju-

gation with amino acids, glycosylation, hydroxylation, carboxylation/decarboxylation, sulfation, esterification and methylation) yielding in several biologically active and inactive derivatives of JA (reviewed by Wasternack and Strnad (2016); Wasternack and Feussner (2018)). One well-known JA derivate is the volatile ester methyl jasmonate (MeJA), can diffuse trough membranes and can act as an airborne signal and mediator of intra- and inter-plant communication (Farmer and Ryan, 1990; Kessler et al., 2006; Baldwin et al., 2006; Tamogami et al., 2012; Seo et al., 2013). For instance, clipped sagebrush (*Artemisia tridentate*) releases a pulse of MeJA that induces resistance against herbivores in nearby (10 - 15 cm) neighbouring wild tobacco plants (*N. attenuata*), which caused under field conditions a reduced leaf damage in these plants compared to plants next to unclipped sagebrush (Karban et al., 2000, 2003). However, neither JA nor MeJA are directly active metabolites, but the amino-acid conjugate jasmonic acid-isoleucine (JA-Ile), particularly the stereoisomer (+)-7-iso-JA-L-Ile, is the actually bioactive compound (Thines et al., 2007; Katsir et al., 2008; Fonseca et al., 2009), generated mainly through a conjugation of JA with the amino acid isoleucine by the jasmonoyl amino acid conjugate synthase (JAR1) (Staswick and Tiryaki, 2004; Suza and Staswick, 2008; Fonseca et al., 2009).

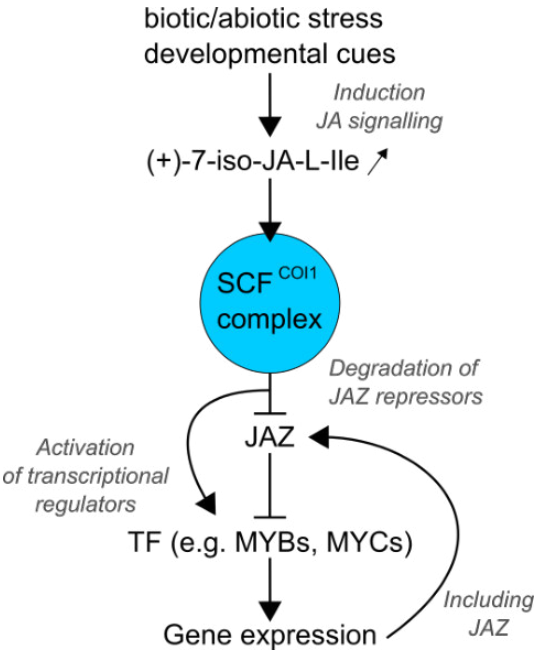

Figure 4: Regulation of jasmonate-induced gene expression. Upon biotic/abiotic stresses or due to developmental cues JA signalling is induced resulting in increased levels of (+)-7-iso-JA-L-Ile. Binding of (+)-7-iso-JA-L-Ile to the SCF ^{C}OI1 ubiquitin ligase complex leads to degradation of jasmonate ZIM-domain (JAZ) repressor proteins, resulting in activation of transcription factors (TF), e.g. MYBs or MYCs, that regulate gene expression like defence-related genes, but also JAZ repressor proteins (negative feedback loop; adapted from Lortzing and Steppuhn (2016); Howe and Jander (2008) and Santner et al. (2009)).

Jasmonate signalling

The core JA signalling cascade comprises several functional modules (see Fig. 4; reviewed by Wasternack and Strnad (2016); Wasternack and Song (2017); Howe et al. (2018). In response to biotic or abiotic stress like wounding / herbivory or due to developmental cues, jasmonate levels, particularly (+)-7-iso-JA-L-Ile, rapidly increase. Then (+)-7-iso-JA-L-Ile binds to the F-box protein COI1 (coronatine insensitive 1), constituent of the ubiquitinproteasome protein degradation machinery a Skp1/Cullin/F-box (SCF^{COI1}) complex with E3 ubiquitin ligase activity (Fonseca et al., 2009; Sheard et al., 2010; Wasternack and Strnad, 2016). Upon binding with (+)-7-iso-JA-L-Ile the SCF^{COI1} complex interacts with JAZ (JASMONATE ZIM DOMAIN) repressor proteins and triggers their degradation by the S26 proteasome (Devoto et al., 2002; Xu et al., 2002; Chini et al., 2007; Song et al., 2011). Degradation of JAZ proteins, which function with co-repressors by repressing positively acting transcription factors, releases repression of transcription factors (e.g. MYC or MYB), resulting in expression of JA-responsive defence related genes (Thines et al., 2007; Chini et al., 2007; Yan et al., 2007; Thireault et al., 2015; Pauwels et al., 2010). In conjunction with JA-responsive genes, the expression of JAZ genes is induced and newly synthesized JAZ repressors dampen the response (negative feedback loop) (Thines et al., 2007; Chini et al., 2007). MYCs (basic helix-loop-helix transcription factors) have a central role in mediating jasmonate responses (Kazan and Manners, 2013; Howe et al., 2018; Erb and Reymond, 2019). For example, MYC2 orchestrates a transcriptional cascade and activates various downstream metabolic pathways involved in plant defence against herbivores (Lorenzo et al., 2004; Dombrecht et al., 2007; Kazan and Manners, 2013; Du et al., 2017). Different MYCs act synergistically to control JA-dependent defences, indicated by mutants lacking MYC(s) which display a higher susceptibility to chewing herbivores (Fernández-Calvo et al., 2011; Schweizer et al., 2013; Song et al., 2014, 2017). Taken together, jasmonates exert their function by large-scale reprogramming of gene expression (Kombrink, 2012). For example, in response to herbivory by Pieris rapae caterpillars, between 67 to 84 % of the induced changes in gene expression of *A. thaliana* were totally or in part controlled by the jasmonate pathway (Reymond et al., 2004).

In decades of research several studies established the indispensable role of JA in plant defense against herbivores (McConn et al., 1997; Kessler et al., 2004; Howe and Jander, 2008). For example the accumulation of direct defence metabolites like phenylpropanoid derivatives as well as defensive proteins are regulated by jasmonates (described later on see 2.3.4). The fact that mutants of plants that

normally resist attacks become remarkably vulnerable without the ability to produce or perceive jasmonates, further underlines their role in plant defence (Howe et al., 1996; McConn et al., 1997; Thaler et al., 2002; Reymond et al., 2004; Li et al., 2005; Kang et al., 2006; Paschold et al., 2007). Responses mediated by JA are involved in the competitive and trophic interactions between various organisms and can cascade up at least four trophic levels affecting predators, parasitoids, or even hyperparasitoids (reviewed by Lortzing and Steppuhn (2016)). For instance, induced indirect defence mechanisms like the secretion of extrafloral nectar which attracts enemies of the feeding herbivores are regulated by jasmonates (Heil et al., 2001; Heil, 2015).

2.3.3.2 Abscisic acid

Abscisic acid (ABA), a sesquiterpene, is an important regulator of plant growth, development, and stress responses, with essential roles among others in stomata closure, cuticular wax accumulation, leaf senescence, bud dormancy, seed germination, osmotic regulation, and growth inhibition (Zhang, 2014; Chen et al., 2020). Moreover, ABA is considered as master regulator of responses to abiotic stresses, such as drought and salt (Lee and Luan, 2012; Chen et al., 2020). Plants synthesize ABA using the carotenoid pathway while catabolism of ABA is controlled by conjugation and catalytic hydroxylation (Nambara and Marion-Poll, 2005; Chen et al., 2020). ABA can for example be glucosylated and thereby inactivated to ABA-glucosyl ester, but this conjugate can be returned to active ABA (Lee et al., 2006; Xu et al., 2014; Liu et al., 2015b). The core ABA signalling constituents comprise recognition by the intracellular receptor PYLs (pyrabactin resistance 1-like) which upon binding form complexes with the clade A PP2Cs allowing the release of the inhibition of SnRK2 protein kinases (Chen et al. (2020); Lee and Luan (2012) and references therein). SnRK2s are then activated by other protein kinases or through autophosphorylation and regulate multiple physiological responses through phosphorylation targets like ion channels, TF, and transporters (Chen et al. (2020); Lee and Luan (2012) and references therein). Moreover, ABA contributes and may even reinforce plant defence to chewing herbivores. Several studies found that herbivore attack or treatment with herbivorous OS increases ABA contents (Erb et al., 2009, 2011; Schäfer et al., 2011; Tooker and De Moraes, 2011; Vos et al., 2013b). The importance of ABA for anti-herbivore defence was further highlighted by analysing mutants with altered ABA biosynthesis or signalling, because ABA deficient mutants fail to induce a full anti-herbivore defence

response and are more susceptible to herbivores (Thaler and Bostock, 2004; Bodenhausen and Reymond, 2007; Dinh et al., 2013; Vos et al., 2013b). ABA-mediated defence responses against herbivores are suggested to be tightly interconnected with JA and it is assumed that ABA can modulate JA-mediated defence responses (Chen and Yu, 2014). ABA e.g. enhances JA-biosynthesis and signalling, resulting in increased transcript levels of herbivore-related genes (Lorenzo et al., 2004; Bodenhausen and Reymond, 2007; Garg et al., 2012). Overexpressing an ABA-responsive transcription factor in tomato plants increases the expression of several JA-dependent genes (Orellana et al., 2010). *N. attenuata* plants with a silenced gene encoding a FAC-regulated protein were found to have an impaired jasmonate and ABA signalling and metabolism, which makes the plants more susceptible to *M. sexta* (Dinh et al., 2013). The authors concluded that the FAC-regulated protein acts as a natural suppressor of ABA catabolism after herbivore attack which in turn activates the full defence profile against herbivores (Dinh et al., 2013). Furthermore, ABA is known to have synergistic effects on the MYC-branch and antagonistic effects on the ethylene response factor (ERF)-branch of transcriptional regulation by JA (Lorenzo et al., 2004; Anderson et al., 2004; Lorenzo and Solano, 2005; Kazan and Manners, 2013; Vos et al., 2013b, 2015).

2.3.3.3 Salicylic acid

Salicylic acid (SA, 2-hydroxy benzoic acid) is a phenolic compound produced in procaryotes and plants (Vlot et al., 2009; Dempsey et al., 2011). For a long time, SA has been applied as medically effective ingredient while its role in plant signalling emerged later so that it was introduced as phytohormone only in the early 1990s (Raskin, 1992; Klessig et al., 2018). Since then, its function as critical plant hormone that regulates defence against biotic and abiotic stress emerged (Klessig et al., 2018; Ding and Ding, 2020). However, besides mediating stress responses, SA also influences numerous aspects of plant growth and development, such as seed germination, vegetative growth, respiration, thermogenesis, flower formation (Rivas-San Vicente and Plasencia, 2011; Klessig et al., 2018). Albeit the most prominent function of SA is represented by its key role as endogenous signal mediating local and systemic plant defence responses against pathogens (Vlot et al., 2009; Dempsey et al., 2011). However, in some cases SA-induced defences are effective against sucking herbivorous insects, such as aphids (Zhang et al., 2015; Züst and Agrawal, 2016).

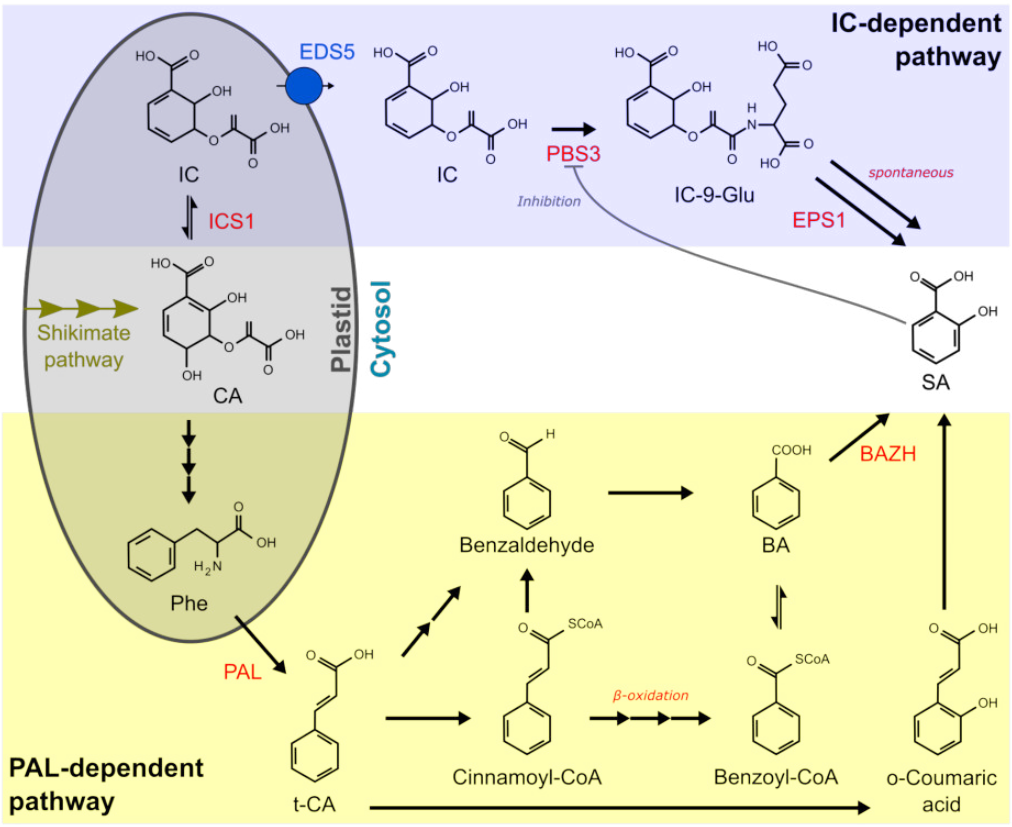

Figure 5: Potential pathways for salicylic acid (SA) biosynthesis in higher plants. Both pathways start with chorismic acid (CA) derived from the shikimate pathway. In the first step of the isochorismate (IC) – dependent pathway (blue box) CA is converted by isochorismate synthase (ICS1) to IC, which is transported to the cytosol by the protein enhanced disease susceptibility 5 (EDS5). In the cytosol IC is conjugated by avrPphB susceptible 3 (PBS3) to isochorismate-9-glutamate (IC-9-Glu) (conjugation under competitive inhibition by SA). Subsequent spontaneous decay results in SA, while in members of the Brassicaceae family, EPS1 (a BAHD acyltransferase-family protein) converts IC-9-Glu to SA. The phenylalanine ammonia-lyase (PAL) – dependent pathway (yellow box) has not been fully resolved. CA is converted to phenylalanine (Phe), which is then converted by PAL to trans-cinnamic acid (t-CA). Subsequently, t-CA is converted via ortho- coumaric acid (o-coumaric acid) to SA. Alternatively, t-CA can be converted via three different routes to benzoic acid (BA) including a β-oxidation route via cinnamoyl Co-A and benzoyl-CoA, a non-oxidative route via cinnamoyl Co-A and benzaldehyde, and a non-oxidative route via benzaldehyde, which is then converted to SA by BA 2-hydroxylase (BAZH) (adapted from Dempsey et al. (2011) and Huang et al. (2019)).

Synthesis of SA

In higher plants, SA can be produced in two distinct pathway branches: the isochorismate (IC)-dependent pathway and the phenylalanine ammonia-lyase (PAL)-dependent pathway, named after the initiating enzyme (see Fig. 5; Dempsey et al. (2011); Huang et al. (2019)). In *A. thaliana*, 10 % of pathogen defence related SA is produced by the PAL-dependent pathway, while 90 % of the SA is derived from the IC-dependent pathway (Serino et al., 1995). However, suppression of PAL expression in tobacco (Pallas et al., 1996) and *Arabidopsis* (Huang et al., 2010) also caused major reduction in SA accumulation. This suggests that the regulatory mechanism and functional significance of the partition of these two branches under different conditions are yet to be elucidated (Torrens-Spence et al., 2019). Several important aspects of SA metabolism and regulation still remain to be addressed, while recent studies (Torrens-Spence et al., 2019; Rekhter et al., 2019) dissected and uncovered missing steps of the IC pathway. Both pathways are initiated in plastids and originate from chorismate (CA), the end product of the shikimate pathway (Dempsey et al., 2011). The first step of the IC pathway (see Fig 5, blue box) is the conversion of CA by isochorismate synthase (ICS1) to IC in the plastid (Wildermuth et al., 2001; Catinot et al., 2008). EDS5 (Enhanced disease susceptibility 5), localized in the plastid membrane, exports IC into the cytosol (Torrens-Spence et al., 2019; Rekhter et al., 2019). Here, IC is conjugated with L-glutamate to isochorismate-9-glutamate (IC-9-Glu) by the cytosolic amidotransferase avrPphB Susceptible3 (PBS3, Torrens-Spence et al. (2019); Rekhter et al. (2019)). Subsequent non-enzymatic decomposition of IC-9-Glu results in SA and 2-hydroxy-acryloyl-N-glutamate (2HNG; Torrens-Spence et al. (2019); Rekhter et al. (2019)). In plants belonging to the Brassicaceae family, EPS1 (a BAHD acyltransferase-family protein) facilitates the production of SA and 2HNG from IC-9-Glu by functioning as an unprecedented isochorismoyl-glutamate A pyruvoyl-glutamate lyase (Torrens-Spence et al., 2019). PBS3 can either accommodate SA or IC, this competitive inhibition provides a feedback mechanism to regulate the IC pathway (Okrent et al., 2009). Ectopic overexpression of ICS1 is required to produce sufficient IC which allow a quantitative displacement of SA from the active site of PBS3 (Okrent et al., 2009).

As mentioned above, several aspects of the SA metabolism and regulation remain unknown, for instance in the PAL-dependent pathway the enzymatic steps downstream of PAL remain largely unresolved (Torrens-Spence et al., 2019). The PAL pathway (see Fig. 5, yellow box) begins with the conversion of chorismate in three steps to L-phenylalanine (Phe, Wildermuth (2006); Dempsey et al. (2011)). PAL

then cleaves NH3 from Phe yielding trans-cinnamic acid (t-CA, Raes et al. (2003); Rohde et al. (2004)). The conversion of t-CA to SA can occur in different biosynthetic routes, on the one hand via the intermediate ortho-coumaric acid, on the other hand via the intermediate benzoic acid (BA, Wildermuth (2006); Dempsey et al. (2011)). Plants can potentially utilize three biosynthetic routes to convert t-CA in BA, including β-oxidation of cinnamoyl Co-A to benzoyl CoA a non-oxidative route from cinnamoyl Co-A to benzaldehyde, and a non-oxidative route in which t-CA is converted directly to benzaldehyde (Wildermuth, 2006; Dempsey et al., 2011). BA is then proposedly catalysed by an inducible BA 2-hydroxylase (BAZH) to SA (Leon et al., 1995).
SA can further undergo several modifications (e.g. glucosylation, methylation, amino acid conjugation, sulfonation or hydroxylation), rendering SA either inactive or allow for fine-tuning of accumulation, function and mobility (Dempsey et al., 2011; Ding and Ding, 2020). For instance, inactivation can be realized by glucosylation, while methylation increases SA´s membrane permeability as well as its volatility, allowing for more effective long-distance signalling (Dempsey et al., 2011). This is especially important, as grafting experiments and other studies revealed that SA is not a generic mobile signal (Métraux et al., 1991; Vernooij et al., 1994; Shulaev et al., 1995).

SA signalling

Signalling downstream of SA is largely regulated by the regulatory protein NON-EXPRESSOR OF PR GENES 1 (NPR1; Vlot et al. (2009); Dempsey et al. (2011)). *Arabidopsis* carries five paralogs of NPR1 (NPR2/3/4, Blade On Petiole 1 (BOP1) and BOP2), of these NPR1/2/3/4 can strongly bind SA in vitro, while BOP1/2 have only weak interactions with SA (Manohar et al., 2015; Castelló et al., 2018; Ding et al., 2018). Recently it has been shown that NPR1 and NPR2 play positive roles in regulating downstream genes in response to SA, while NPR3 and NPR4 seem to serve as negative regulators (Castelló et al., 2018; Ding et al., 2018). However, it is still not fully understood how NPRs are regulated in response to SA accumulation and if all NPR1 homologs would undergo similar biochemical processes (Ding and Ding, 2020). For NPR1 it is presumed that in an uninduced state, NPR1 is present in the cytosol as an oligomer formed through intermolecular disulfide bonds (Mou et al., 2003; Tada et al., 2008). Upon induction, a biphasic change in cellular reduction potential occurs resulting in reduction of NPR1 from its inactive oligomeric form to its active monomeric form (Mou et al., 2003; Tada et al., 2008). This monomeric NPR1 is then translocated to the nucleus, where

it interacts with TGA transcription factors (Mou et al., 2003; Birkenbihl et al., 2017). This interaction subsequently causes an activation of a large set of defence-related genes also including genes coding for PATHOGENESIS-RELATED (PR) proteins and WRKY transcription factors (van Loon et al., 2006; Rushton et al., 2010; van Verk et al., 2011).
As described above, phytohormones are embedded in a complex network and crosstalk with other phytohormones which determines as a hole the outcome of the signalling network. Considerable evidence suggests an antagonistic interaction SA- and JA-signalling pathways, as numerous studies have shown an antagonistic interaction between SA- and JA-mediated plant responses (Erb et al., 2012; Thaler et al., 2012; Pieterse et al., 2012). For example, in lima bean herbivory of the SA-inducing and phloem-feeding sweetpotato whitefly (*Bemisia tabaci*) negatively affected JA biosynthesis and JA-dependent indirect defence responses induced by the twospotted spider mite (*Tetranychus urticae*) on the same plant (Zhang et al., 2009). This resulted in a reduced attractiveness to predatory mites, which would be attracted in absence of whiteflies by a volatile blend and kill the spider mites (Zhang et al., 2009). Therefore, elevated SA levels which antagonize JA-mediated plant defences are considered to be beneficial for chewing herbivores (Bruessow et al., 2010). However, a few studies also revealed neutral or synergistic interactions between SA and JA (Mur et al., 2006; Hilfiker et al., 2014; Rostás et al., 2013; Van Oosten et al., 2008). In tobacco (*Nicotiana tabacum*) and *A. thaliana*, cotreatment with various concentrations of SA and JA suggested that the reciprocal antagonism of SA and JA signalling is dose dependent and occurs at higher doses, while lower concentrations caused a synergistic enhancement of genes involved in JA- and SA-mediated defence (Mur et al., 2006).

2.3.3.4 Other defence related phytohormones

Besides its diverse roles in plant growth and development, e.g. by influencing meristem activity, branching and developmental transitions, CKs are also involved in response to biotic and abiotic stresses (Werner and Schmülling, 2009; Kieber and Schaller, 2018; Cortleven et al., 2019). Endogenous levels of CK can be increased by the plant in interaction with a variety of organisms, e.g. bacteria, fungi, parasitic nematodes and herbivorous insects (Cortleven et al., 2019; Akhtar et al., 2019; Giron et al., 2013). Furthermore, CKs have an active role in regulating plant defence responses against herbivores (Giron et al., 2013; Schäfer et al., 2015; Dervinis et al., 2010). Albeit, during the interaction with the plant the organisms

can also supply exogenous CKs to manipulate the host plant to create 'green islands' that increase the nutritional value of infested tissues (Engelbrecht et al., 1969; Brütting et al., 2018).
Often considered as 'aging' hormone due to its role in accelerating developmental processes such as ripening, senescence, and abscission, the phytohormone ET also regulates many other aspects of growth and development (Abeles et al., 2012). In the context of plant defence against insect herbivory, JA and ET are suggested to act antagonistically. Silencing an ET-synthesising enzyme in rice reduces ET production and resistance to the chewing herbivore *Chilo suppressalis* but increases resistance to the phloem feeder *Nilaparvata lugens* (Lu et al., 2014). *Arabidopsis* ET-insensitive mutants were more resistant to *S. littoralis* and *S. exigua* (Bodenhausen and Reymond, 2007; Lu et al., 2014). ET-stabilized transcription factors interact with the JA-activated transcription factor MYC2 resulting in an inhibition of JA-related gene expression and defences against herbivores, providing a molecular mechanism for the suggested ET/JA antagonism (Lu et al., 2014).

2.3.4 Induced anti-herbivore defences

Perception of the herbivore and subsequent processing of the information in elaborate signalling cascades causes ultimately the synthesis or activation of distinct defensive compounds or metabolites. Herbivore-attacked plants can for example induce the biosynthesis of specialized compounds and metabolites, also referred to as secondary metabolites, which can directly target biological systems unique to the herbivore, e.g. the nervous, digestive, and endocrine system (Rosenthal and Berenbaum, 2012).
A diverse group of compounds involved in defence, but also in growth, structural support, and survival, are phenylpropanoids (Vogt, 2010). The phenylpropanoid pathway serves as rich source of metabolites, as plant phenylpropanoid-derived compounds comprise monolignols, flavonoids, various phenolic acids, and stilbenes (Fraser and Chapple, 2011; Liu et al., 2015b) with diverse roles in anti-herbivore defence (Appel, 1993; Lattanzio et al., 2008; Salminen et al., 2011). Jasmonate-regulated MYB transcription factors (see 2.3.3.1) have key functions in regulation of the synthesis of phenylpropanoid-derived compounds (Gaquerel et al., 2014; Liu et al., 2015a). For example in the wild tobacco, *N. attenuata*, two major phenylpropanoid-polyamine conjugates, caffeoylputrescine (CP) and dicaffeoylspermidine (DCS), with important roles in plant defence against leaf-chewing

herbivores increase dramatically in response to herbivore attack also regulated by NaMYB8 (Kaur et al., 2010). Similar to the SA biosynthesis, the widely branched phenylpropanoid pathway is located downstream of the shikimate pathway (Vogt, 2010). The phenylpropanoid pathway is initiated with three mandatory reactions, collectively referred to as the general phenylpropanoid pathway, catalysed by phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), and 4-coumaroyl CoA-ligase (4CL), converting the end product of the shikimate pathway phenylalanine to 4-coumaroyl CoA (see Fig. 6, blue; Vogt (2010); Fraser and Chapple (2011)). In higher plants, 4-coumaroyl CoA probably represents the most important branchpoint within the central phenylpropanoid pathway and provides the basis for all subsequent branches, i.e. the starting point for the biosynthesis of phenylpropanoid compounds and a variety of other metabolites (Vogt, 2010; Fraser and Chapple, 2011). The basic flavonoid skeleton of three aromatic rings is generated by the enzymes chalcone synthase (CHS) and chalcone isomerase (CHI) from 4-coumaroyl CoA, form biosynthesis of flavonoids and anthocyanins (see Fig. 6; Koes et al. (2005)). The enzyme hydroxycinnamoyl CoA:shikimate/quinate hydroxycinnamoyltransferase (HCT), catalyses two steps within the phenylpropanoid metabolism. First, HCT catalyses the transfer of the p-coumaroyl group of 4-coumaroyl CoA to shikimate respectively quinate forming p-coumaroyl shikimate respectively p-coumaroyl quinate (Hoffmann et al., 2004). Following the 3´ hydroxylation of p-coumaroyl shikimate by p-coumarate 3-hydroxylase (C3H) to form caffeoyl shikimate (Schoch et al., 2001; Franke et al., 2002) HCT catalyses then the transfer of the caffeoyl moiety back onto Coenzyme A (Vogt, 2010; Fraser and Chapple, 2011). In parallel, p-coumaroyl quinate can be converted by C3H to Chlorogenic acid (CGA), alternatively caffeoyl quinate, which can also be further catalysed by HCT to caffeoyl CoA (Schoch et al., 2001; Franke et al., 2002). An alternative route for the formation of CGA can occur via conversion of cinnamic acid to cinnamoyl D-Glucose (catalysed by UDP-glucose:cinnamate glucosyl transferase (UGCT)) and caffeoyl D glucose followed by the catalysation with quinate by hydroxycinnamoyl glucose:quinate hydroxycinnamoyl transferase (HCGQT) to CGA (Niggeweg et al., 2004). In Solanaceae, CGA and rosmarinic acid (caffeoyl phenyl lactic acid) are the predominant soluble phenylpropanoids (Vogt, 2010). CGA has a broad spectrum of anti-herbivore activity (Elliger et al., 1981; Leiss et al., 2009; Lee et al., 2017; Liu et al., 2017b). Moreover, detrimental effects of other phenolic compounds produced from the phenylpropanoid pathway on herbivore performance have been shown for numerous plant-insect interactions (Dixon et al., 2002; Kaur et al., 2010; Salminen et al., 2011; War et al., 2012).

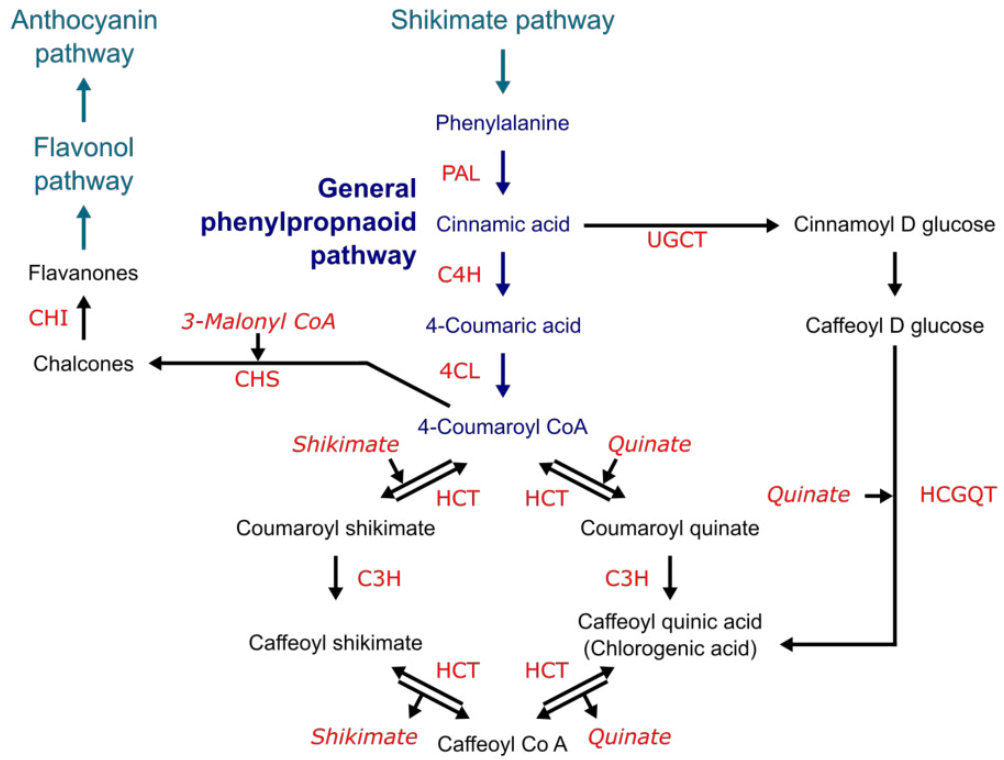

Figure 6: Phenylpropanoid pathway. Phenylalanine from the shikimate pathway is converted by phenylalanine ammonia-lyase (PAL) to cinnamic acid which is subsequently converted by cinnamate 4-hydroxylase (C4H) to 4-coumaric acid. 4-coumaroyl:CoA-ligase (4CL) then catalyses 4-coumaric acid to 4-coumaroyl CoA. Chalcone synthase (CHS) then converts 4-coumaroyl CoA with 3-malonyl CoA to chalcones and chalcone isomerase (CHI) further to flavanones, basis for the biosynthesis of flavonoids and anthocyanins. 4-coumaroyl CoA can also be converted by hydroxycinnamoyl-CoA shikimate hydroxycinnamoyl transferase (HCT) to coumaroyl shikimate respectively coumaroyl quinate, who are further converted by p-coumarate 3-hydroxylase (C3H) forming caffeoyl shikimate respectively caffeoyl quinate (chlorogenic acid, CGA). HCT further catalyses the reaction of caffeoyl shikimate respectively caffeoyl quinate to caffeoyl CoA. Alternatively, cinnamic acid can be converted to cinnamoyl D-Glucose by UDP-glucose:cinnamate glucosyl transferase (UGCT), which is further converted to caffeoyl D glucose followed by the catalysation with quinate by hydroxycinnamoyl glucose:quinate hydroxycinnamoyl transferase (HCGQT) to form CA (adapted from Hoffmann et al. (2004) and Payyavula et al. (2013)).

Another part of direct inducible defences are jasmonate-inducible defensive proteins, such as anti-nutritive proteinase inhibitors (PIs) or polyphenol oxidases (PPOs; Kessler and Baldwin (2002a); Chen (2008); War et al. (2012); Fürstenberg-Hägg et al. (2013)).

Digestive enzyme inhibitors as defence mechanism were first discovered by Green and Ryan (1972). The authors revealed that in response to insect attack potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*) plants rapidly accumulate PIs, while the induction was mediated by a systemic signal which could be activated by wounding (Green and Ryan, 1972). Since then, PIs have been extensively studied for their roles in plant defence. As abundant proteins in reproductive, storage and vegetative tissues, PIs are an important strategy of natural plant defence against phytophagous insects, particularly against lepidopteran insects (War et al., 2012; Parde et al., 2012; Jadhav et al., 2016). The generally accepted mode of action is that PIs inhibit digestive proteases, which catalyse the hydrolytic cleavage of peptide bonds in insect guts resulting in amino acid deficiencies and thereby developmental delay, mortality and/or reduced fecundity (Gatehouse, 2011; Zhu-Salzman and Zeng, 2015). However, through the long coevolution with their host plants, insects also have adapted sophisticated mechanisms to circumvent antinutritional effects of dietary challenges (Zhu-Salzman and Zeng, 2015).

Another group of anti-nutritive proteins are PPOs (Constabel and Barbehenn, 2008; War et al., 2012). These enzymes catalyse the oxidation of phenolic compounds to reactive and polymerizing quinones, which decrease the nutritive value and/or impair the nutrient uptake by crosslinking with nucleophilic side chains of proteins and fee amino acids (Kessler and Baldwin, 2002a; Chen, 2008; War et al., 2012; Fürstenberg-Hägg et al., 2013). Furthermore, PPOs can produce oxidative stress in the gut lumen and produced ROS could have toxic effects on herbivores (War et al., 2012; Bhonwong et al., 2009). Indication for an involvement of PPOs in plant anti-herbivore defence is given by the observation that insect growth suppression was found when herbivores were fed by PPO supplemented artificial diet (Felton et al., 1992) and the high stability of ingested, active PPOs in insect guts (Chen et al., 2005). Furthermore, in distinct plant insect interactions the induction of PPO activity and herbivore performance has been reported to be negatively correlated (Bhonwong et al., 2009; Sethi et al., 2009).

2.4 Plant responses to insect oviposition

The first contact of plants and herbivorous insects often precedes the actual attack, as the herbivorous insects often deposit their eggs directly on various organs of the host plant of the larvae (Hilker and Fatouros, 2015, 2016). The mode of oviposition thereby varies vastly among insect species, ranging from singly or gregariously laid eggs to lose or tight attachment of the eggs to the leaf surface, insertion of eggs in cavities after scratching the leaf cuticle or egg deposition after the mesophyll tissue is wounded (Reymond, 2013; Hilker and Fatouros, 2015).
However, oviposition indicates a particularly high risk of herbivory for the plant. After perception of the insect eggs and subsequent signalling to process the information (see 2.4.1) the plants could induce defence responses targeting the eggs which reduce or prevent the impending herbivory (see 2.4.2). Moreover, oviposited plants might prime the anti-herbivore defence response to the subsequent feeding herbivores (see 2.4.3).

2.4.1 Perception of insect eggs and subsequent signalling

In order to react appropriately to insect eggs and potentially inherent danger, plants need to perceive the eggs. Similar to perception of DAMPs and HAMPs, plants are expected to perceive egg derived or associated elicitors, referred to as egg associated molecular patterns (EAMPs), which are assumed to be located in secretions released by oviposition that cover the eggs and the interspace between the eggs and the plant (Hilker and Fatouros, 2015). So far, only a few EAMPs have been identified. The first described egg-associated elicitors, referred as "bruchins" (C22-C24 long-chain α,ω-diols esterified at one or both ends with 3-hydroxypropanoic acid), were isolated and characterized in the interaction of bruchid weevils (*Bruchus pisorum*) and pea (*Pisum sativum*) (Doss et al., 1995, 2000). In *A. thaliana*, accessory reproductive gland (ARG) secretions of *P. brassicae* covering the eggs evoke responses in a dose dependent manner similar to those after oviposition suggesting that these secretions contain the elicitor (Paniagua Voirol et al., 2020). Other studies with *A. thaliana* and *Brassica oleracea* found that the ARG secretions of *P. brassicae* and *P. rapae* females contain the active elicitor only after mating, as the active elicitors (i.e. *P. brassicae*: benzyl cyanide, *P. rapae*: indole) are antiaphrodisiacs received by the females from males and released with the eggs onto the plant (Fatouros et al., 2008, 2009; Blenn et al., 2012). Recently, phosphatidylcholines (PCs) released from *P. brassicae* eggs were

identified as EAMPs in *Arabidopsis*, as these conserved molecules (primarily C16 to C18 fatty acyl chains with various levels of desaturation) diffuse out of the eggs and induce plant responses comparable to oviposition or treatment with egg extract (Stahl et al., 2020). Presumably proteinaceous elicitors were found in the oviduct secretions covering the eggs of the pine sawfly (*Diprion pini*) and the elm leaf beetle (*Xanthogaleruca luteola*) (Meiners and Hilker, 2000). In addition, *Pinus sylvestris* is able to perceive the sex pheromone of *D. pini* and prime its defence response against the eggs accordingly (Bittner et al., 2019). It is assumed that plants perceive EAMPs via specific plasmamembrane bound receptors (Reymond, 2013), although heretofore no such receptor has been identified. However, Gouhier-Darimont and colleagues demonstrated that in *A. thaliana* leaves an L-type lectin receptor kinase (LecRK-I.8) is an early component of *P. brassicae* egg perception (Gouhier-Darimont et al., 2013, 2019). LecRK-I.8 could also be involved of PCs originating from *P. brassicae* eggs, because responses in the knock-out lecrk-I.8 mutant PC treatment as well as oviposition or treatment with egg extract were drastically reduced, although not fully abolished (Stahl et al., 2020).

After perception, plants deploy a signalling cascade to transduce and process information to induce adequate countermeasures (Hilker and Meiners, 2011; Reymond, 2013; Hilker and Fatouros, 2015), similar to the signalling cascade in response to herbivory (see 2.3.2). Several plant species accumulate ROS (see 2.3.2 Processing of the herbivore signal) in leaf tissue beneath oviposited eggs (Little et al., 2007; De Puysseleyr et al., 2011; Kim et al., 2012; Bittner et al., 2017; Geuss et al., 2017). In *Arabidopsis*, superoxide and hydrogen peroxide accumulate in response to oviposition, but this accumulation is independent two NAPDH oxidases (Gouhier-Darimont et al., 2013). ROS production in leaf tissue beneath *S. exigua* eggs correlated with the accumulation of several peroxidase gene transcripts in *S. dulcamara*, while NADPH oxidase gene expression was not altered (Geuss et al., 2017).

Signalling by phytohormones are known to play a role in mediating plant responses to insect eggs, although knowledge is only fragmentary (Reymond, 2013; Hilker and Fatouros, 2015). It is not surprising that oviposition by certain insect species which inflict wounding during oviposition is associated with accumulation or subsequent signalling of the wound hormone JA (see 2.3.3.1). When pine and elm trees are treated with JA, these trees emit similar volatile blends as trees oviposited by herbivore species that wound plant tissue during oviposition (Meiners and Hilker, 2000; Hilker et al., 2005). Moreover, elm leaf beetle oviposition on elm trees and of oviposition of an omnivorous pirate bug on tomato, which both include epi-

dermal wounding during oviposition, induce expression of JA biosynthesis genes (De Puysseleyr et al., 2011; Altmann et al., 2018; Büchel et al., 2012). However, JA-related plant responses to herbivore eggs do not necessarily require ovipositional wounding. Lepidopteran herbivore oviposition, which is not associated with wounding, on *S. dulcamara* and tomato plants for instance, locally triggers the expression of JA-responsive genes although JA levels remain unaltered (Kim and Felton, 2013; Geuss et al., 2017).
Furthermore, SA (see 2.3.3.3) is assumed to play a major role in plant responses to oviposition. In various plant-insect interactions, oviposition or treatment of leaves with egg extract caused an accumulation of SA or induction of SA-related transcripts (Bruessow et al., 2010; Gouhier-Darimont et al., 2013; Hilfiker et al., 2014; Geuss et al., 2017; Bonnet et al., 2017; Lortzing et al., 2019). In addition, several plant species induced SA-related transcripts for example PR genes like PR1 (Little et al., 2007; Bruessow et al., 2010; Gouhier-Darimont et al., 2013; Hilfiker et al., 2014; Geuss et al., 2017; Bonnet et al., 2017; Bittner et al., 2019; Lortzing et al., 2019). Especially in the leaf tissue directly beneath the eggs, a high SA accumulation and associated transcriptional induction was detected in *S. dulcamara* and *A. thaliana* (Little et al., 2007; Bruessow et al., 2010; Geuss et al., 2017). Accompanied by JA- and SA-related transcript accumulation, oviposition triggers a substantial transcriptional reprogramming (Reymond, 2013). Transcriptome analyses of elm, *A. thaliana*, *B. nigra* and *S. dulcamara* in response to oviposition for instance revealed cross-species induction of genes involved in defence to pathogens, response to oxidative stress and phenylpropanoid metabolism (Little et al., 2007; Firtzlaff et al., 2016; Bonnet et al., 2017; Geuss et al., 2017; Altmann et al., 2018; Drok et al., 2018).

2.4.2 Plant defence against insect eggs

To solve problems before they arise, plants can induce defence responses against the highly vulnerable insect eggs and prevent or reduce the impending herbivory (reviewed in Hilker and Fatouros (2015, 2016)). Plants can mount various defence responses directly targeting the eggs that may result in dropping, crushing, desiccation or intoxication of the insect eggs (Hilker and Fatouros, 2015, 2016). To do so, plants can induce growth of neoplasms (limited non-meristematic growth) in the leaf tissue under the eggs leading to detachment (Doss et al., 1995, 2000; Petzold-Maxwell et al., 2011; Geuss et al., 2017). Oviposition can further induce growth responses that physically affect the eggs and thereby reduce egg survival,

like oviposition by the leaf beetle *Pyrrhalta viburni* on stems of *Viburnum* species which elicits tissue growth at the oviposition site that displaces the egg cap, partially crushes the eggs and encases egg masses (Desurmont and Weston, 2011). In response to insect eggs, several plant species exhibit chlorotic or necrotic responses accompanied by the production of ROS at oviposition sites which are paralleling a hypersensitive response (e.g. Little et al. (2007); Petzold-Maxwell et al. (2011); Kim et al. (2012); Fatouros et al. (2014); Bittner et al. (2017); Geuss et al. (2017); Gouhier-Darimont et al. (2013); Griese et al. (2020)). For example, in pine needles oviposited by *D. pini* a locally egg-induced ROS accumulation with HR-like symptoms occurs which results in reduced hatching from the eggs (Bittner et al., 2017). Also, in the bittersweet nightshade *S. dulcamara* oviposition induced accumulation of the ROS hydrogen peroxide, which was shown to directly act as an ovicidal metabolite increasing egg mortality (Geuss et al., 2017). Moreover, plants can induce the production of other ovicidal compounds like, for instance, benzyl benzoate at the oviposition sites of the planthopper Sogatella furcifera on rice plants (Seino et al., 1996; Suzuki et al., 1996).

In addition to directly targeting the eggs, plants can further attract predators or parasitoids of the insect eggs as indirect defence by changing the leaf odor or the leaf surface chemistry (Hilker and Fatouros, 2016). Such indirect defence responses against the eggs are often highly specific with respect to the plant and insect species (Hilker and Fatouros, 2015). In several plant insect interactions, emissions of oviposition induced plant volatiles (OIPVs) were shown to attract egg parasitoids (e.g. Meiners and Hilker (2000); Hilker et al. (2005); Fatouros et al. (2008, 2009); Büchel et al. (2011); Blenn et al. (2012); Fatouros et al. (2014)). For instance, oviposition by the elm leaf beetle (*Xanthogaleruca luteola*) on leaves of *Ulmus minor* induce the emission of OIPVs, especially terpenoid volatiles, which attracts the specialised egg parasitoid of *X. luteola*, the eulophidwasp (*Oomyzus gallerucae*) (Büchel et al., 2011). However, plants can not only attract parasitoids that kill the herbivore eggs, OIPVs can also cause an early attraction of larval parasitoids (Bruce et al., 2010; Fatouros et al., 2012; Pashalidou et al., 2015b). In addition to volatile emission, oviposition induces changes in the chemistry of leaf surface waxes that tend to retain parasitoids on leaves with herbivore eggs (Blenn et al., 2012; Fatouros et al., 2005).

Direct or indirect plant defences targeting the eggs are not mutually exclusive. For instance, *B. nigra* plants reducing egg survival by exhibiting a hypersensitive response-like necrosis at the oviposition site (direct defence) also attracted egg parasitoids (*Trichogramma* spp.) by OIPVs (indirect defence) (Fatouros et al.,

2014). Furthermore, in some plant insect interactions OIPVs or other oviposition induced changes in the leaf odor can cause deterrence of further oviposition (Hilker et al., 2005; Fatouros et al., 2009, 2012; Blenn et al., 2012).

2.4.3 Priming of anti-herbivore defence by oviposition

Besides inducing defences which target the eggs, plants can take the insect oviposition as a warning cue for imminent herbivory and prime the following stress response, i.e. the subsequent anti-herbivore defence against the feeding larvae. In this context, oviposition represents the priming stimulus and the larval feeding the triggering stimulus/stress. In parallel to defence priming by volatiles, oviposition-mediated priming of anti-herbivore defence is assumed to improve the defence response resulting in a benefit for the primed plant (see 2.2.4).
But why not directly mounting defences upon oviposition? Although it is probable that herbivory will occur after oviposition, direct or indirect defences targeting the insect eggs (see 2.4.2) or unfavourable abiotic conditions may prevent larval hatching and subsequent herbivory. Opposite to high costs associated with directly inducing anti-herbivore defences in response to oviposition, costs of priming are assumed to be be relatively small before the actual attack occur (Hilker et al., 2016; Martinez-Medina et al., 2016). Consequently, oviposition priming could enable the plants to prepare themselves for a more efficient defence against the larvae but save costs in all occasions when larvae do not hatch. Moreover, a major drawback of induced defence responses, i.e. the lag time until produced defence against the herbivore has full effectiveness (Cipollini et al., 2003; Karban, 2011), might be curtailed when responding to stimuli that reliably predict herbivory (Hilker et al., 2016; Martinez-Medina et al., 2016; Hilker and Schmülling, 2019). An earlier or faster defence response (see Fig. 1) due to oviposition-mediated priming after the initial larval feeding could be advantageous for the plant.
The effect of prior oviposition by various insect species (especially Lepidoptera, but also Hemiptera, Hymenoptera or Coleoptera) on the anti-herbivore defence was examined in various plants ranging from annual herbaceous plants of Brassicaceae, Fabaceae or Solanaceae to a perennial shrub and two tree species (see Tab. 1). Furthermore, a study investigating the effect of prior oviposition on larval performance of *P. brassicae* on different Brassicaceae species, found a reduced larval weight after seven days of larval feeding when larvae feed on prior oviposited plants (not included in Tab. 1 because differences between oviposited and non-

oviposited plants were pooled and not separated for each plant species; Griese et al. (2020). Almost all studies found a diminished or impaired performance (mortality, weight, and/or development) of herbivores feeding on prior oviposited plants or those treated with egg-extract compared to herbivores feeding on plants exposed to feeding only (see Tab. 1, highlighted red). Although found in different interactions, the observation that oviposition by *M. brassica* did not cause an effect on larval performance of conspecific larvae or larvae of *P. brassicae*, while oviposition of *P. brassicae* did affect larval performance of both herbivore species, indicate some kind of species specificity (Pashalidou et al., 2013). The missing effect of oviposition priming on larval performance on larvae of the tobacco specialist *M. sexta* (Bandoly et al., 2016) is probably based on the high specialization of the herbivore to the plant (see 2.5.2). Interestingly, *M. sexta* larvae feeding on oviposited plants had a reduced antimicrobial activity in their haemolymph, which could be associated with a reduced pathogen resistance and might resemble an indirect plant defence via entomopathogens (Bandoly et al., 2016). The observation that oviposition-mediated priming can enhance indirect defences was shown in the interaction of *B. nigra* and *P. brassicae*. Oviposition by *P. brassicae* caused an earlier attraction of larval parasitoids (Pashalidou et al., 2015c) and under field conditions higher parasitism rates compared to plants exposed to larval feeding only (Pashalidou et al., 2015b).

Compared to the effect of oviposition mediated priming on the herbivore, the consequences of oviposition mediated priming for the plant received much less attention (see Tab. 1, highlighted with a yellow background). Probably associated with the impaired herbivore performance, plants which experienced oviposition before larval feeding had less feeding damage (Bandoly et al. (2015); Geiselhardt et al. (2013); Drok et al., unpublished). Further knowledge on consequences of oviposition priming for the plant fitness is sparse. Consequently, adequate proof for the assumed improved plant fitness of oviposition primed plants compared to non-primed plants (Hilker et al. (2016); Martinez-Medina et al. (2016), see 2.2.4) is missing. Black mustard (*B. nigra*) plants exposed to oviposition and larval feeding by *P. brassicae* had a greater increase in plant height and flower earlier compared to plants which were exposed to larval feeding only (Pashalidou et al., 2013). Furthermore, the number of seeds from plants exposed to oviposition and larval feeding by *P. brassicae* were higher than of untreated control plants (Pashalidou et al., 2015b).

While most studies described comparable effects caused by the primed response on the organismic level (i.e. an impaired or diminished herbivore performance), rela-

tively little is known about the molecular or physiological mechanisms involved in causing such a primed response (see Tab. 1, highlighted with a blue background). On the phytohormonal level, changes caused by oviposition priming are not fully understood. In two Brassicaceae (*A. thaliana* and *B. nigra*), SA levels of oviposited and feeding induced plants were higher than in plants exposed to larval feeding only (Bonnet et al., 2017; Lortzing et al., 2019; Valsamakis et al., 2020). However, in other plant species no feeding induced SA accumulation was detected. A few studies with mutants impaired in phytohormonal accumulation or signalling revealed the relevance of phytohormonal signalling for oviposition-mediated priming (see Tab. 1, highlighted with a yellow background). In *A. thaliana* mutants impaired in SA or JA accumulation or signalling, the role of SA and JA signalling for facilitating an oviposition-mediated impaired herbivore performance was highlighted (Lortzing et al., 2019; Valsamakis et al., 2020). Deficiency of JA-related NaMYB8 in the wild tobacco also abolishes the effect of prior oviposition on feeding larvae (*S. exigua*: larval mortality, *M. sexta*: reduced antimicrobial activity), indicating an involvement of JA (Bandoly et al., 2015, 2016). However, in *S. dulcamara* and *N. attenuata* no altered phytohormonal induction was detectable between oviposited or non-oviposited plants after 24 h of larval feeding (Bandoly et al., 2016; Geuss et al., 2018). In contrast, a higher JA accumulation and an increased expression of JA-related transcripts in oviposited compared to non-oviposited plants were found in tomato plants shortly (30 min and 1 h) after simulated herbivory (Kim and Felton, 2013). Also, phytohormonal and transcriptional induction in Arabidopsis and elm further points to an earlier or faster response to larval feeding after oviposition (Altmann et al., 2018; Valsamakis et al., 2020).

A recently published study Lortzing et al. (2020) compared transcriptomic data from previously published experiments (see Tab. 1) investigating oviposition-mediated priming in *N. attenuata* (Drok et al., 2018), *A. thaliana* (Lortzing et al., 2019), *U. minor* (Altmann et al., 2018) and *S. dulcamara* (Geuss et al., 2018, 2017). Using Generally Applicable Gene set Enrichment (GAGE) on gene ontology terms, the authors found a considerable overlap in the transcriptomic responses to both eggs and larval feeding (Lortzing et al., 2020). This overlap comprised gene sets related to several phytohormones and to the phenylpropanoid biosynthesis pathway, of which specific branches were activated in different plant–insect combinations (Lortzing et al., 2020). Such a pattern was also observed when considering oviposition mediates changes on the metabolite level. Different phenylpropanoid derivatives were higher induced in prior oviposited plants upon feeding compared

to feeding induced plants without oviposition (Bandoly et al., 2015, 2016; Austel et al., 2016; Geuss et al., 2018; Lortzing et al., 2019). For example, caffeoylputresine, a phenylpropanoid-polyamine conjugate, was higher induced in oviposited and feeding exposed *N. attenuata* plants and found to be responsible for the reduced performance of *S. exigua* on oviposited plants (Bandoly et al., 2015, 2016). In *A. thaliana* flavonol levels in oviposited and feeding-damaged plants were higher induced (Lortzing et al., 2019). Beside phenylpropanoids, metabolite analyses also found feeding-induced glycosinolates (Geiselhardt et al., 2013; Lortzing et al., 2019), leaf volatiles (Pashalidou et al., 2015c) or proteinase inhibitor activity (Bandoly et al., 2015, 2016) affected by prior oviposition.

Table 1: Studies investigating oviposition-mediated priming of anti-herbivore defence. An experimental result was referred as oviposition-mediated effect or change when the considered parameter was significantly different expressed between plants exposed to larval feeding or simulated herbivory without (T) and with prior oviposition (PT) (* = difference between control (C) and PT plants, no difference between T and PT or C and T plants). Parameters connected to the ecological effect are coloured red, physiological parameters are coloured blue. Parameters related to plant growth, performance or reproduction are highlighted with a yellow background, results of mutant studies are highlighted with a blue background.

Plant species	Herbivore species	Oviposition-mediated effect	References
Solanaceae			
Nicotiana attenuata	*Manduca sexta (Lepidoptera)*	Reduced antimicrobial activity in larval haemolymph, deficiency of NaMYB8 abolishes the effect on antimicrobial activity, increased content of phenylpropanoid derivatives (caffeoylputrescine)	Bandoly et al. (2016)
Nicotiana attenuata	*Spodoptera exigua Lepidoptera)*	Higher mortality, reduced larval weight, lower feeding damage, retarded larval development, Increased accumulation of phenylpropanoid derivatives (caffeoylputrescine), higher TPI activity, increased expression of a transcription factor regulating PPC (NaMYB8), deficiency of NaMYB8 abolishes the effect on mortality	Bandoly et al. (2015)
		When oviposited by *M. sexta*: Higher mortality, reduced larval weight, higher TPI activity, deficiency of NaMYB8 abolishes the effect on mortality, when oviposited by conspecific: Higher mortality	Bandoly et al. (2016)
Solanum lycopersicum	*Helicoverpa zea (Lepidoptera)*	Higher expression of genes encoding for defence proteins (protease inhibitor), stronger JA accumulation	Kim et al. (2012)
Solanum dulcamara	*Spodoptera exigua (Lepidoptera)*	When larvae were encaged on single leaves: Higher mortality (beginning) and reduced larval weight; when larvae were released to the hole plant: Higher mortality (until pupation) and higher larval weight, altered transcriptional regulation (microarray) and changes of primary metabolite contents (phenylpropanoids)	Geuss et al. (2018)
Solanum dulcamara	*Acrolepia autumnitella (Lepidoptera)*	Reduced larval size (correlated with larval weight), prolonged time until pupation, lower feeding damage	Drok et al., unpublished

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Plant species	Herbivore species	Oviposition-mediated effect	References
Brassicaceae			
Arabidopsis thaliana	*Pieris brassicae (Lepidoptera)*	Reduced larval weight, higher mortality, lower feeding damage, reduced level of glucosinolates and suppressed feeding-induced expression of genes related to glucosinolate metabolism	Geiselhardt et al. (2013)
		Reduced larval weight, increased SA level, altered transcriptional regulation (Microarray), increased levels of flavonoids (phenylpropanoid) deficiency of genes involved in SA accumulation or signalling abolish the effect on larval weight (pr5, sid2, pad4 and ald1 mutants)	Lortzing et al. (2019)
		Reduced larval weight, increased SA level, increased JA-Ile level (after 3h of larval feeding, effect vanished after 12h of feeding), increased ABA levels (after 12h of feeding), In mutants of sid2 (impaired SA accumulation) and jar1-1 (reduced JA-Ile accumulation) the effect on larval weight is abolished	Valsamakis et al. (2020)
		Reduced larval and pupal weight, Prolonged time until pupation	Paniagua Voirol et al. (2020)
Brassica nigra	*Pieris brassicae (Lepidoptera)*	Under field conditions: Reduced larval and pupal weight, reduced parasitoid and hyperparasitoid weight, higher parasitism rates Higher number of seeds*	Pashalidou et al. (2015b)
		Earlier attraction of parasitoids, reduced parasitoid weight	Pashalidou et al. (2015c)
		Reduced larval weight (prior treatment with egg extract), altered transcriptional regulation (Microarray), Increased SA level, reduced feeding-induced expression of JA-related genes, higher expression of SA-related genes (although higher induced by egg extract treatment alone)	Bonnet et al. (2017)
		Under laboratory and semi-field conditions: Reduced larval weight, Increased plant height, earlier flowering	Pashalidou et al. (2013)
		Reduced larval weight, higher mortality, prolonged time until pupation	Pashalidou et al. (2015a)
		Reduced larval weight (in response to oviposition and to volatiles of oviposited plants)	Pashalidou et al. (2020)
Brassica nigra	*Mamestra brassicae (Lepidoptera)*	When oviposited by *P. brassicae*: Reduced larval weight; Plants exposed to oviposition by *P. brassicae* and larval feeding by *M. brassicae*: Increased plant height, Larval performance not affected when oviposited by conspecific	Pashalidou et al. (2013)
Sinapis arvensis	*Pieris brassicae (Lepidoptera)*	Reduced larval weight, higher mortality, prolonged time until pupation	Pashalidou et al. (2015a)
Moricandia moricandioides	*Pieris brassicae (Lepidoptera)*	Reduced larval weight, higher mortality, prolonged time until pupation	Pashalidou et al. (2015a)
Brassica oleracea	*Pieris brassicae (Lepidoptera)*	Reduced larval weight, higher mortality, prolonged time until pupation	Pashalidou et al. (2015a)
		Reduced larval weight (in response to oviposition and to volatiles of oviposited plants)	Pashalidou et al. (2020)

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Plant species	Herbivore species	Oviposition-mediated effect	References
Fabaceae			
Vicia faba	*Halyomorpha halys (Hemiptera)*	Reduced nymph weight, higher and more rapid transcriptional induction of JA-dependent genes, increased induction of PR1 transcripts	Rondoni et al. (2018)
Pinaceae			
Pinus sylvestris	*Diprion pini (Hymenoptera)*	Reduced larval, cocoon and adult female weight, higher larval mortality, reduced fecundity of females (next generation)	Beyaert et al. (2011)
Ulmaceae			
Ulmus minor	*Xanthogaleruca luteola (Coleoptera)*	Higher larval mortality, reduced larval and adult weight, lower number of females, male-biased shift in the sex ratio of adults in the 2nd generation (field conditions), increased consumption of phenylpropanoid derivatives (robinin), higher content of phenylpropanoid derivatives (robinin)*	Austel et al. (2016)
		Altered transcriptional regulation (RNAseq): points to an earlier or faster response to larval-feeding	Altmann et al. (2018)

2.5 Study system

Two different solanaceous plant species were examined in this thesis in interaction with generalist and specialist herbivores (see Fig. 7). On the one side, *S. dulcamara*, the perennial bittersweet nightshade was investigated in interaction with the specialist leaf miner *A. autumnitella* and the generalist beet armyworm *S. exigua*. On the other side, the annual wild tobacco (*N. attenuata*) was examined in interaction with the generalist *S. exigua* and in interaction with the tobacco specialist *M. sexta* (tobacco hornworm).

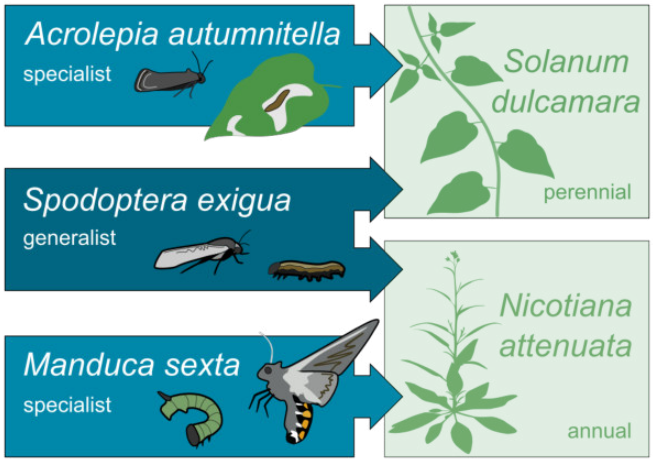

Figure 7: Overview of the model systems. *S. dulcamara* (bittersweet nightshade) was investigated in interaction with the specialist leaf miner *A. autumnitella* and the generalist herbivore *S. exigua* (beet armyworm). *N. attenuata* (wild tobacco) was examined in interaction with *S. exigua* and the tobacco specialist *M. sexta* (tobacco hornworm).

2.5.1 The bittersweet nightshade and its interaction with herbivores

As the name reveals, *S. dulcamara* (Linnaeus), the bittersweet nightshade, belongs to the Solanaceae and is a close relative to important crop species like potato, eggplant, pepino or tomato (Lester, 1991; Amiryousefi et al., 2018). This perennial plant is native to greater parts of Eurasia and Northern Africa, but it also occurs as an invasive species in Northern America, Australia and New Zealand (Howell, 2008; Knapp, 2013). *S. dulcamara* is a polymorphic and phenotypically plastic species, characterized by intraspecific genetic and phenotypic variation within and among populations (D'Agostino et al., 2013; Zhang et al., 2016b; Geuss et al., 2017; Calf et al., 2018, 2019). High levels of phenotypic plasticity in response to abiotic conditions allow this species to thrive in urban areas as well as in undisturbed natural sites either as a bush or a winding climber (see Fig. 8 a), with habitats ranging from relatively dry sandy coastal areas to regularly inundated floodplains (Dawood et al., 2014; Calf et al., 2018; Visser et al., 2016). The purple flowers are

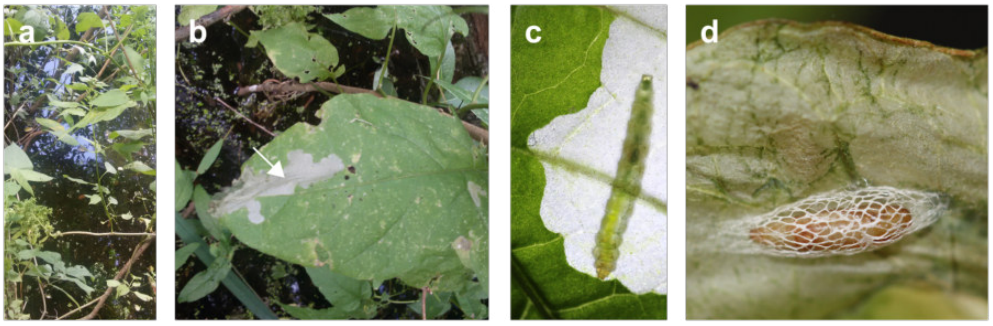

Figure 8: The bittersweet nightshade *S. dulcamara* and one of its herbivores, *A. autumnitella*. (a) *S. dulcamara* in its natural habitat, (b) Feeding damage by *A. autumnitella* (typical feeding damage denoted with a white arrow) and other herbivores on *S. dulcamara* leaves in the field, (c) Feeding *A. autumnitella* larvae, (d) Pupae of *A. autumnitella* silk net cage.

mostly pollinated by bumblebees (*Bombus* spp.), as their buzzing behaviour is essential for releasing the pollen from the anther cone (Calf et al., 2012). Pollinated flowers convert into a green, matured red globular berry.

S. dulcamara plants at undisturbed natural sites typically show small holes and other evidence of herbivory (see Fig. 8 b), indicating for an adapted herbivore community. In Central Europe, this herbivore community mainly consists of beetles, but also specialist lepidopteran herbivores, like *Acrolepia autumnitella* (Curtis) (Calf et al., 2012). Larvae of these small moths feed as leaf miners from *S. dulcamara* mesophyll of leafs, resulting in clear blotch mines (see Fig. 8 b white arrow). Larvae dispose its faeces outside of the mine and throughout development they mine into several leaves. When the larvae are fully grown, they crawl out of the mine and pupate in a delicate silk net cage (see Fig. 8 d). *A. autumnitella* has two generations per year and larvae can be found from June to September (Calf et al., 2012).

To cope with these herbivores, the bittersweet nightshade evolved a vast array of direct and indirect defences. Ecological research in this context dates back almost 40 years when the defence of the plant against the Colorado potato beetle (*Leptinotarsa decemlineata*) was investigated, as adults and larvae of this species also feed and successfully reproduce on *S. dulcamara* (Hare, 1983). Since then, several studies investigated interactions of *S. dulcamara* with herbivores, ranging from gall-mites (e.g. Westphal et al. (1981); Bronner et al. (1991b,a); Westphal et al. (1991)) to herbivorous beetles (e.g. Viswanathan and Thaler (2004); Viswanathan et al. (2005, 2007); Lortzing et al. (2016)) or even slug herbivory (Calf et al., 2018, 2019; Lortzing et al., 2016; Calf et al., 2020)).

In response to herbivory, *S. dulcamara* exhibits a remarkable indirect defence mechanism. Following herbivory on leaves, petioles, and stems, *S. dulcamara* secretes without specific structures wound secretions which are functionally equivalent to extrafloral nectar secretions involved in indirect defence (Lortzing et al., 2016). These wound secretions attract ants which protect plants from slug feeding and stem herbivory by flea beetle larvae (Lortzing et al., 2016). Additionally, *S. dulcamara* is equipped with several direct defences. Like several other *Solanum* species, *S. dulcamara* produces steroidal glycolalkaloids (GA), which are highly toxic and deterrent to many organisms (Eich, 2008; Milner et al., 2011; Calf et al., 2018, 2019). These alkaloids appear to play a key role in defence against gastropods, as variation in slug preference correlated with variation in GA between different accessions from the Netherlands (Calf et al., 2018, 2019). Also, in exposure to the natural herbivore community plants with low GA level received highest gastropod feeding, while flea beetles preferred to feed on plants with high GA contents (Calf et al., 2019). Furthermore, *S. dulcamara* induces the production of defensive proteins, like for example PIs, in response to larval herbivory by the beet armyworm (*S. exigua*, will be introduced later on, see 2.5.2) (Geuss et al., 2018; Nguyen et al., 2016), a generalist herbivore with a wide host range (Greenberg et al., 2001). Additionally, beetle herbivory was shown to induce biosynthesis of defensive proteins (Viswanathan and Thaler, 2004; Viswanathan et al., 2007).
Within one of these studies, also the plant´s vascular architecture was examined. By inserting a water-soluble dye (rhodamine-B) to a petiole and tracking its distribution through the vascular system, one can visualize the vascular connectivity between leaves (Viswanathan and Thaler, 2004). The three next younger leaves

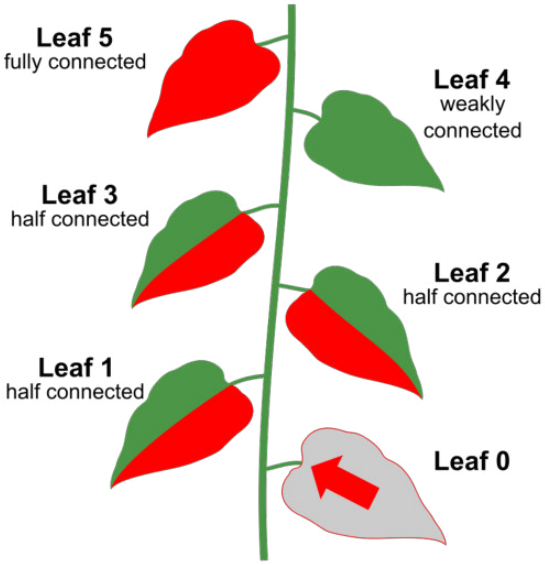

Figure 9: Illustration of the vascular connection between leaves of *S. dulcamara*. The vascular connection of the leaf 0 in relation to leaves 1 to 5 in red. Leaves 1 to 3 are half connected with leaf 0, while leaf 4 is only weakly connected. Leaf 5 is fully connected with leaf 0 (adapted from Viswanathan and Thaler (2004)).

(leaf 1-3) showed half connectivity to the dye treated leaf (leaf 0, see Fig. 9). While the fourth leaf did only show a weak vascular connectivity, the fifth leaf showed full connectivity to the dye treated leaf (leaf 0, see Fig. 9). Interestingly, also the larval performance on leaves with distinct vascular connection differed (Viswanathan and Thaler, 2004). Feeding of adult three-lined potato beetle (*Lema trilinea*) on one *S. dulcamara* leaf decreased the mass gain of subsequently feeding larvae on the leaf five positions higher (vascularly fully connected to the feeding leaf), while larvae feeding on the fourth leaf (weakly connected to the feeding leaf), gained more mass than larvae feeding on previously undamaged plants (Viswanathan and Thaler, 2004).
But the defences of *S. dulcamara* can also be used by the herbivore to defend themselves. Tortoise beetles (*Plagiometriona clavata*) for example deposit feces on their back as shield barrier against predators and incorporation of e.g. steroidal alkaloids from their diet into their fecal shield makes them even more unappetizing for predators (Morton and Vencl, 1998; Vencl et al., 1999).

2.5.2 The wild tobacco and its interaction with herbivores

Nicotiana attenuata (Torr. Ex Watson), the wild tobacco belongs, as well as *S. dulcamara*, to the genus Solanum. This postfire annual is an ephemeral member of the annual community in burned sagebrush, blackbrush and pinyon-juniper forests of the Great Basin Desert in the USA. Seeds can rest dormant in the soil for up to 150 years (Baldwin et al., 1994; Preston and Baldwin, 1999), until they sense smoke cues from the burned biomass, respond to compounds from unburned litter and initiate germination synchronously (Baldwin et al., 1994; Schwachtje and Baldwin, 2008). This synchronization allows the species to exploit the ephemeral but nutrient-rich herbivore- and competitor-poor environments after fires (Wright and Bailey, 1982; Baldwin et al., 1994; Whelan, 1995). Consequently, *N. attenuata* plants grow under high intra- but low interspecific competition. With proceeding postfire succession the temporal window of growth opportunity for *N. attenuata* is quite short because herbivores and competitors quickly recolonize the burned habitats and populations decline with the rise of immigrating competitors and disappearance of ash (Baldwin, 1998). However, as herbivores have to migrate and recolonize the burned region and establish a new population, their occurrence and abundance highly variable and unpredictable.

A wide range of herbivores from different taxa, feeding guilds and with varying diet spectrum attack the wild tobacco. For example, sucking herbivores like mirid bug *Tupiocoris notatus*, phloem feeding aphids from genera *Myzus* or stem-borers like the tobacco stalk-borer *Trichobaris mucorea* colonize the wild tobacco (Glawe et al., 2003; Heidel and Baldwin, 2004; Diezel et al., 2011). Two of the most abundant lepidopteran herbivores in the Great Basin Desert (USA) are the leaf-chewing larvae of the tobacco hornworm *M. sexta* and larvae of the beet armyworm *S. exigua*, which are furthermore major defoliators of *N. attenuata* in this native habitat (Steppuhn et al., 2004; Zavala and Baldwin, 2004).

The generalist herbivore *S. exigua* (Hubner; Lepidoptera, Noctuidae), also known as beet army worm or small mottled willow moth, is polyphagous and feeds from foliage and fruits of a wider range of different host plants (Greenberg et al., 2001; Azidah and Sofian-Azirun, 2006). Nocturnal moths lay their eggs in clutches (5-300 eggs) that can be covered with fibres or threads (Skudlik et al., 2005). Larvae hatch after three or four days and pass through five to eight instars in their life span of two week (Greenberg et al., 2001; Azidah and Sofian-Azirun, 2006). Larvae pupate in the soil and the adults emerge after one and a half to two weeks, so that a life cycle lasts three to four weeks. Occasionally, larval feeding by S. exigua can be responsible for half of the canopy lost to herbivores in wild populations (Steppuhn et al., 2004).

Another major defoliator of *N. attenuata* is the tobacco hornworm *M. sexta* (Linnaeus; Lepidoptera, Sphingidae), as the name reveals an herbivore specialised on tobacco, although it also feeds on other solanaceous plants like tomato (Yamamoto and Fraenkel, 1960; de Boer and Hanson, 1984). The geographic distribution of *M. sexta* ranges from Canada to Argentina and matches with that of *N. attenuata* (King and Saunders, 1984). In contrast to *S. exigua*, *M. sexta* moths lay single eggs, sometimes up to five, on the abaxial side of leaves. After three to five days, larvae hatch and pass through five to six larval instars, while one larva can completely defoliate up to ten mature *N. attenuata* plants during its development (Kessler and Baldwin, 2001). Due to this vast defoliation-behaviour, *M. sexta* larvae are responsible for most of the leaf damage in native North American populations (van Dam et al., 2001; Kessler and Baldwin, 2002b). Larvae pupate in the soil and the nocturnal moths feed also on the nectar from the flowers of *N. attenuata*. A life cycle lasts 30 to 50 days and two to four generations can occur in a year.

N. attenuata displays a remarkable array of inducible direct and indirect defences in response to resist herbivore attack. One of the most prominent defensive compounds of *N. attenuata* is the eponymous neurotoxin nicotine (Schmeltz, 1971; Steppuhn et al., 2004). However, due to its close relationship with *N. attenuata*, *M. sexta* evolved resistance to nicotine (Morris, 1983). Larvae can tolerate doses of nicotine that are fatal to unadapted herbivores, although larvae grow more slowly on high-nicotine diet (Appel and Martin, 1992; Wink and Theile, 2002). Such a tolerance is achieved through a high detoxification capacity for nicotine and mechanisms to rapidly excrete dietary nicotine (Wink and Theile, 2002; Snyder et al., 1994). Interestingly, *M. sexta* might even be better defended against by dietary nicotine against its parasitoid *Cortesia congregata*, which suffers a higher mortality when parasitizing larvae feeding on high- rather than low-nicotine diets (Thorpe and Barbosa, 1986).

Another inducible direct defence of *N. attenuata* are Trypsin protease inhibitors (TPIs) which function as anti-digestive plant defence (van Dam et al., 2001; Glawe et al., 2003; Zavala et al., 2004b). Despite the fact that TPIs and nicotine have different physiological targets, they can act synergistically because nicotine prevents compensatory feeding that *S. exigua* larvae exhibit in response to induced TPIs (Steppuhn and Baldwin, 2007). Also, other compounds function as direct defence and play an important role in defence against chewing herbivores, for example other phenylpropanoid-polyamine conjugates (PPCs) such as caffeoylputresine (CP) or dicaffeoylspermidine (Kaur, et al., 2010) and terpenoids such as diterpene glycosides (Jassbi et al., 2008). *N. attenuata* produces many secondary metabolites, most of which are elicited by or related to jasmonates. Silencing the genes responsible for JA, TPI or nicotine biosynthesis produces plants with diminished resistance to herbivores (Halitschke and Baldwin, 2003; Steppuhn et al., 2004; Zavala and Baldwin, 2004). In addition to direct defences against herbivorous enemies, *N. attenuata* also mounts several indirect defences in response to herbivory like for example the release of a bouquet of volatile organic carbons (VOCs), green leaf volatiles or volatile terpenoids. VOCs emission can reduce the herbivore load on the plant by more than 90 % in nature, as this emission attracts a generalist predator and reduces herbivore oviposition rates (Kessler and Baldwin, 2004).

2.6 Main research questions and thesis outline

The aim of this doctoral thesis was to examine oviposition-mediated priming of plant anti-herbivore defences in two solanaceous plant species in their interaction with generalist and specialist lepidopteran herbivores. Particularly, the dissertation focusses on investigating the dynamics of the primed state in the bittersweet nightshade, *S. dulcamara* (see 2.6.1) and on investigating the fitness consequences of oviposition priming for the wild tobacco, *N. attenuata* (see 2.6.2). Experiments were preferably realized in a full-factorial experimental setup (see Fig. 10). This experimental design allows to investigate the primed plant response in relation to that induced by herbivory alone. In this setup, the effects of single treatments, i.e. plants that experienced only oviposition (primed (P)) or only herbivory (triggered (T)), can be compared with effects of combining both treatments, i.e. plants exposed to oviposition and herbivory (primed and triggered (PT)).

		Priming stimulus	
		untreated	*oviposition*
Triggering stimulus	*untreated*	control (C)	primed (P)
	natural or simulated herbivory	triggered (T)	primed and triggered (PT)

Figure 10: General treatments in a full-factorial priming experiment. Four different treatment groups result from combining two different stimuli (priming stimulus = oviposition, triggering stimulus = larval feeding or simulated herbivory).

2.6.1 Dynamics of the primed state in *S. dulcamara*

Experience and memory of a priming stimulus can transform the plant into a primed state, prepared for an improved stress response in the near future (see 2.2.4 Priming of plant defence). However, knowledge of the temporal dynamics of plant responses to oviposition in context of priming respectively the primed state is

limited. Therefore, the first part of this dissertation aims to examine the phytohormonal and transcriptional regulation of *S. dulcamara* in response to oviposition and/or larval feeding by the generalist herbivore *S. exigua* and the specialist leaf-miner *A. autumnitella*. In this part, the following research questions were addressed:

What are the temporal dynamics of potential regulators after oviposition within the phase of egg exposure and after removal of the eggs?
To mount a primed stress response, plants need to establish a primed state after perception of the priming stimulus ("onset" of the primed state) and maintain it until the triggering stimulus occurs. Under conditions where the subsequent triggering does not occur, plants may at some point forget ("offset" of the primed state) and switch back to a non-primed state. Consequently, the primed state is subjected to temporal dynamics. Most studies investigated physiological responses to insect eggs only at one time point (Hilfiker et al., 2014; Bandoly et al., 2015, 2016; Geuss et al., 2017; Bonnet et al., 2017; Geuss et al., 2018; Lortzing et al., 2019; Paniagua Voirol et al., 2020), while only a few studies examined responses at different time points within the egg incubation time (Bruessow et al., 2010; Beyaert et al., 2011; Firtzlaff et al., 2016; Altmann et al., 2018; Valsamakis et al., 2020). Consequently, the dynamics of the primed state respectively the temporal kinetics of oviposition-induced responses remain largely unknown.
The bittersweet nightshade is an adequate model system to investigate plant responses to oviposition, as this plant exhibit severe local reactions to insect eggs (Geuss et al., 2017) and furthermore oviposition impairs performance of subsequently feeding *S. exigua* larvae associated with a transcriptional and metabolic reshaping of the response to herbivory (Geuss et al., 2018). Also, the specialist herbivore *A. autumnitella* is impaired in development when feeding on oviposited *S. dulcamara* plants (Drok et al., unpublished).
Consequently, the first aim of this dissertation was to examine the temporal dynamics of the primed state after oviposition by the generalist herbivore *S. exigua* and the specialist herbivore *A. autumnitella* on *S. dulcamara*. Therefore, responses of oviposited (primed) leaves were compared with those of untreated control leaves from individual plants. To decipher how the priming signal is conveyed in the signalling networks, the accumulation of phytohormones (particularly the temporal pattern of SA induction) and the transcriptional regulation of several genes involved in defence were analysed at time points within the natural egg incubation time (to observe the "onset" of the primed state, establishment of the response), at a time point when larvae would hatch (to observe the primed

response at the timepoint the triggering should occur) and at time points after removal of the eggs at the time point corresponding to larval hatching (to follow the "offset" of the primed state, relaxation of the response).

Does oviposition-mediated defence priming alter the onset of plant responses to feeding larvae? Most studies investigating oviposition priming described ecological effects on the herbivore, while mechanisms facilitating such an improved defence are still poorly understood (see 2.4.3, Tab. 1). An improved defence response due to priming may be based on altered signalling during the onset of the defence response, for instance the primed response could be earlier or faster than a non-primed response (Fig. 1, Hilker et al. (2016)). However, previous studies investigating oviposition mediated priming of anti-herbivore defences did not find indication for an altered feeding-induced phytohormonal accumulation between plants with or without prior oviposition, although these studies measured phytohormonal contents only after 24 hours of larval feeding (Geuss et al., 2018; Bandoly et al., 2015). But especially the onset of an induced defence response could be affected by priming.
Thus, in a full-factorial setup phytohormonal and transcriptional levels of oviposition primed or non-primed *S. dulcamara* plants were examined at time points early after herbivory by *S. exigua* started. In one experimental setup oviposited and non-oviposited plants were exposed to natural herbivory, i.e. the leaf material was harvested and analysed after the initial 4 h of larval feeding. In another more standardised experimental setup, the leaf material was harvested and analysed one hour after simulated herbivory. Differences in the responses between oviposited and non-oviposited plants, at these time-points could indicate that oviposition priming facilitates an earlier or faster defence response.

2.6.2 Fitness consequences of oviposition priming for *N. attenuata*

Defence priming is postulated as an adaptive strategy, that allows plants to increase their resistance to herbivores at low costs and to thereby enhance plant fitness (Martinez-Medina et al., 2016). However, knowledge regarding the effect of oviposition priming on the plant fitness is largely missing. Hence, the second part of this thesis aims to scrutinize the fitness consequences of oviposition priming for the annual plant *N. attenuata* in interaction with the generalist herbivore *S. exigua* and the tobacco specialist *M. sexta*. Within this part, the following research questions were addressed:

How does oviposition affect the plant fitness of herbivore attacked and unattacked plants? To test the assumption that oviposition priming constitutes an adaptive value for plant fitness, the consequences of oviposition priming on growth and plant fitness were investigated in relation to the consequences of induced defence. In *N. attenuata*, oviposition by *S. exigua* and *M. sexta* caused a primed defence induction (i.e. PPCs like CP, in case of *S. exigua* also TPI activity) when larval feeding ensued (Bandoly et al., 2015, 2016). However, only larvae of the generalist herbivore *S. exigua* are affected in their larval performance, when oviposition preceded larval feeding (Bandoly et al., 2016). Whether this higher defence induction due to oviposition priming imposes fitness costs and whether the effect of priming on the herbivore performance results in fitness benefits that may outweigh potential costs was not investigated before.
Therefore, full factorial priming experiments with both herbivores were conducted, in which growth (stalk length) and fitness parameter (flowering, number of capsules and seed weight) of oviposited and non-oviposited plants were examined with and without larval feeding. In an additional experiment, herbivory was simulated to discriminate between the effects of leaf tissue lost to the herbivores from the effects of the primed defence induction. Subsequently results were considered with regard to fitness consequences of (a) natural and simulated herbivory, respectively, of (b) oviposition alone and of (c) herbivory preceded by oviposition.
Growth or fitness consequences of herbivory (induced defence) were denoted in differences between unattacked (C) and herbivory-attacked (T) plants and compared to the differences between unattacked and attacked plants that were previously exposed to oviposition (P and PT plants). If oviposition priming benefits the plant, the fitness of oviposited plants exposed to larval feeding (PT) would be expected to be enhanced compared to plants exposed to larval feeding only (T). This outcome would signify a net benefit of the priming, despite potentially higher investments in plant defence that can be expected from the increased plant defence induction in oviposited plants. To examine whether oviposition priming increases the costs of defence production, it was investigated whether priming has a negative impact on fitness of plants that are induced but not damaged by the herbivorous larvae, i.e. by using an induction treatment that simulates herbivory. Furthermore, differences between untreated (C) and oviposited (P) plants can signify, whether the response to only the priming stimulus, i.e. oviposition, would be costly for the plant. If priming would constitute an adaptive value for the plant, such a negative fitness impact caused by oviposition would be expected to be vanishingly low in all occasions at which the herbivore eggs do not hatch (e.g. due to predation or

parasitation). Hence, oviposited plants which are not exposed to larval feeding should have a similar growth and fitness as untreated control plants.

Does oviposition priming affect the fitness of plants that regrow after complete shoot removal? In the field, herbivory of highly adapted hornworms like *M. sexta* can cause vast defoliation of *N. attenuata* plants, as these larvae consume several plants during their development (Kessler and Baldwin, 2002b; van Dam et al., 2001). To cope with such massive herbivore damage on aboveground plant parts, *N. attenuata* can allocate photoassimilates to the roots upon herbivore attack where these assimilates are stored and used for regrowth processes when the herbivore threat has passed (Schwachtje et al., 2006). These increases in root reserves of induced plants caused a delayed senescence and prolonged flowering after regrowth (Schwachtje et al., 2006). Therefore, it was investigated whether such an induced tolerance mechanism is also enhanced by oviposition priming.
To assess if oviposition affects the regrowth capacity of *N. attenuata*, priming experiments were conducted including oviposition and/or larval feeding by *M. sexta* (or corresponding mock treatment) as in previous experiments. But after a phase of herbivory, all aboveground plant parts were removed and subsequently fitness of regrown plants was examined. If the tolerance responses facilitating the ability to regrow were more pronounced in plants exposed to oviposition followed by larval feeding, these plants may show an enhanced fitness compared to plants exposed to single treatments (oviposition or larval feeding alone).

How does the plant developmental stage affect the primed and induced tolerance abilities? During ontogeny plants pass though different developmental stages with distinct physiological states with varying deployment, distribution, and turnover of assimilates between diverse tissues of the plant as well as a spatiotemporally distinct expression of defence or tolerance traits (Boege and Marquis, 2005). For example, the accumulation of the defensive metabolite caffeoylputresine (CP) in *N. attenuata* plants follow a complex developmental pattern as high levels in the vegetative tissues during establishment phase at rosette and early elongating stages clearly shifted toward reproductive tissues after flowering and capsule development (reproductive phase) (Kaur et al., 2010). Consequently, also the allocation of assimilates to the roots (Schwachtje et al., 2006), which is probably also closely linked to factors affected by plant ontogeny like the photosynthetic capacity, could follow a developmental pattern.
Aiming to further dissect a possible effect of oviposition priming on tolerance responses (i.e. the enhanced transport and storage of assimilates in the roots) which affect the ability to regrow after defoliation, different developmental stages

of *N. attenuata* were examined for their regrowth capacity. Therefore, the previous experiments with defoliation were repeated with plants in young rosette stage (four-week-old plants at timepoint of oviposition) and plants in flowering state (eight-week-old plants at timepoint of oviposition). If the observed ability of oviposition priming to facilitate an improved fitness after regrowth is dependent on storage of assimilates in the roots, plants in different developmental stages should give different outcomes than the results with early elongating plants.

3 Material and methods

3.1 Plant culture

3.1.1 *Solanum dulcamara*

Solanum dulcamara L. (Solanaceae) were propagated vegetatively via stem cuttings from plants originating from different populations in the vicinity of Berlin (locations: Siethen, Langes Luch, Henningsdorf; coordinates of the populations listed in Tab. A1). Stems of six to eight-week-old plants were cut into pieces of ca. 6 cm, containing two leaf nodes. The cuttings were potted with one node within and one above the soil in 0.69 l or 0.89 l pots (same size within one experiment) filled with potting soil (Einheitserde® Classic Topferde, type T, Uetersen, Germany) enriched with fertilizer (Triabon® 16+8+12(+4+9), COMPO EXPERT GmbH, Münster Germany, 2 g l^{-}, components listed in Tab. A2) covered with a approximately one cm thick layer of sand (grain size 2–4 mm) to prevent fungus gnat infestation. Plants were grown in a greenhouse (16/8 light/dark cycle, 24 (± 10) ℃, Exp. 1 - 4: sodium lamps, type: SON-T Pia Plus 400 W lamps in SON-KE lights, DH Licht, Wuelfrath, Germany; Exp. 5 & 6: metal-halide lamp, type: MGR-E 315-CDM, 315 W, DH Licht, Wuelfrath, Germany) with ample water supply. When required, nematodes (*Steinernema feltiae*, Katz Biotech AG, Baruth Germany) and predatory mites (*Hypoaspis miles* or *Amblyseius cucumeris*, Katz Biotech AG, Baruth Germany) were added as pest control.

3.1.2 *Nicotiana attenuata*

Nicotiana attenuata Torr. ex Watson (Solanaceae) were grown from seeds of inbred lines from a field collection in the Great Basin Desert (Utha, USA). Seeds were sterilized and smoke-germinated on agar plates (0.6 % Agar Agar (Carl Roth GmbH + Co. KG, Karlsruhe Germany), 0.36 % Gamborg B5 (Duchefa Biochemie, Haarlem, Netherlands)). Plants grew in a greenhouse (24 ℃(16/8 light/dark cycle, 24 (± 10) ℃; Exp. 7, 10 & 12: sodium lamps, type: SON-T Pia Plus 400 W lamps in SON-KE lights, DH Licht, Wuelfrath, Germany; Exp. 8, 9, 11 & 13 - 15: metal-halide lamp, type: MGR-E 315-CDM, 315 W, DH Licht, Wuelfrath, Germany). After 7-10 days, seedlings were transferred to propagation trays (Ø 4 cm, 6 cm hight) filled with potting soil (Einheitserde® Type T). Three-week-old plants

were planted in 0.89 l pots. When required, nematodes (*Steinernema feltiae*, Katz Biotech AG, Germany) and predatory mites (*Hypoaspis miles* or *Amblyseius cucumeris*, Katz Biotech AG, Germany) were added as pest control.

3.2 Insect rearing

3.2.1 *Acrolepia autumnitella*

Acrolepia autumnitella Curtis (Acrolepiidae) larvae were reared from larvae initially collected from native *S. dulcamara* populations in Grunewald (Berlin, 52°27'48.8"N; 13°15'12.2"E). Larvae were reared on *S. dulcamara* plant material (grown as described, see 3.1.1) throughout their development. Plant material was placed in vented plastic boxes (20 x 20 x 9 cm or 40 x 59 x 43,5 cm) either provided as small twigs or single leaves kept fresh in an Eppendorf tubes (2 ml) or falcon tubes (50 ml) filled with tab water, or as whole *S. dulcamara* plants. Pupae were collected from the larvae boxes and transferred to a flight cage (plastic box 38 x 44 x 31 cm equipped with gaze windows). Adults were kept in these flight cages with a 20 % honey solution provided on cotton wool as food. For oviposition, a small *S. dulcamara* twig was placed for 24 h in the fight cage. The twig with eggs was then placed in a vented plastic box, where larvae could hatch and were used for further rearing.

3.2.2 *Spodoptera exigua*

Spodoptera exigua Hübner (Noctuidae) were reared as described in Bandoly et al. (2015) in a climate chamber (24 °C; 16:8 L:D). Larvae were fed *ad libitum* on a bean flour based artificial diet (components listed in Tab. A3) whereas a part of the rearing was temporary reared on a wheat germ-based artificial diet (components listed in Tab. A4) in vented plastic boxes (approx. 50 larvae per box after the larvae reached the 3rd instar, 22 x 15 x 4 cm). For pupation a crumpled tissue was placed in the boxes and pupae were collected in separate boxes. Moths were reared in flight cages (45 x 45 x 60 cm) supplied with 20 % honey solution and paper tissue for oviposition. Parts of the tissue with egg clutches were cut off, placed in a larvae box and used for further rearing.

3.2.3 *Manduca sexta*

Manduca sexta L. (Sphingidae) were reared as described in Trauer and Hilker (2013) in a climate chamber (24 °C; 16:8 L:D). Larvae were fed ad libitum on on a wheat germ-based artificial diet (composition see Tab. A4). Eggs, larvae and pupae were kept in vented plastic boxes of different sizes (approx. 100 eggs, 100 L1 or 50 L2 instars in 20 x 20 x 9 cm boxes; approx. 30 L3 or 30 L4 instars in 30 x 19.5 x 20.5 cm boxes; 10–20 L5 instars in 46 x 30 x 17 cm boxes; 3–5 wandering L5 instars or 10 pupae separated by sexes in 15 x 6 x 17.5 cm boxes). Moths were reared in flight cages (60 x 45 x 90 cm) supplied with 20 % honey solution and a tobacco leaf on the top of a glass jar wrapped with parafilm for oviposition. Moths lay eggs on the parafilm while landing on the tobacco leaf. Eggs could easily be removed from the parafilm and were used for further rearing.

3.3 Experimental setup

3.3.1 Experiments examining the dynamics of the primed state in *S. dulcamara*

Within the first part of this dissertation, the initial aim was to examine the dynamics of the primed state in *S. dulcamara* plants.

Experiment 1 & 2: Temporal pattern of phytohormonal and transcriptional responses of *S. dulcamara* to *S. exigua* oviposition. The first two experiments were conducted to investigate the effect of oviposition by the generalist herbivore *S. exigua* on the phytohormonal and transcriptional accumulation in leaves of *S. dulcamara* at different time points after oviposition. Therefore, in both experiments the 5th fully developed leaf of four-week-old *S. dulcamara* plants from the population in Siethen (one genotype; grown as described, see 3.1.1) were either exposed to oviposition by *S. exigua* or left as untreated control (conducted as described, see 3.4.1). To determine the temporal pattern of the responses to oviposition, leaf tissue of the oviposited leaf or the corresponding leaf of control plants was harvested 2, 4, 6, 8 and 10 days (Exp. 1, see Fig. 11 a) or one and three days (Exp. 2, see Fig. 11 b) after oviposition from individual plants in aluminum bags, flash-frozen in liquid nitrogen and stored at -80 °C until phytohormonal and transcriptional analysis. To reduce differences due to different abiotic conditions, harvest was executed at the same day for all time points (see Fig. 11 a & b). Eggs

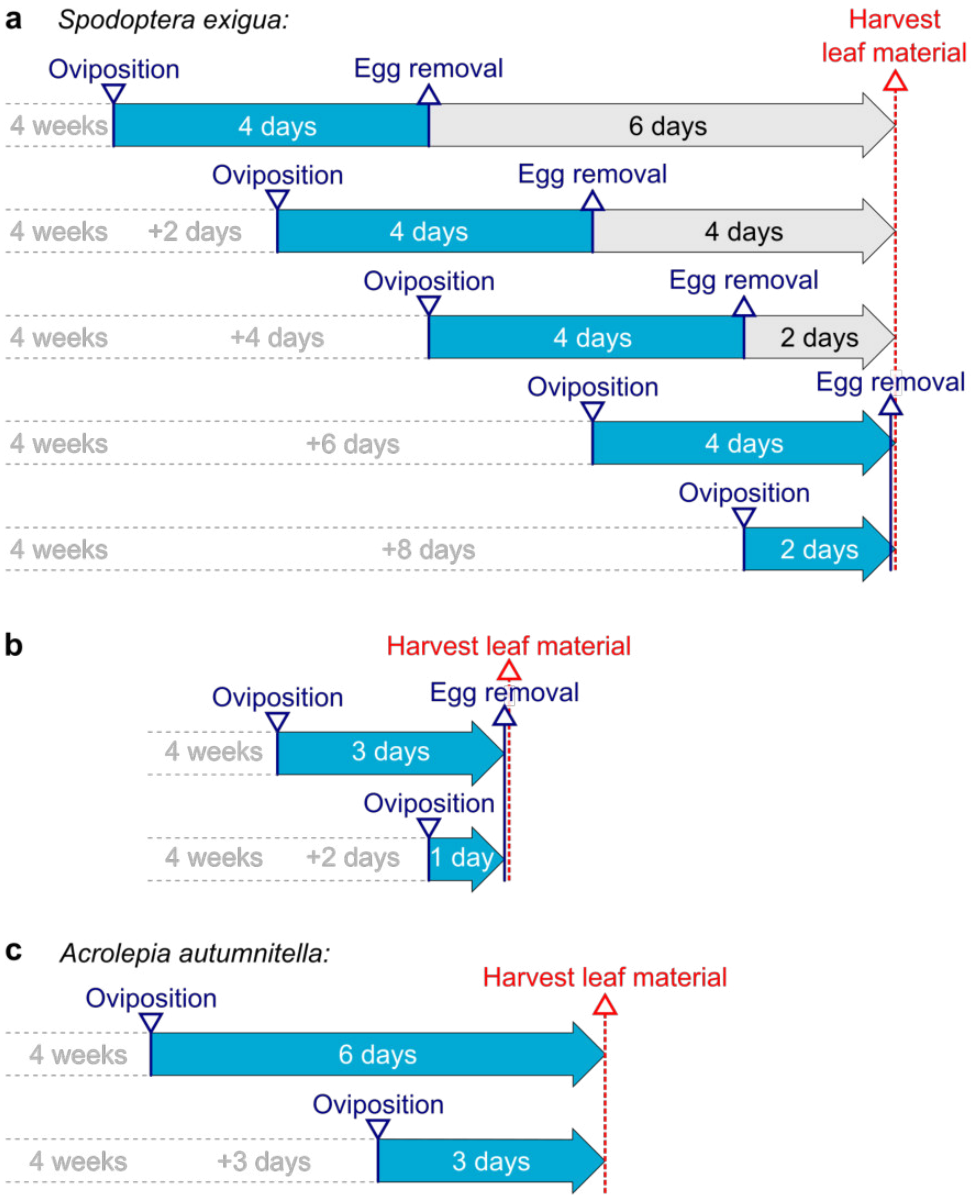

Figure 11: Illustration of the experimental setup of the (a) first, (b) second, and third (c) experiment. To determine the temporal pattern of the responses to oviposition, leaf tissue of the oviposited leaf or the corresponding leaf of control plants was harvested in the first experiment (a) 2, 4, 6, 8 and 10 days or (b) 1 and 3 days after oviposition by *S. exigua*. In the third experiment (c) leaf material was harvested in a similar procedure 3 and 6 days after oviposition by *A. autumnitella*. Harvest was executed on the same day for all time points from individual plants. In the experiments with *S. exigua*, eggs remained on the leaf for four days (time with eggs on the plant: blue) or were removed right before harvest (Exp. 1: 2 and 4 days after oviposition; Exp. 2: both time points). In the third experiment, eggs were not removed before harvest.

remained on the plant after oviposition and were carefully removed using a fine paintbrush immediately before leaf harvest for the first two timepoints and 4 days after oviposition (corresponding to the timepoint when larvae would hatch) for the remaining time points.

Experiment 3: Temporal pattern of phytohormonal and transcriptional responses of *S. dulcamara* to *A. autumnitella* oviposition. In parallel to the experiments with *S. exigua* (Exp. 1 & 2), the third experiment was realized to investigate the effect of oviposition by the specialist leaf miner *A. autumnitella* on the phytohormonal and transcriptional accumulation in leaves of *S. dulcamara* within the natural egg incubation time. Therefore, four-week-old *S. dulcamara* plants (Siethen population, two genotypes; grown as described, see 3.1.1) were either exposed to oviposition by *A. autumnitella* or left as untreated control. Oviposition respectively the mock treatment for control plants (conducted as described, see 3.4.1) was performed on the first fully developed leaf. Eggs remained on the plant. To ascertain the temporal pattern of the transcriptional and phytohormonal responses to oviposition, a stripe of leaf tissue of the oviposited leaf or the corresponding leaf of control plants was harvested three and six (end of the natural egg incubation time of *A. autumnitella*) days after oviposition from individual plants (see Fig. 11 c) in screw cap tubes containing matrix, flash-frozen in liquid nitrogen and stored at -80 °C until phytohormonal and transcriptional analysis. To reduce differences due to different abiotic conditions, harvest was executed at the same day for both time points (see Fig. 11 c).

In order to study the dynamics of the primed response in the beginning of the larval attack, the aim for another set of experiments was to examine the effect of oviposition and natural / simulated herbivory during the onset of the defence response

Experiment 4 & 5: Phytohormonal and transcriptional responses to oviposition and herbivory in the beginning of the attack. The next experiments were conducted to investigate the phytohormonal and transcriptional accumulation in leaves of *S. dulcamara* during the onset of the triggered response after natural herbivory, with and without prior oviposition. Therefore, two full-factorial priming experiments with oviposition (priming stimulus) and/or four hours of larval feeding (triggering stimulus) by *S. exigua* were conducted (see Fig. 12 a). Four weeks old *S. dulcamara* plants (Siethen population, Exp. 4: two genotypes, Exp. 5: one genotype; grown as described, see 3.1.1) were compiled in replicates according to plant height to exclude ontogenetic differences within the

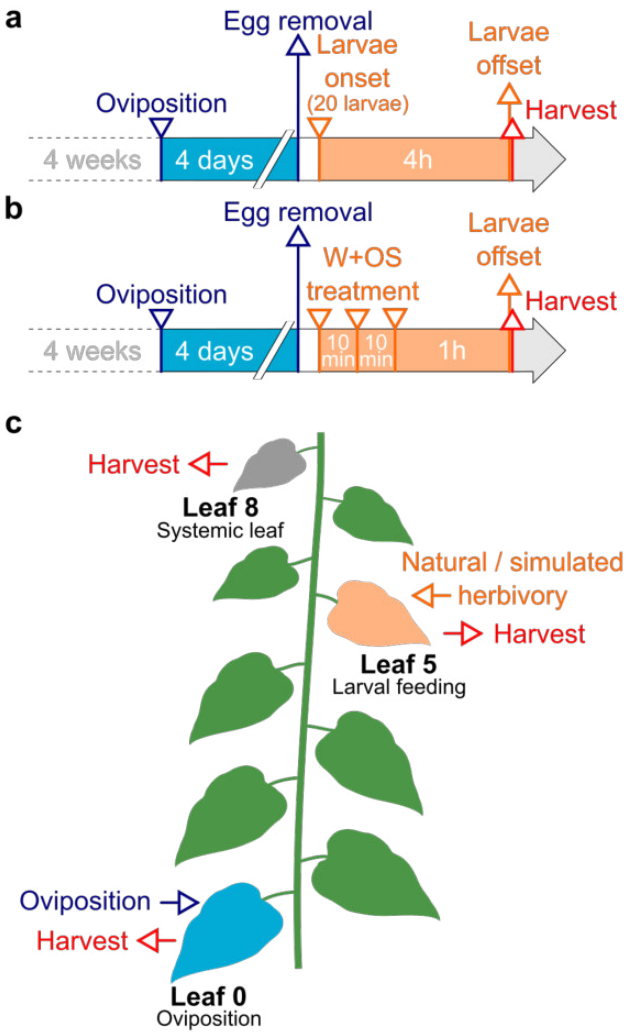

Figure 12: Illustration of the experimental setup of the full-factorial priming experiments with (a) natural and (b) simulated herbivory, as well as the (c) spatial separation of the treatments on the plant. To investigate the onset of the defence response after oviposition in *S. dulcamara*, four-week-old plants were exposed to oviposition by *S. exigua* at leaf 0. Following four days eggs were removed and (a) 20 larvae were applied to the plant (natural herbivory) or a W+OS treatment was applied with a time gab of ten minutes (simulated herbivory) at leaf 5. After (a) four hours of larval feeding or (b) one hour after the last W+OS treatment, material of leaf 0, 5 and 8 was harvested for phytohormonal and transcriptional analyses. (c) Oviposition resp. the priming stimulus was applied to leaf 0, while the triggering stimulus, i.e. natural or simulated herbivory, was applied to the vascularly fully connected leaf five leaf positions higher (leaf 5).

replicate. Plants either remained untreated (control, C), were treated only with oviposition by *S. exigua* (primed, P), were exposed only to four hours of larval feeding by *S. exigua* (triggered, T) or received a combination of both oviposition and short-time larval feeding by *S. exigua* (primed and triggered, PT). To exclude intermixtures of the direct local responses of *S. dulcamara* to the eggs (see 2.4.2) and changes associated with the onset of responses to larval feeding, oviposition and larval feeding were spatially separated and applied to different, but vascularly fully connected leaves (larval feeding/triggering were exposed to the leaf five positions higher than oviposition/priming, see Fig. 12 c). Oviposition (P and PT plants) was exposed to the 5th fully developed leaf (conducted as described before, see 3.4.1). Eggs remained on the plant for four days and were then removed using a fine paintbrush and water. The triggering stimulus comprised larval feeding by 20 third instar larvae (Exp. 3) or two third instar and 18 second instar larvae (Exp. 4; procedure as described, see 3.4.2). To ensure a direct start of larval feeding af-

ter onset, larvae were starved for 24 hours before the experiment. Application of larval feeding was implemented lagged (plants within a replicate were treated at the same time) from morning till noon. After four hours, larvae were removed and the oviposition leaf (leaf 0), the larval feeding leaf (leaf 5) and a younger systemic leaf (leaf 8) were harvested for phytohormonal and transcriptional analysis in aluminum bags, flash-frozen in liquid nitrogen and stored at -80 °C until further use.

Experiment 6: Phytohormonal and transcriptional accumulation after oviposition and simulated herbivory during the onset of the response. To examine responses during the onset of the triggered response with a more standardized triggering stimulus than larval feeding, a full-factorialexperiment with oviposition as priming stimulus and/or simulated herbivory as triggering stimulus were conducted. Therefore, four-week-old *S. dulcamara* plants (Siethen and Langes Luch population, each population five genotype; grown as described, see 3.1.1) were compiled in replicates according to plant height to exclude ontogenetic differences within the replicate. Plants were either left as untreated control plants (C) or were exposed to oviposition by *S. exigua* (plants of the treatments P and PT) or/and mechanical wounding followed by application of *S. exigua* larval oral secretion (W+OS treatment, plants of the treatments T and PT). Oviposition was applied at the 5^{th} fully developed leaf (conduced as described, see 3.4.1). Eggs remained on the plant for four days and were then removed using a fine paintbrush and water. As in the previous experiments with larval feeding (Exp. 4 and 5), the triggering stimulus was applied to the leaf five leaf positions higher than the leaf exposed to oviposition or corresponding leaves (see Fig. 12 c). After egg removal, the W+OS treatment (conducted as described before, see 3.4.2), each consisting of two rows (one on each side) of wounding per leaf in parallel to the mid vein, was repeated three times in intervals of ten minutes (see Fig. 12 b, 18 b). Application of the W+OS treatments were conducted lagged (plants within a replicate were treated at the same time) from morning till noon. One hour after the last W+OS treatment, the leaves were harvested into screw cap tubes containing matrix, flash-frozen in liquid nitrogen and stored at -80 °C until phytohormonal and transcriptional analysis.

3.3.2 Experiments examining the fitness consequences of oviposition priming for *N. attenuata*

The second part of this dissertation aims to investigate the fitness consequences of oviposition priming for *N. attenuata* plants.

Experiment 7 & 8: Growth and fitness consequences of oviosition and larval feeding by *S. exigua*. To investigate the fitness consequences of oviposition priming for *N. attenuata* in the interaction with the generalist herbivore *S. exigua*, two experiments (Exp. 7 and 8, see Fig. 13) were conducted. Five-week-old *N. attenuata* plants (grown as described, see 3.1.2) were matched in replicates according to their ontogeny (by size and elongation state). Treatments of one experiment (Exp. 7) comprised untreated control plants (C) and plants exposed to larval feeding by *S. exigua* either without (T) or with prior oviposition (PT). The other experiment (Exp. 8) was conducted in a full-factorial setup (treatments C, P, T and PT). To account for the known dependency of defensive compounds and leaf ontogeny (Van Dam et al., 2001), always the second youngest source leaf was exposed to oviposition by *S. exigua* or received the mock treatment (see 3.4.1). Eggs remained on the plant for three days (Exp. 7) or four days (Exp. 8) and were then gently removed with a soft brush without damaging the leaf surface. The removal of eggs allowed to standardize the onset of larvae, that started after egg removal by transfer of 20 (Exp. 7) or 25 (Exp. 8) *S. exigua* neonate larvae on the oviposition leaf. Subsequently, plants with treatments comprising *S. exigua* herbivory were exposed to 12 (Exp. 8) or 15 (Exp. 7) days of larval feeding, while plants without were handled equally with empty cages (conduced as described, see 3.4.2). The number of larvae per plant was counted every second day for four (Exp. 7) or five (Exp. 8) times to assess larval perfomance. Additionally, larval weigt (each larvae individually weighed) was measured after eight and twelve days of feeding in experiment eigth (balance: Semi-micro balances, SM 1265Di, VWR International GmbH, Darmstadt Germany). Growth and fitness parameters were recorded every day (conduced as described, see 3.7) until the plant fully withered after the watering ceased.

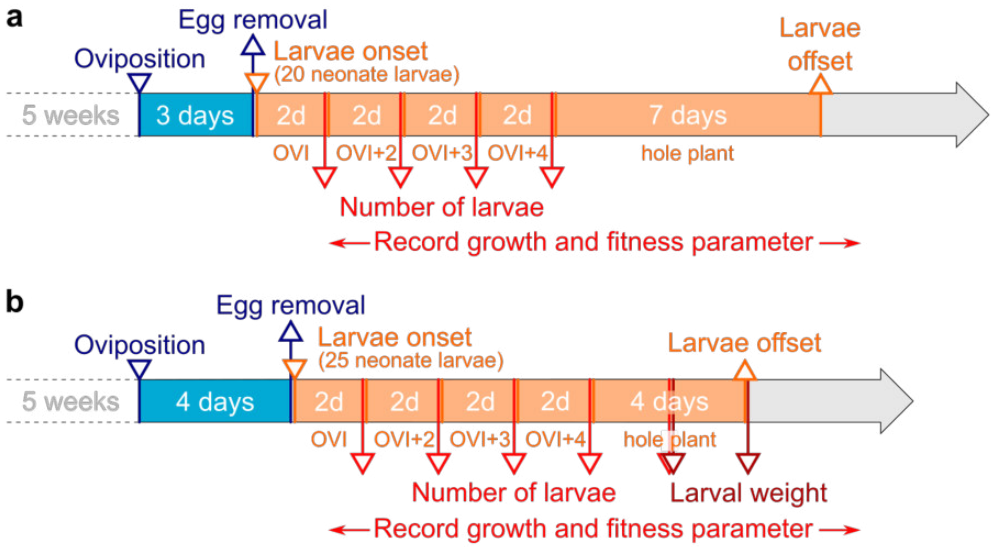

Figure 13: Illustration of the experimental setup of the two fitness experiments with *S. exigua* (Exp. 7 and 8). To investigate the fitness consequences of oviposition and larval feeding by *S. exigua*, five-week-old *N. attenuata* plants were exposed to oviposition and three (Exp. 7; a) respectively four (Exp. 8; b) days after oviposition, eggs were removed and 20 (Exp. 7; a) respectively 25 (Exp. 8; b) *S. exigua* neonate larvae were applied. Larvae were placed on the plants using clip cages, which were moved to the next older leaf every second day until larvae were released to the hole plant (leaf exposed to larval feeding denoted below bar). Plants were exposed to herbivory for 15 (Exp. 7; a) respectively 12 (Exp. 8; b) days. Plant fitness parameter were recorded every day.

Experiment 9: Growth and fitness consequences of oviosition and larval feeding by *M. sexta*. To investigate the fitness consequences of oviposition priming in the interaction of *N. attenuata* with the specialist herbivore *M. sexta*, a full factorial priming experiment was conducted (see Fig. 14). Five-week-old *N. attenuata* plants (grown as described, see 3.1.2) were matched in replicates according to their ontogeny (by size and elongation state). The experiment was conducted as full-factorial priming experiment, i.e. comprised all four treatments (C, P, T and PT). Oviposition by *M. sexta* or the mock treatment (conducted as described, see 3.4.1) was exposed to the second youngest source leaf, to account for the dependency of induction of defensive compounds and leaf ontogeny (Van Dam et al., 2001). Eggs remained on the plant for three days and were then gently removed with a soft brush without damaging the leaf surface (egg removal was executed in the evening). Egg removal was performed to standardize the onset of larvae, that started within 14h (next morning) after egg removal by transfer of two *M. sexta* neonate larvae on the plant. Subsequently, plants with treatments comprising herbivory were exposed to nine days of larval feeding, while

plants without were handled equally with empty cages (conducted as described, see 3.4.2). Growth and fitness parameters were recorded every day (conducted as described, see 3.7) until the plant fully withered after the watering ceased.

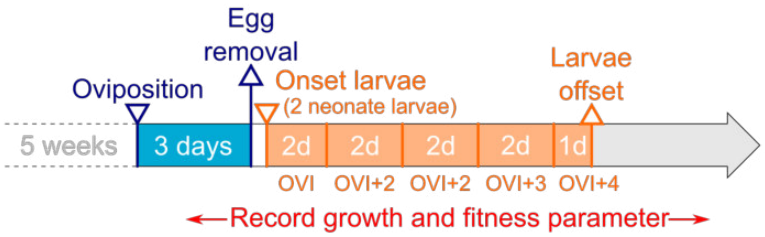

Figure 14: Illustration of the experimental setup of the fitness experiment with *M. sexta* (Exp. 9). To investigate the fitness consequences of oviposition priming, five-week-old *N. attenuata* plants were exposed to oviposition, eggs were removed after three days (evening) and at the next day (morning) two *M. sexta* neonate larvae were applied. Larvae were placed on the plants using clip cages or fine netting bags, which were moved to the next older leaf (leaf exposed to larval feeding denoted below bar). Plants were exposed to herbivory for nine days. Plant fitness parameter were recorded every day.

Experiment 10: Growth and fitness consequences of oviposition and simulated herbivory. To investigate the fitness consequences of oviposition priming on *N. attenuata* plants in absence of the herbivore, a full-factorial priming experiment, i.e. experiment comprised four treatments (C, P, T and PT) with simulated herbivory as triggering stimulus was conducted (see Fig. 15). Five-week-old *N. attenuata* plants (grown as described, see 3.1.2) were matched in replicates according to their ontogeny (by size and elongation state). Oviposition by *M. sexta* or the mock treatment (conducted as described, see 3.4.1) was exposed to the second youngest source leaf to account for the dependency of induction of defensive compounds and leaf ontogeny (Van Dam et al., 2001). Eggs remained on the plant for four days and were then gently removed with a soft brush without damaging the leaf surface. Within 14 h after egg removal the plants with treatments comprising a triggering stimulus received a W+OS treatment at the oviposition leaf (conducted as described, see 3.4.2). At that day the W+OS treatment was then repeated two times with a time gab of 3 h between the treatments (in total three W+OS treatments per leaf). In the same manner, the next two younger leaves were treated in the next two days. Growth and fitness parameters were recorded every day (conducted as described, see 3.7) until the plant fully withered after the watering ceased.

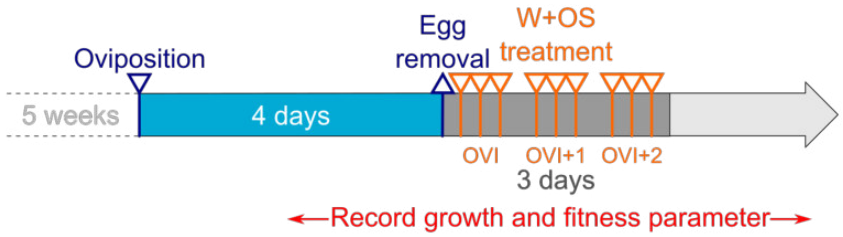

Figure 15: Illustration of the experimental setup of the fitness experiment with simulated herbivory (Exp. 10). Five-week-old *N. attenuata* plants were exposed to oviposition by *M. sexta*, eggs were removed after four days and plants were exposed to three W+OS treatments per day (time gab between the treatments ca. 3 h) for the next three days. The three treatments per day were applied to one leaf, starting with the oviposition leaf (next two younger leaves were treated in following two days). Plant fitness parameter were recorded every day.

Experiment 11 to 13: Effect of oviposition and larval feeding before defoliation on fitness of regrown plants. To examine the effect of oviposition and larval feeding before complete removal of all aboveground plant parts on the fitness of regrown *N. attenuata* plants, three independent experiments were conducted. The experiments were conducted in a full-factorial setup (Exp. 11: only C, T, and PT), comprising *M. sexta* oviposition and larval feeding followed by defoliation were conducted (see Fig. 16). In this context, also different developmental stages of *N. attenuata* plants were investigated with regard to their capacity to regrow. In two experiments (Exp. 11 & 12), early elongating *N. attenuata* plants (four- to five-week-old, grown as described, see 3.1.2) were used for the experiment starting with oviposition by *M. sexta* (conducted as described, see 3.4.1), while in Exp. 13 young rosette plants (four-week-old plants, not jet elongating) and flowering plants (eight-week-old plants) were exposed to oviposition. The second youngest source leaf was exposed to oviposition, with exception of elongated flowering plants in experiment thirteen where the second last rosette leaf (source) was exposed to oviposition. After four days, eggs were removed and two neonate larvae (Exp. 12 & 13) or three third instar larvae (Exp. 11) were applied for a phase of seven days of *M. sexta* herbivory (conducted as described, see 3.4.2). To increase herbivore damage, two additional third instar larvae were added to the first larvae after two days of feeding (Exp. 12 & 13). After the phase of larval feeding, larvae were removed and plants were exposed to defoliation. Therefore, all plant parts were removed with a scalpel so that only 3 cm of the main shoot remained without any leaves (see Fig. 16 b). Afterwards, plants remained under the same conditions as before and were allowed to regrow. Growth and fitness parameters of the regrown plants were recorded every day (conducted as described, see 3.7) until the plant fully withered after the watering ceased.

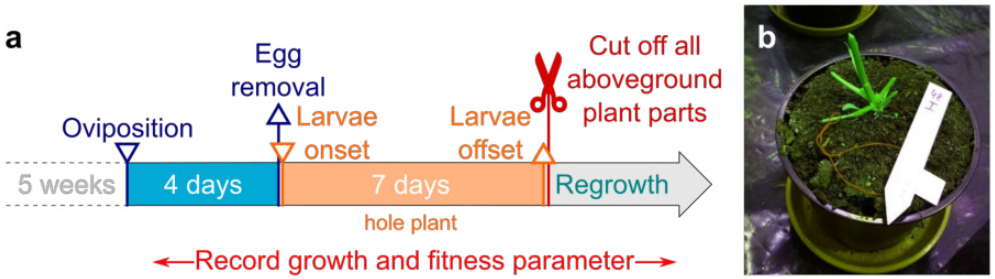

Figure 16: Fitness experiments with defoliation: (a) Illustration of the experimental setup and (b) picture of the plant after defoliation (Exp. 11 - 13). (a) Five-week-old *N. attenuata* plants were exposed to oviposition bby *M. sexta*, eggs were removed after four days and larvae were applied. Larvae (two respectively three larvae, additional larvae to increase damage after two days) were allowed to feed on the plant for seven days. After this phase of herbivory, plants were exposed to defoliation (complete removal of all aboveground plant parts). Plants were then allowed to regrow and plant fitness parameter were recorded every day.

3.4 Experimental procedures

3.4.1 Plant exposure to oviposition

Oviposition by *A. autumnitella* To implement oviposition on a standardized leaf position, five mated *A. autumnitella* moths (male and females) were put with an exhauster into a vented clip cage (Ø 10 x 10 cm) placed around the first fully developed leaf (see Fig. 17). Plants designated for a treatment without oviposition received an empty clip cage on the corresponding leaf position. The clip cages were placed on the plants for approximately 5 h. Then clip cages were removed and eggs were counted.

Oviposition by *S. exigua* To achieve oviposition by *S. exigua*, the hole selected leaf for oviposition was encaged with a gauze bag (12 x 14,5 cm) overnight. For plants of the treatments including oviposition, the cages contained 5-8 (equal number of moths within one experiment) mated *S. exigua* moths (males and females), while plants of the treatments excluding oviposition remained empty. After one night, bags were removed and eggs counted. Considering the natural egg clutch size, plants with less than 15 eggs were excluded for treatments with oviposition.

Oviposition by *M. sexta* Oviposition by *M. sexta* was implemented as described in Bandoly et al. (2016), with a few adaptations. Plants were positioned around gauze cages (76 x 42 x 42 cm), the selected leaf through inserted in slots

of the cage. Plants of the treatments including oviposition were exposed to cages containing approximately 10-15 mated female *M. sexta* moths, while plants of the treatments excluding oviposition were exposed to empty cages. To prevent overloads of eggs on the leaves, the moths were observed from dusk on and plants were removed from the cage directly after oviposition event. By this approach, plants with 1-3 eggs were obtained, a similar egg load as under natural conditions.

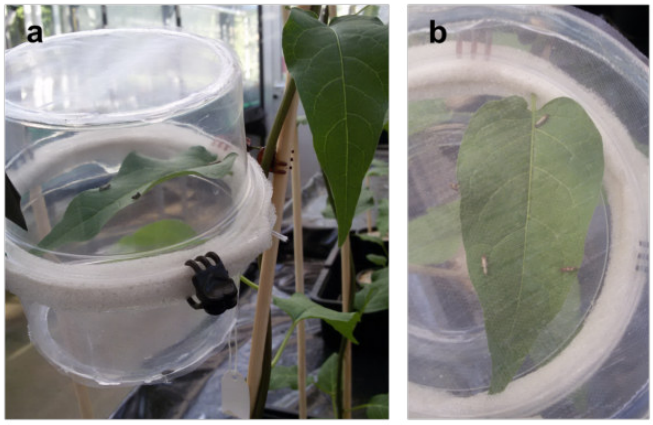

Figure 17: Experimental procedure *A. autumnitella* oviposition. (a) *S. dulcamara* in the greenhouse with clip cages with *A. autumnitella* moths for oviposition. (b) *A. autumnitella* moths in clip cage on *S. dulcamara* leaf.

3.4.2 Plant exposure to natural and simulated herbivory

Natural herbivory by *S. exigua* Larval feeding by *S. exigua* was implemented by applying 20 (Exp. 3, 4 & 7) or 25 (Exp. 8) unfed neonate larvae on the selected leaf of *S. dulcamara* or *N. attenuata* plants. Larvae were kept on the leaf using vented clip cages (Ø 6.5 x 2.5 cm; see Fig. 18 a). Control plants received empty clip cages at the same leaf position. To warrant enough leaf material for larval feeding, cages were positioned one leaf position higher usually every second day. To implement subsequent larval feeding on the hole plant (Exp. 7 & 8: after 8 days), plants were encaged completely using a gaze cage (33.5 x 33.5 cm, height 140 or 95 cm) and larvae of the clip cages were released to the hole plant.

Natural herbivory by *M. sexta* Larval feeding by *M. sexta* was implemented by applying two neonate larvae (Exp. 9, 12 & 13; Exp. 12 & 13: after 2 days of feeding two additional third instar larvae were added to increase herbivory) or three third instar larvae (Exp. 3) on the plant. Larvae were encaged on selected leaves using vented clip cages (Ø 6.5 x 2.5 cm) or gauze bags (12 x 14,5 cm). To warrant enough leaf material cages were moved depending on the instar usually every or every second day. To implement larval feeding on the hole plant, larvae

were kept on the plant using gaze cages (33.5 x 33.5 cm, height 140 or 95 cm) enclosing the hole plant.

Simulated herbivory To obtain a standardized elicitation, simulated herbivory was implemented as treatment by wounding the leaf in parallel to the mid-vein with a pattern wheel and immediate application of *M. sexta* or *S. exigua* oral secretions (OS) on *N. attenuata* respectively *S. dulcamara*. OS, that contain fatty acid-amino acid conjugates as elicitors, was collected from 3rd to 5th instar larvae that had been feeding on *N. attenuata* (*M. sexta*) or *S. dulcamara* (*S. exigua*) leaf material. OS was collected with a Teflon tube in 5 ml glass vials (cooled on ice) that were connected to a vacuum pump. Solid particles were removed from the OS by centrifugation and stored the OS supernatant (1:3 diluted with water) at -20 °C. Using a pattern wheel puncture wounds were inflicted in rows parallel to the mid-vein and instantly 10 µl OS was added into the wounds (see Fig. 18 b). The treatment was repeated several times with a certain time gap depending on the experiment.

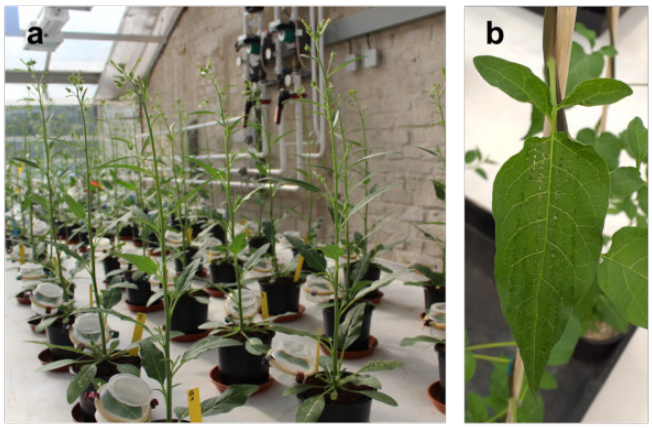

Figure 18: Experimental procedures. (a) *N. attenuata* in the greenhouse with clip cages. (b) *S. dulcamara* leaf after application three W + OS treatments.

3.5 Phytohormone quantification

Extraction of phytohormones was implemented according to Wang et al. (2007) with some adaptations. When harvested in alu bags, leaf material was homogenized by grinding with mortar and pestle in liquid nitrogen before extraction and approximately 100 mg of the leaf powder were filled in screw cap tubes (2 ml) containing matrix (Zirconox®, 2.8 - 3.3 mm, Mühlmeier Mahltechnik Germany). When directly harvested in screw cap tubes (2 ml) with matrix, samples were homogenized (two times 20 seconds at 4.5 m/sec) in a FastPrep homogenizer (MP Biomedicals, model: FastPrep®-24 Instrument, Eschwege Germany) and weighted

before extraction. To avoid defreeze the samples were stored on liquid nitrogen immediately before and after weighting and homogenization. For the extraction, samples were put on ice and 1 ml extraction buffer (ethylacetate containing phytohormone deuterated standard mix (2 µl per ml ethylacetate) comprised of 10 ng/µl of D4-SA, D6-ABA, D6JA-Ile and 30.2 ng/µl D6-JA (High Purity Compounds, Cunnersdorf Germany)) was added to the frozen leaf tissue samples. Then, samples were homogenized (two times 20 seconds at 4.5 m/sec) again, centrifuged (4 ℃/ 5 min / 16.2 g; VWR International GmbH, model: Micro Star 17R, Darmstadt Germany) and the supernatant of each sample was transferred into a new tube (2 ml). Subsequently, the pellet was re-extracted by adding 1 ml pure ethyl-acetate. Following homogenization (two times 20 seconds at 4.5 m/sec) and centrifugation (4 ℃/ 5 min / 16.2 g) the supernatants of both extraction steps were combined and concentrated to honey-like viscosity (not complete dry) in a vacuum concentrator (Eppendorf Germany, model: Concentrator 5301) at room temperature. Thereafter 400 µl re-eluation buffer (70 % MeOH, 30 % H2O and 0.1 % formic acid) was added to each tube and the samples were shaken on a Vortex (Scientific Industries, model: Vortex-Genie 2 T, Bohemia New York, USA or neoVortex® shaker, model: D-6012, neoLab, Germany) for 10 minutes at room temperature and maximum speed. Finally the samples were centrifuged (4 ℃/ 10 min / 16.2 g) and 200 µl of each sample were transferred into a GC/HPLC vial (volume 2 ml) equipped with an inlay. The samples were stored up to one night at 4 ℃ until measurement with tandem mass spectrometry.

To control for purity of solvents and technical malfunctions of the extraction and measurement, different blanks were prepared. Blanks contained no leaf material and were handled equally to the samples, but go through different lengths of the extraction protocol: Two blanks go through all steps of the extraction, one with and one without the previous homogenization including matrix, while both extraction steps of the third blank were performed with pure ethyl-acetate (no phytohormone deuterated standard). None of the blanks indicate impurities or malfunctions in the following measurement.

The phytohormones of intrest (SA, ABA, JA and JA-Ile) were seperated, identified, and quantified using the Ultra-high-Performance-Liquid-Chromatography (UPLC) coupled to a Time Of Flight Mass Spectrometer (Q-ToF-ESI) (Synapt G2-S HDMS; Waters®, Milford, Massachusetts, USA). Phytohormones were separated in the UPLC system (instruments from AQUITY™, Waters, Milford Massachusetts USA) with a C_{18} column (Aquinity UPLC BEH-C18, Ø 2.1 mm x 5 cm, particle size 1.7 µm). As eluents served water (A) and methanol (B), both containing 0.1 %

formic acid in a gradient mode with constant flow of 250 µl per minute at 30 °C. The injection volume for each sample was 7 µl. The elution started at 30 % eluent B 1 minute isocratic, followed by a linear gradient to 90 % eluent B in 3.5 minutes, which then remained at 90 % eluent B for 3.5 minutes. Then eluent B fell back to 30 % in 1 minute in pressure equilibrated for 3 minutes (equilibration time between the runs). Separated compounds were subjected to electrospray ionization (ESI) at following conditions: capillary voltage, 2.50 kV; nebulizer 6.0 bar; desolvation gas flow rate 500 l/hour, N_2 served as desolvation gas; source temperature 80 °C; desolvation temperature 150 °C. Compounds were detected by tandem mass spectrometry scanning the full mass spectrum of compounds between 50–600 m/z. Phytohormonal compounds were annotated according to their parent $[M\text{-}H]^-$ ion and a diagnostic daughter ion as well as according to co-elution with their deuterated derivatives (internal standard). Characteristic parent/daughter ions for the analyzed phytohormones are: SA (m/z 137 and 93), ABA (m/z 263 and 153), JA (m/z 209 and 59), JA-Ile (m/z 322 and 130), and their deuterated derivatives: D4-SA (m/z 141 and 97), D6-ABA (m/z 269 and 159), for D6-JA (m/z 215 and 59), D6-JA-Ile (m/z 328 and 130). The phytohormones were quantified according to the peak area of the daughter ions of the plant-derived phytohormones relative to the daughter ions of the internal standards by using MassLynx™ Software (version 4.1, Waters, Milford Massachusetts USA). Concentrations per sample were normalized according to the fresh weight of the leaf tissue samples.

3.6 Quantification of gene expression

3.6.1 RNA isolation, quality check and quantification

The RNA extraction was implemented according to Oñate-Sánchez and Vicente-Carbajosa (2008) with some adaptations. Leaf material (approximately 25 - 35 mg) for transcriptional analyses was either harvested in alu bags, then leaf material was homogenized by grinding with mortar and pestle in liquid nitrogen before extraction and filled in screw cap tubes (2 ml) containing matrix, or directly harvested in screw cap tubes (2 ml) containing matrix. Initially, the leaf tissue samples were homogenized (two times 20 seconds at 4.5 m/sec, FastPrep homogenizer), while samples were flash-frozen in liquid nitrogen between both homogenization steps (this step was skipped when samples were harvested in alu bags). 600 µl cell lysis solution (2 % SDS, 68 mM sodium citrate, 132 mM citric acid, 1 mM EDTA;

manufacturer of all chemicals Carl Roth GmbH + Co. KG, Karlsruhe Germany) was added to each sample on ice. Subsequently, the samples were shortly vortexed and incubated at room temperature for 2 minutes. Afterwards 200 µl precipitation solution (4 M NaCl, 16 mM sodium citrate, 32 mM citric acid; manufacturer of all chemicals Carl Roth GmbH + Co. KG, Karlsruhe Germany) was added and the samples were mixed by inverting. Following 5 minutes incubation on ice, the samples were centrifuged (4 °C / 10 min / 16.2 g). Then the supernatant was transferred into a new tube and this tube was again centrifuged (4 °C / 10 min / 16.2 g). Once more the supernatant was transferred into a new tube. Next, 600 µl isopropanol was added and the samples were mixed by inverting. After centrifugation (4 °C / 2 min / 16.2 g), the supernatant was poured off and the RNA pellet was washed with 800 µl ethanol (70 %). Thereafter, the samples were air dried at room temperature and the pellet was suspended in 30 µl DNase-free water and shortly vortexed. Until further use the isolated RNA was stored at -80 °C.
To examine the quality of the extracted RNA and to determine contaminations, the isolated RNA samples were checked via gel electrophoresis. For this purpose, 2 µl of each sample was mixed with 8 µl DNase free water (Carl Roth GmbH + Co. KG, Karlsruhe Germany) and 2 µl ROTI®Load DNA-tricolor (6x concentrated, Carl Roth GmbH + Co. KG, Karlsruhe Germany) loading dye in 0.2 ml reaction vials and incubated for 10 min at 70 °C in a PCR Cycler (Mastercycler® gradient, Eppendorf) to denature RNA followed by a short cool down on ice for some seconds. Samples were then loaded into a 1.8 % agarose gel (100 ml 0.5xTAE buffer, 1.8 g agarose, 5 µl ROTI® GelStain (Carl Roth GmbH + Co. KG, Karlsruhe Germany)). After an electrophoretic separation (Mupid® -One, Eurogentec, Seraing Belgium; 100 V, 30 min) the gel was photographed under UV-light (Transilluminator 20x20 M Basic, 302 nm, VWR).
To exclude interfering DNA, the TURBO DNA-free™ Kit (Thermo Scientific™) was used according to the manufacturer (rigorous DNase treatment). In brief, 0.1 sample volume 10x TURBO DNase buffer and 1 µl TURBO DNase was added to each sample and gently mixed. Then the samples were incubated at 37 °C for 30 min (AccuTherm Microtube Shaking Incubator, Labnet), then another 1 µl TURBO DNase was added to each sample, gently mixed and again incubated for 30 min at 37 °C. Thereafter, 0.2 sample volume DNase inactivation reagent was added and the tubes were mixed. During the following incubation (5 min, room temperature), the samples were repeatedly mixed by flicking. Subsequently, samples were centrifuged (4 °C / 2 min / 16.2 g) and the supernatant containing the RNA was transferred into a new tube.

To quantify the isolated RNA, 2 µl of each sample was placed at a µDrop Plate (Thermo Scientific™, N12391, Schwerte Germany) and measured photometric with Multiscan™ GO Microplate Spectrophotometer (Thermo Scientific™, Schwerte Germany). The concentration was calculated through the measured absorption at a wavelength of 260 nm, the optical density (OD) of the ascertainable nucleic acid (OD_{RNA}= 40) and the optical density of the µDrop Plate (0.52 mm) as follows: A_{260} * 40 * $^{10}/_{52}$ = x ng RNA μl^{-1}.

3.6.2 Reverse transcription

Complementary DNA (cDNA) was synthesized either using the Reverse Transcriptase Core kit (used in Exp. 1; Reference: RT-RTCK-03, Eurogentec, Seraing Belgium) or the Biozym cDNA Synthesis Kit (used in Exp. 2 - 6 ;Biozym Scientific, Hessisch Oldendorf Germany). To synthesize cDNA with the Reverse Transcriptase Core kit, the different components of the kit were mixed (for each sample/ 10 µl reaction: 1 µl 10x reaction buffer, 2 µl 25 mM $MgCl_2$, 2 µl 2.5 mM dNTP, 0.25 µl random nonamer, 0.2 µl RNAse Inhibitor, 0.25 µl EuroScriptRT) and 4.05 µl template-RNase free water mix (components individually adjusted according to the RNA concentration, total RNA 200 ng / 10 µl reaction) in reaction vials (400 µl). During all steps the reagents and probes were placed on ice. The vials were then gently mixed, spined down and placed into a PCR thermocycler (T100™ Thermal Cycler, Bio-Rad Laboratories, Feldkirchen Germany or Mastercycler® gradient, Eppendorf, Hamburg Germany, temperature program: 10 min 25 °C, 30 min 48 °Cand 5 min 95 °C). Afterwards, the samples were diluted with water to 50 ng μl^{-} and stored at -80 °C until further use. To synthesize cDNA with the Biozym cDNA Synthesis Kit, the different components of the kit were mixed (for each sample/ 10 µl reaction: 2 µl 5x reaction buffer, 1 µl dNTP Mix, 0.25 µl RNase inhibitor, 0.5 µl Biozym reverse transcriptase, 0.25 µl oligo (dT) primer (10 µM), 0.25 µl random hexamer (25 µM)) and 5.75 µl template-RNase free water mix (components individually adjusted according to the RNA concentration, total RNA 500 ng / 10 µl reaction). During all steps the reagents and probes were placed on ice. The vials were then gently mixed and spined down. Reverse transcription was then executed in a PCR thermocycler (temperature program: 10 min 30 °C , 30 min 50 °C and 5 min 99 °C). Afterwards the probes were diluted with water to 50 ng μl^{-} and stored at -80 °C until further use.

3.6.3 Quantitative real time polymerase chain reaction

To quantify the expression rates of genes involved or corresponding to phytohormone signalling or plant defence, real time polymerase chain reactions (qPCR) were used utilizing either the qPCR Core kit for SYBR™ Green 1 (used in Exp. 1 ;Reference: RT-SN10-05, Eurogentec, Seraing Belgium) or the Biozym Blue S'Green qPCR Mix (used in Exp. 2 - 6 ;Biozym Scientific, Hessisch Oldendorf Germany). The qPCR technique enables the quantification of new synthesized transcripts of a specific gene or template ('gene of interest' (GOI)) relative to reference genes, involved in core metabolism and not affected by the treatments. To do so, the fluorescence accumulation is detected during thermocycling utilizing a double stranded DNA intercalating dye. For each biological sample, three (Exp. 4) or two (Exp. 1 - 3, 5, & 6) technical replicates were implemented.
To use the qPCR Core kit for SYBR™ Green 1, different components of the kit (for each qPCR sample / technical replicate: 1 µl 10x reaction buffer, 0.7 µl 50 mM $MgCl_2$, 0.4 µl 5 mM dNTP, 0.05 µl HotGoldStar, 0.3 µl diluted SYBR, 1 µl forward primer, 1 µl reverse primer, 1.55 µl water) were mixed. For each GOI and reference gene a separate reaction mix was prepared with specific primer pairs obtained from Eurofins Genomics GmbH (primer sequences listed in Tab. A5). For each sample 6 µl reaction mix and 4 µl cDNA template (50 ng μl^{-}) was combined in a 96-well plate (400 µl, white), carefully mixed and spun down. For the qPCR run, the 98-well plate was then placed into the Real-time thermocycler (Mx3005P QPCR System, Agilent Technologies, US) with the following temperature program: 2 min 50 °C, 10 min 95 °C, 40-cycles: 15 sec. 95 °C, 1 min 60 °C, thereafter 50 °C.
To use the Biozym Blue S'Green qPCR Mix kit, the different components of the kit (for each qPCR sample / technical replicate: 5 µl master mix, 0.4 µl forward primer, 0.4 µl reverse primer) were mixed. For each GOI and reference gene a separate reaction mix was prepared with specific primer pairs obtained from Eurofins Genomics GmbH (primer sequences listed in Tab. A5). For each sample 5.8 µl reaction mix and 4.2 µl cDNA template (50 ng μl^{-}) was combined in a 96-well plate (400 µl, white), carefully mixed and spun down. For the qPCR run, the 98-well plate was then placed into the real-time thermocycler (CFX Connect Real-Time System, Bio-Rad Laboratories, Feldkirchen Germany) with the following temperature program: 2 min 95 °C , 40-cycles: 5 sec 95 °C, 30 sec 60 °C, thereafter 50 °C.
To check for contaminations or malfunctions, a meltcurve with continuous fluorescence monitoring was implemented at the end of the qPCR run (settings: 5 sec

65 ℃– 95 ℃, increment: 0.5 ℃). Samples without a clear dissociation curves were excluded from further calculations.
The amount of fluoresce is proportional to the amount of newly synthesized target DNA and by plotting the recorded fluorescence signal of each sample against the number of cycles, a sigmoid function can be obtained. The output variable for each qPCR-sample, the C_T (threshold cycle)-value, represents the number of cycles at a threshold level of fluorescence, i.e. the level where the fluorescence exceeds the background fluorescence and where all functions are situated in the exponential phase (Mülhardt, 2009). To determine the C_T-value of each GOI and reference gene based on the same threshold, although eventually measured on different plates, LinRegPCR (version 2017.1; each GOI or reference gene as amplicon group) program was used. Due to the fact that the three implemented technical replicates of each biological sample can exhibit variation, an outlier correction was implemented when three technical replicates were executed. A technical replicate was excluded from further calculations, when its C_T-value was more than three-times further distant to the median C_T of all three technical replicates than the other two technical replicates. After this correction, the mean value of the technical replicates from one biological sample was calculated. To obtain the relative quantity (RQ), the mean PCR efficiency (evaluated by LinRegPCR) was raised by the exponent of the C_T-value. To quantify the expression of each GOI relative to reference genes, the mean RQ-value of the used reference genes were calculated for each biological sample. This mean value was then used to normalize the corresponding RQ of each GOI, to obtain the NRQ (normalized RQ) value of each GOI. A subsequent log transformation to the NRQ data, yielding in the $\log_2$NRQ, brings the values back to the C_T-scale. For a better comparison between treatments, the $\log_2$NRQ-values were normalized to the mean of the $\log_2$NRQ-values of the control treatment.

3.7 Measurement of plant growth and fitness

Growth of *N. attenuata* was monitored by examining the stalk length. The plant fitness was determined by examining flowering (number of flowers, number of open flowers and duration of flowering) and seed production (number of capsules and seed weight). The number of flowers was counted each day by counting only flowers that were opening for the first time (flowers were marked with a small plastic

ring thereafter to prevent repeated counting). Furthermore, the total number of open flowers per day was counted (cumulatively counted number of flowers) to obtain the days with open flowers (duration of flowering). After flowers converted into capsules, these were counted and cut from the plant shortly before opening. After the cut off capsules released the seeds, seed weight was determined without capsule envelope (balance: Semi-micro balances, SM 1265Di, VWR International GmbH, Darmstadt Germany).

3.8 Statistical analysis

All statistical analyses were performed with the R software, version 3.6.1 (R Core Team, 2019). In all experiments treatments were assigned randomly either between all experimental plants or between size-matched plants within a biological replicate. Common diagnostic plotting techniques were used to evaluate if the data met assumptions of the respective statistical analysis and if quality of models was sufficient. These included: boxplots, qq-plots, plotting residuals vs. fitted values, estimated random intercepts vs. random intercepts, and estimated residuals vs. residuals. If required homogeneity of variances was furthermore tested with F- or Levene-test. In case the data did not meet the requirements of the respective parametric tests, they were either transformed (transformations: inversed, square-root, inversed-square-root, square-root or $\log_2$) or an alternative statistical analysis was used. All summaries of the applied statistical tests or models described below are provided in the appendix as supporting information (see B.2.2, Tab. A6 - A20).
Within the first experiments with *S. dulcamara* assessing the temporal dynamics of responses to oviposition (Exp. 1 - 3) two sample t-test or Welch two sample t-test were used to compare phytohormonal respectively transcriptional level between oviposited and corresponding untreated control plants within the corresponding time point.
In the full-factorial priming experiments with *S. dulcamara* examining the phytohormonal and transcriptional changes during the onset of the response to larval feeding (Exp. 4 - 6), linear mixed models (LMMs) were used (function "lmer" in package "lme4", Bates et al. (2014)). These models included priming (oviposition), triggering (larval feeding / simulated herbivory) and their interaction as fixed factors and the replicate block as random factor (blocked experimental design). The p-values for a comparison between the treatments were calculated using the function "lsmeans" which compute least-squares means for specified factors or

factor combinations of a LMM (package "lsmeans", Lenth (2016)). In the first experiment investigating the fitness consequences of oviposition and larval feeding by *S. exigua* for *N. attenuata* (Exp. 7) two sample t-test were used to check for differences the corresponding parameters (number of open flowers per plant, duration of flowering, cumulative number of open flowers per plant, number of capsules per plant and seed weight per plant). Differences between the treatments in larval survival, stalk length and the number of open flowers per plant were compared with t-test within the corresponding measurement time point.
In the following experiment with *N. attenuata* and *S. exigua* (Exp. 8), differences in larval survival and mean larval weight per plant between larvae on oviposited and non-oviposited plants (T and PT plants) in experiment eight were compared using two sample t-test. The effect of priming (oviposition) and triggering (larval feeding) as well as their interaction on plant fitness parameters (number of flowers per plant, duration of flowering, number of capsules per plant and seed weight per plant) in this experiment (Exp. 8) and the subsequent experiments (Exp. 9 and 10) were assessed with two-way ANOVAs. In parallel, the effect of priming, triggering and their interaction on stalk length and the number of open flowers per plant were determined using two-way ANOVA, while only the data of the respective time point were compared with one another. To compare the growth of the plants in experiment seven and eight, the growth rate (cm stalk length per day) per plant was calculated from three successive stalk length measurements during a comparable growth phase (see Fig.A5, grey boxes), as the plants grew lagged about five days. Differences in the growth rate between both experiments were assessed with Wilcoxon rank sum test with continuity correction.
To compare the fitness of regrown *N. attenuata* plants after oviposition and larval feeding before defoliation (Exp. 11 and 12), results of both experiments were combined and the effect oviposition and larval feeding on plant fitness parameters (number of flowers per plant, duration of flowering and number of capsules per plant) were assessed using generalized linear mixed models (GLMMs, function "glmer" in package "lme4", Bates et al. (2014)). To examine the effect on the total seed weight, LMMs were used. These GLMMs with poisson error distribution and a logit link function respectively the LMMs included the replicate as random factor, while priming priming (oviposition), triggering (larval feeding) and their interaction were included as fixed factors. The p-values for a comparison between the treatments were calculated using the function "lsmeans" which compute least-squares means for specified factors or factor combinations of a GLMM (package "lsmeans", Lenth (2016)). Within the experiment investigating the fitness of re-

grown plants which were exposed to defoliation in different developmental stages (Exp. 13), the effect of priming (oviposition) and triggering (larval feeding) as well as their interaction on plant fitness parameters (number of flowers per plant, duration of flowering, number of capsules per plant and seed weight per plant) were assessed with two-way ANOVA. In this analysis only the data of one of the two developmental stages were compared with each other. For a treatment wise comparison of the seed weight a post-hoc Tukey test for multiple comparisons of means was used.

4 Results

4.1 Dynamics of the primed state in *S. dulcamara*

4.1.1 Temporal pattern of phytohormonal responses to *S. exigua* oviposition

To examine the temporal dynamics of potential regulators after oviposition within and after the phase of egg exposure, two independent experiments were conducted to compare the phytohormonal accumulation at different time points after oviposition by *S. exigua* (Exp. 1 & 2). In the first experiment, responses in oviposited leaves of *S. dulcamara* were compared to those in untreated control leaves at five time points (2, 4, 6, 8 and 10 days after oviposition; while eggs were removes four days after oviposition) within and after the natural egg incubation time of *S. exigua* (which is arround 3 to 4 days), while in the second experiment two time points within the phase of egg exposure (one and three days after oviposition) were considered.

During the phase of egg exposure oviposition caused a SA induction. In the first experiment, oviposition by *S. exigua* caused an approximately threefold increase of SA two days after oviposition (Fig. 19 b). Four days after oviposition by *S. exigua*, SA contents in oviposited leaves were still higher than in corresponding control leaves, although on a lower level. Also in the second experiment, SA levels in oviposited plants were higher than in control plants one day after oviposition (Fig. 19 a). Three days after oviposition, SA contents in oviposited leaves tended to be higher in oviposited plants than in control plants (Two Sample t-test, $t(14)$=-1.503, p=0.155; Fig. 19 a). At the time points after egg removal (6, 8 and 10 days after oviposition respectively 2, 4 and 6 days after egg removal; Exp. 1), no differences in SA levels between oviposited leaves and control leaves were detected. In both experiments, contents of ABA in oviposited leaves remained unaltered compared to those of control plants at the time points during the phase of egg exposure (Fig. 19 c & d). Moreover, eight days after oviposition (four days after egg removal) ABA levels were not differentially induced in oviposited and control leaves. However, six days after oviposition (two days after egg removal) ABA levels were slightly higher in oviposited leaves (Welch Two Sample t-test, $t(13.972)$=-1.667,

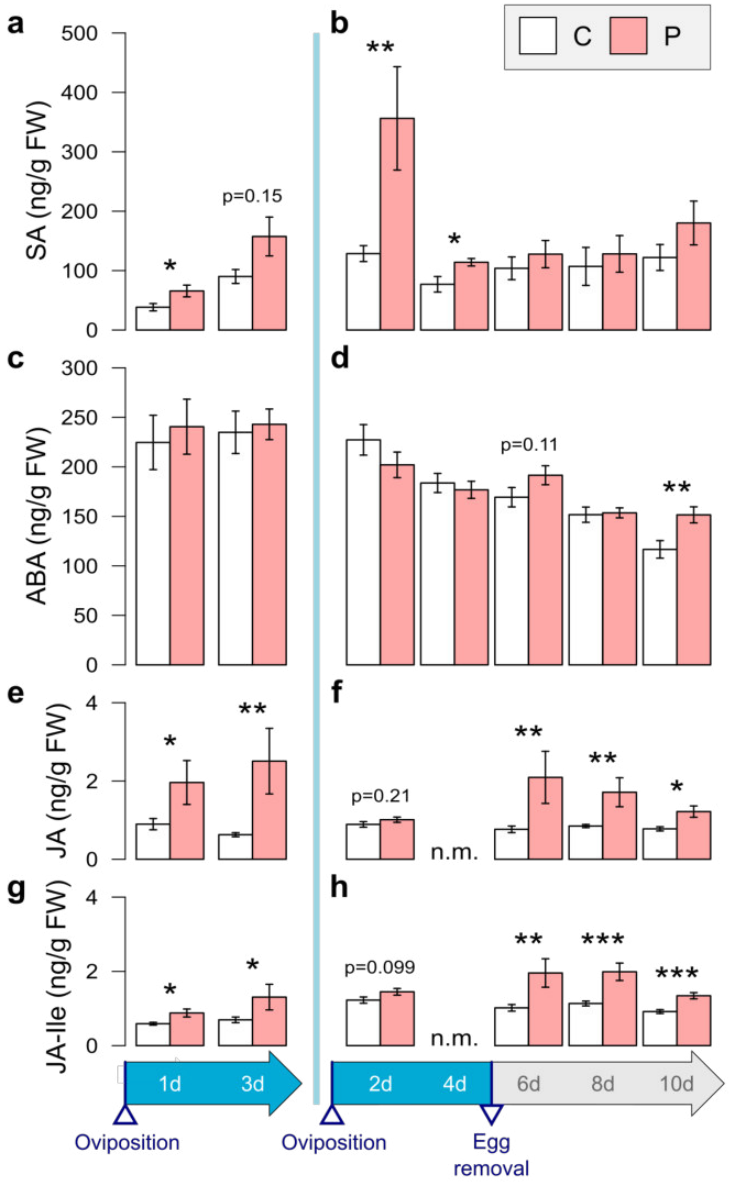

Figure 19: *S. dulcamara* responds to oviposition by *S. exigua* with a phytohormonal induction. Levels of (a & b) salicylic acid (SA), (c & d) abscisic acid (ABA) (e & f) jasmonic acid (JA) and (g & h) jasmonic acid-isoleucine (JA-Ile) in leaves local to oviposition (eggs, rose bars) or corresponding leaves of non-oviposited plants (control, white bars). Leaf material was harvested (a, c, e & g) 1 and 3 days or (b, d, f & h) 2, 4, 6, 8 and 10 days after oviposition from individual plants. Eggs were removed right before harvest or 4 days after oviposition. Bars represent mean ± SEM. (f & h) JA and JA-Ile contents 4 days after oviposition were not measured. N = (a, c, e & g) 8-10 / (b, d, f & h) 7-12. Asterisks indicate significant differences according to (a, c, d, g, h) two sample t-test or (b, e, f) Welch two sample t-test (detailed information on the statistics used see Tab. A6): */**/*** ($p < 0.05/0.01/0.001$).

p=0.118). At the last measurement time point ten days after oviposition (six days after egg removal) contents of ABA in oviposited leaves were increased compared to those in untreated control leaves (Fig. 19 d).
Contents of jasmonates, i.e. JA and JA-Ile, were induced in oviposited leaves on a low level. During the phase of egg exposure, JA and JA-Ile contents were higher in oviposited leaves compared to those of control plants one and three days after oviposition in the second experiment (Fig. 19 e & g). In the first experiment, contents of JA in oviposited leaves did not differ from those of control plants two days after oviposition (Welch Two Sample t-test, t(11.68)=-1.305, p=0.217, Fig. 19 h), while JA-Ile were by trend higher at this time point (Two Sample t-test, t(13)=-1.777, p=0.099; Fig. 19 h). Four days after oviposition, jasmonates were not measured. At the time points after egg removal (6, 8 and 10 days after oviposition), contents of JA and JA-Ile were higher in oviposited leaves than in non-oviposited control leaves at the time points (Fig. 19 f & h). However, at all time points in both experiments the induction level of JA and JA-Ile ranged on a low level close to the detection limit at around 2 ng per mg FW.

4.1.2 Temporal pattern of transcriptional responses to *S. exigua* oviposition

In order to investigate the temporal dynamics of potential regulators after oviposition, also responses on the transcriptional level were considered. Therefore, a set of stress- and defence-related genes were analysed for their transcriptional induction in oviposited and non-oviposited leaves at the different time points after oviposition by *S. exigua* (Exp. 1 & 2).
Overall, a differential expression of considered genes in response to oviposition was detectable up to ten days after oviposition (six days after egg removal), while all analysed genes exhibit highest accumulation four days after oviposition (Fig. 20). Transcript levels of *PRX2* (Fig. 20 a) and *PG4* (Fig. 20 d) in the first experiment were not differentially induced in oviposited leaves compared to those in control leaves two days after oviposition. But four, six and eight days after oviposition transcript level of both genes were higher in oviposited leaves than in control leaves, with highest transcriptional levels four days after oviposition. In the second experiment, expression of *PG4* was downregulated in oviposited plants relative to control plants one day after oviposition, while three days after oviposition transcript levels were higher indued in oviposited leaves than in control leaves (Fig. 20 c).

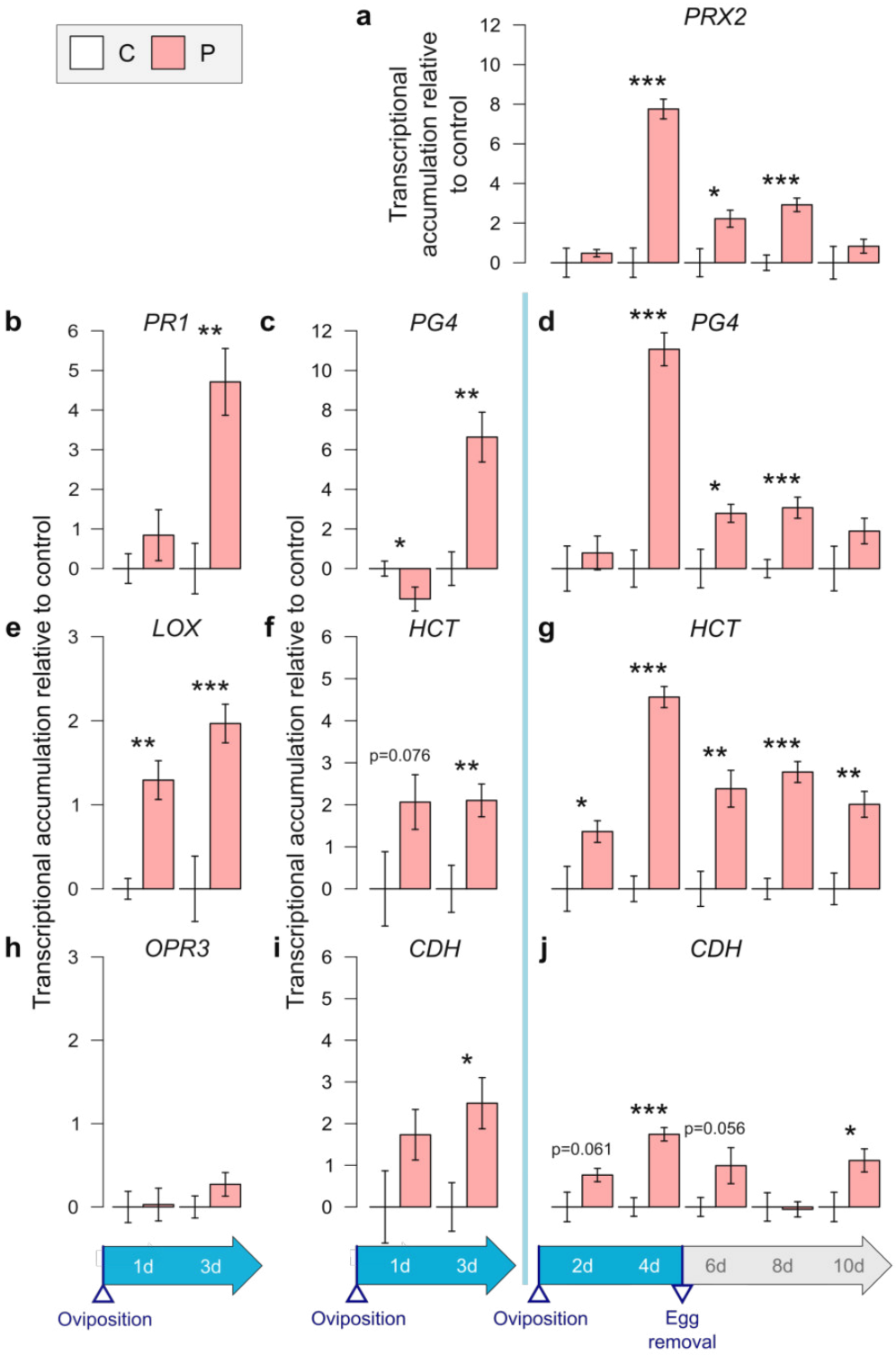
C
P
a
PRX2
Transcriptional accumulation relative to control
b
PR1
c
PG4
d
PG4
e
LOX
f
HCT
p=0.076
g
HCT
h
OPR3
i
CDH
j
CDH
p=0.061
p=0.056
Transcriptional accumulation relative to control
1d
3d
Oviposition
1d
3d
Oviposition
2d
4d
6d
8d
10d
Oviposition
Egg removal

Figure 20: Temporal pattern of transcript accumulation of selected *S. dulcamara* genes in response to oviposition by *S. exigua*. Transcript levels of (a) *peroxidase 2* (*PRX2*), (b) *pathogenesis-related gene 1* (*PR1*), (c & d) *polygalacturonase 4* (*PG4*), (e) *lipoxygenase* (*LOX*), (f & g) *hydroxycinnamoyl-CoA shikimate/quinate-hydroxycinnamoyl transferase* (*HCT*), (h) *12-oxophytodienoate reductase 3* (*OPR3*) and (i & j) *cytokinin oxidase/dehydrogenase* (*CDH*) in leaves local to oviposition (eggs, rose bars), or corresponding leaves of non-oviposited plants (control, white bars). Leaf material was harvested (b, c, e, f, h & i) 1 and 3 days or (a, d, g & j) 2, 4, 6, 8 and 10 days after oviposition from individual plants. Eggs were removed right before harvest or after 4 days. Transcriptional expression was normalized to the average expression of the reference gene ELF1 and CAC, presented as $\log_2$NRQ value (relative to control of each time point). Two technical replicates were conducted per biological replicate. Bars represent mean ± SEM, N = (b, c, e, f, h & i) 8-10 / (a, d, g & j) 7-8. Two technical replicates were conducted per biological replicate. Asterisks indicate significant differences according to (a & d) Welch two sample t-test or (b, c, e, f, g, j, h & i) two sample t-test (detailed information on the statistics used see Tab. A7): */**/*** ($p<$ 0.05/0.01/0.001).

Expression of *HCT* were higher in oviposited leaves than in control leaves at all time points in both experiments (Fig. 20 f & g), while one day after oviposition (Exp. 2) transcript levels of *HCT* were only by trend higher in oviposited leaves than in control leaves (Two Sample t-test, $t(18)$=-1.881, p=0.076). In the first experiment, transcript levels of *CDH* were by trend higher induced in oviposited leaves compared to untreated control leaves two days (Two Sample t-test, $t(14)$=-1.971, p=0.068) and six days after oviposition (Two Sample t-test, $t(14)$=-2.078, p=0.056; Fig. 20 j). Four and ten days after oviposition, as well as three days after oviposition in the second experiment, *CDH* transcripts were higher expressed in oviposited leaves than in non-oviposited control leaves (Fig. 20 i & j). Eight days after oviposition, transcript level of *CDH* were not differentially altered.
At both considered time points in the first experiment, transcript level of *LOX* were higher in oviposited leaves than in control leaves (Fig. 20 e), while *OPR3* were not differentially expressed (Fig. 20 h).

4.1.3 Temporal pattern of phytohormonal and transcriptional responses to *A. autumnitella* oviposition

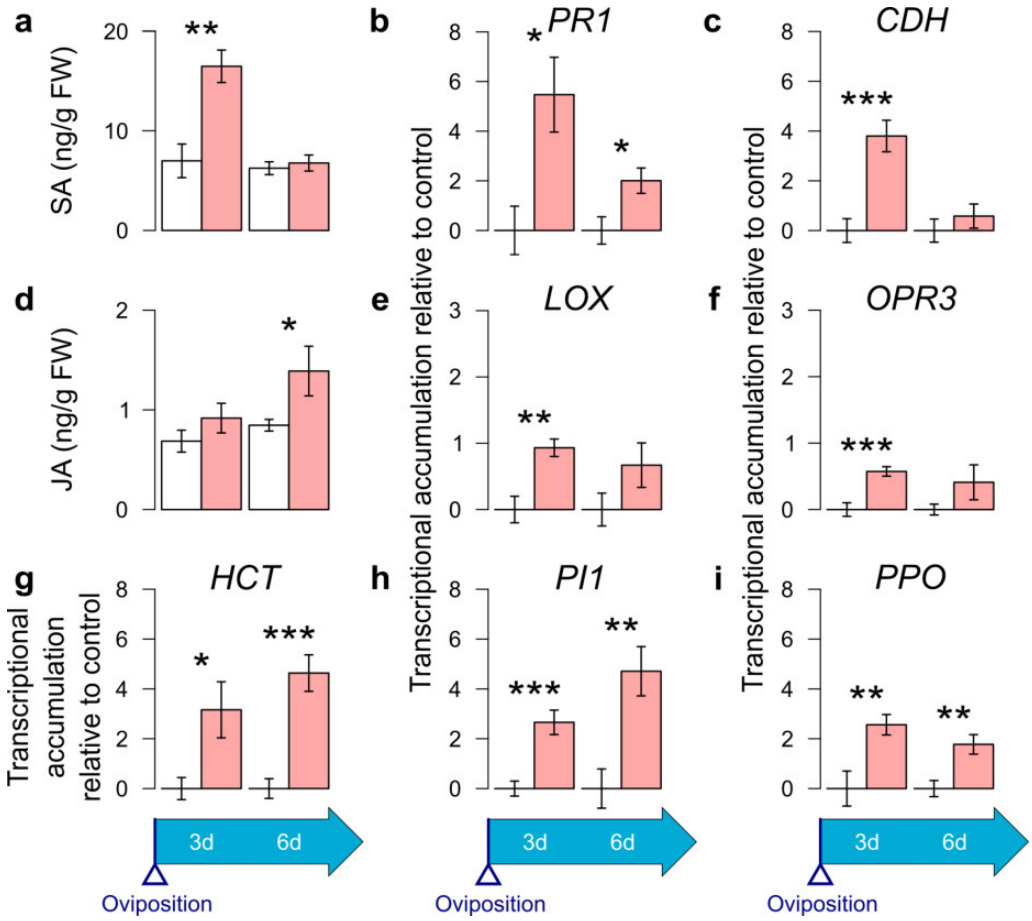

Figure 21: *S. dulcamara* responds to oviposition by *A. autumnitella* with a (a & b) phytohormonal and (c & d) transcriptional induction. Levels of the phytohormones (a) salicylic acid (SA) and (d) jasmonic acid (JA), as well as transcript levels of (b) *pathogenesis-related protein 1* (*PR1*), (c) *cytokinin oxidase/dehydrogenase* (*CDH*), (e) *lipoxygenase 3* (*LOX*3), (f) *12-oxophytodienoate reductase 3* (*OPR3*), (g) *hydroxycinnamoyl-CoA shikimate/quinate-hydroxycinnamoyl transferase* (*HCT*), (h) *protease inhibitor 1*(PI1) and (i) *polyphenol oxidase* (*PPO*) in leaves local to oviposition (eggs, rose bars) or corresponding leaves of non-oviposited plants (control, white bars) harvested either three or six days after oviposition from individual plants. Transcriptional accumulation is presented as $\log_2$ NRQ value (relative to control of each time point), normalized to average expression of the reference genes ELF1, CAC and GAPDH. Bars represent mean ±SEM. N = 5-9. Two technical replicates were conducted for transcriptional measurements. Asterisks indicate significant differences according to two sample t-test (detailed information on the statistics used see Tab. A8): */**/*** ($p<$ 0.05/0.01/0.001).

The aim of the third experiment was to investigate the temporal dynamics of phytohormonal and transcriptional changes of *S. dulcamara* in response to oviposition by the specialist leaf-miner *A. autumnitella* (Exp. 3). Therefore, leaf material was analysed at two time points (three and six days) after oviposition within the natural egg incubation time of *A. autumnitella* (which is around 6-7 days) and compared to untreated control leaves.
On the phytohormonal level, oviposition by *A. autumnitella* caused an increase of SA levels only at the first measurement time point. Three days after oviposition, contents of SA in oviposited leaves were higher than those in untreated control leaves (Fig. 21 a). However, differences in SA levels between oviposited and non-oviposited control leaves were not detectable six days after oviposition. Contents of JA were not induced three days after oviposition, but induced at the second measurement time point around the end of the egg incubation time (Fig. 21 d). Six days after oviposition JA levels in oviposited leaves were higher than those in control leaves, although this induction ranged on a low level (1.5 ng per mg FW). In contrast, concentrations of ABA (Fig. A1 b) and JA-Ile (Fig. A1 a) did not differ between oviposited and non-oviposited control leaves at both considered time points after oviposition.
On the transcriptional level, oviposition by *A. autumnitella* caused an induction of different defence related genes. Transcripts of *PR1* , *HCT*, *PI*1 and *PPO*were higher expressed in oviposited leaves than in non-oviposited leaves at both time points (Fig. 21 b, g, h & i). A higher transcript accumulation of *CDH*, *LOX*and *OPR3* in oviposited leaves compared to control leaves was only detectable three days after oviposition, while six days after oviposition no differences in expression were detectable (Fig. 21 c, e & f).

4.1.4 Phytohormonal and transcriptional responses to oviposition and natural herbivory in the beginning of the attack

To assess how possible oviposition-mediated responses to larval feeding are regulated during the beginning of the larval attack, two full-factorial experiment with oviposition (priming stimulus) and a short phase of larval feeding (triggering stimulus) were conducted (Exp. 4 & 5). Four days after oviposition by *S. exigua* eggs were removed and 20 third instar *S. exigua* larvae were applied for four hours of feeding to the leaf five positions higher (vascularly fully connected) than the leaf exposed to oviposition.

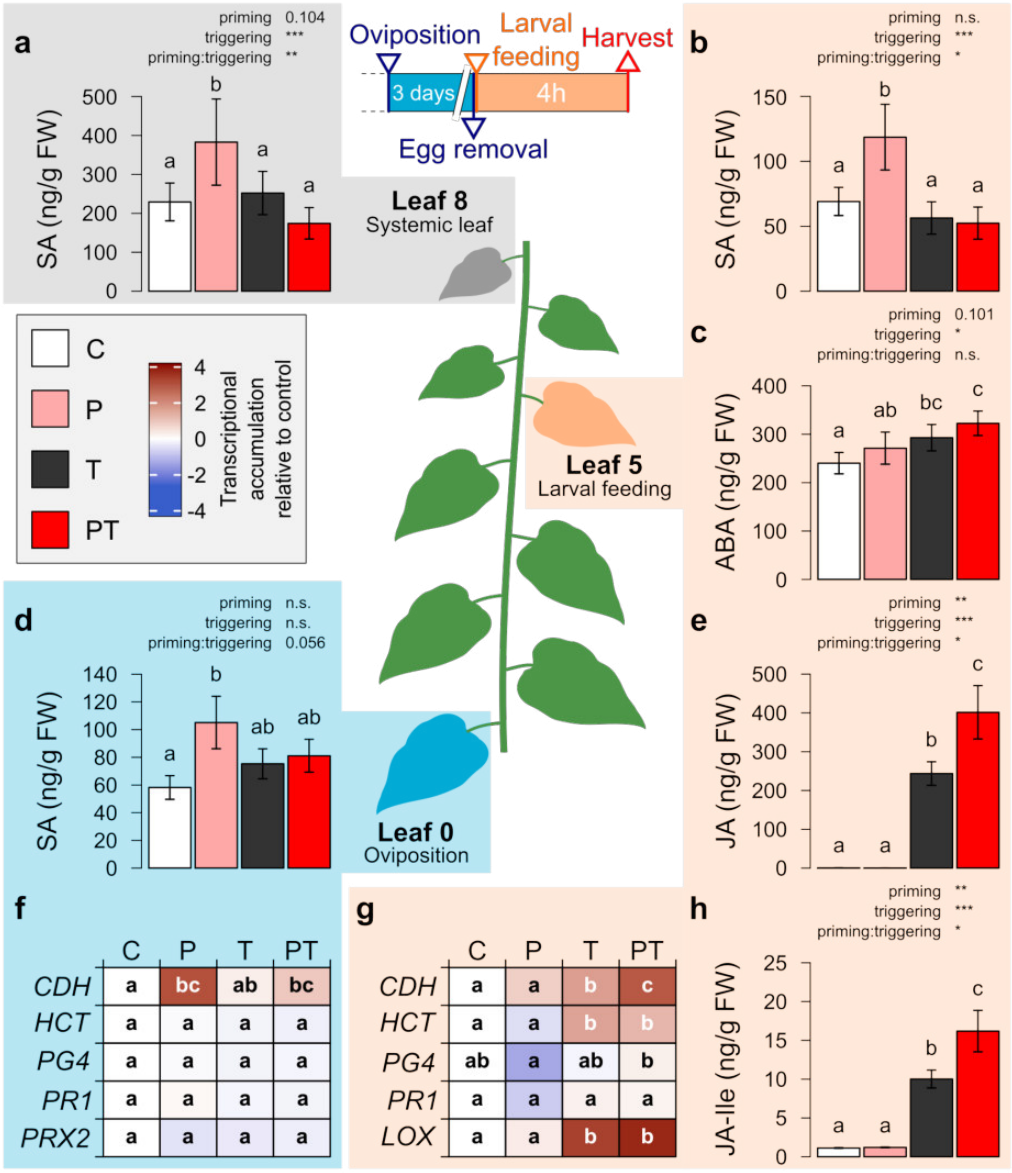

Figure 22: *S. dulcamara* responds on a phytohormonal and transcriptional induction to oviposition and larval feeding by *S. exigua* in different leaf positions. Plants were either kept untreated (C), exposed to oviposition only (P), larval feeding only (T) or a combination of both (PT). Four days after oviposition eggs were removed and 20 neonate larvae were applied to the leaf five leaf positions higher (vascularly fully connected). After four hours of larval feeding, leaf material of the oviposited leaf (leaf 0), the leaf exposed to larval feeding (leaf 5) and a young systemic leaf (leaf 8) was harvested and analysed for phytohormonal and transcriptional contents (no transcriptional analyses of material from leaf 8). Bars represent mean ± SEM levels of salicylic acid (SA) in (a) leaf 8, (b) leaf 5 and (d) leaf 0, as well as the contents of (c) abscisic acid (ABA), (e) jasmonic acid (JA) and (h) jasmonic acid-isoleucine (JA-Ile) in leaf 5. Transcriptional induction of *CDH*, *HCT*, *PG4*, *PR1*, *PRX2* and *LOX*in leaf material of (f) leaf 0 or (g) leaf 5 are displayed in heatmaps, presenting the $\log_2$ NRQ value relative to control, normalized to average expression of the reference genes ELF1. N = 6-8. Three technical replicates were conducted for transcriptional measurements. Different letters indicate significant differences according to LMMs (detailed information on the statistics used see Tab. A9), $p <$ 0.05.

In the first experiment, only the interaction of priming and triggering caused an almost significant effect (p=0.056) on SA levels in the leaf exposed to oviposition (leaf 0; (Fig. 22 d)). However, in a treatment wise comparison oviposition caused a SA induction, as leaves exposed exclusively to oviposition had higher SA levels than control plants, while the other treatments caused intermediate level. In this leaf position, contents of the other measured phytohormones (ABA, JA and JA-Ile) were unaltered in differentially treated plants (Fig. A2 a - c). On the transcriptional level, only transcripts of *CDH* were differentially induced in this leaf position, while transcript level of *HCT*, *PG4* , *PR1* and *PRX2* were not altered (Fig. 22 f). Transcript accumulation of *CDH* was higher induced in leaves exposed exclusively to oviposition compared to leaves of control plants or leaves exposed exclusively to larval feeding.

In the leaf exposed to larval feeding or corresponding leaves in the same position (leaf 5), SA contents were significantly affected by larval feeding and the interaction between priming and triggering (Fig. 22 b), while priming alone had no effect. In a treatment wise comparison, plants which were exclusively exposed to oviposition (on the leaf five positions below) exhibit a higher SA accumulation than plants of the other treatments (Fig. 22 b). In this leaf position (leaf 5), the accumulation of ABA reveals a staircase-shaped induction pattern which further suggests an additive induction pattern. ABA contents in this leaf position were affected by larval feeding and slightly affected by oviposition (priming stimulus, p=0.101) while the interaction had no significant effect (Fig. 22 c). In a treatment wise comparison, ABA levels after oviposition (without larval feeding) were slightly higher than those of control plants (p= 0.12), whereas larval feeding (without oviposition) caused a further higher increase of ABA levels, which were significantly different to those of control plants but insignificant to those of oviposited plants (p=0.407, Fig. 22 c). The combination of those two treatments (PT plants) caused even slightly higher ABA levels than after larval feeding without prior oviposition (p=0.139). The ABA contents of the combination treatment were about the same level as those after larval feeding increased about the induced level of the oviposition treatment, indicating for an additive effect. In the this leaf position local to larval feeding, jasmonates indicated a primed feeding-induced induction. Contents of JA and JA-Ile were clearly induced by larval feeding (Fig. 22 e & h). In addition, oviposition (priming stimulus) and the interaction of priming and triggering had a significant effect on the JA and JA-Ile accumulation. The higher feeding-induced JA and JA-Ile levels of oviposited plants compared to non-oviposited plants (Fig. 22 e & h) indicate for

a primed jasmonate induction. In this leaf position, transcripts of *CDH*, *HCT* and *LOX* were clearly feeding-induced and not altered by oviposition alone (Fig. 22 g). However, oviposition before larval feeding caused higher transcript accumulation of *CDH* than larval feeding alone, indicating for a primed jasmonate induction (Fig. 22 g). In contrast, the transcript level of *HCT* and *LOX* were not affected by oviposition in combination with larval feeding. Transcript levels of *PR1* were not altered in this leaf position (Fig. 22 g). In response to oviposition (on the leaf five positions below), transcript level of *PG4* were slightly downregulated in this leaf position (leaf 5) compared to transcript level of plants exposed to oviposition and larval feeding (Fig. 22 g). Albeit the difference in *PG4* accumulation between exclusively oviposited plants and control (p=0.074) respectively exclusively feeding exposed plants (p=0.099) were almost significant.
In the systemic leaf three positions above the leaf exposed to larval feeding (leaf 8), larval feeding (triggering) and the interaction between the priming and triggering had a significant effect on the SA accumulation (Fig. 22 a), while oviposition (priming) alone had only a slight effect on the SA accumulation (p=0.104, Fig. 22 a). But in a treatment wise comparison, plants exclusively exposed to oviposition had higher SA level in this leaf position than plants of the other treatments (Fig. 22 a). Contents of ABA, JA and JA-Ile remained unaffected in this leaf position (Fig. A2 d - f).

In the second full factorial priming experiment with oviposition and a short phase of larval feeding by *S. exigua* (Exp. 4) only the phytohormonal induction in the leaf exposed to larval feeding (leaf 5) was analysed.
Contents of SA remained unaffected by oviposition or larval feeding within this experiment (Fig. A3 a). Larval feeding (triggering) clearly induced ABA, JA and JA-Ile level in the leaf local to larval feeding (Fig. A3 b - d). Oviposition (priming) had a slight effect on JA (p=0.095) and JA-Ile (p=0.102) accumulation, while it did not affect ABA accumulation. The interaction of priming and triggering did not significantly affect ABA, JA or JA-Ile accumulation. Remarkably, feeding-induced level of JA and JA-Ile were clearly higher (JA levels ca. 1000 – 1600 ng / g FW, JA-Ile levels 55 – 30 ng / g FW) than in the previous experiment (Exp. 4) where levels were approximately three to two times lower (Fig. 22 e & h).

4.1.5 Phytohormonal and transcriptional accumulation after oviposition and simulated herbivory during the onset of the response

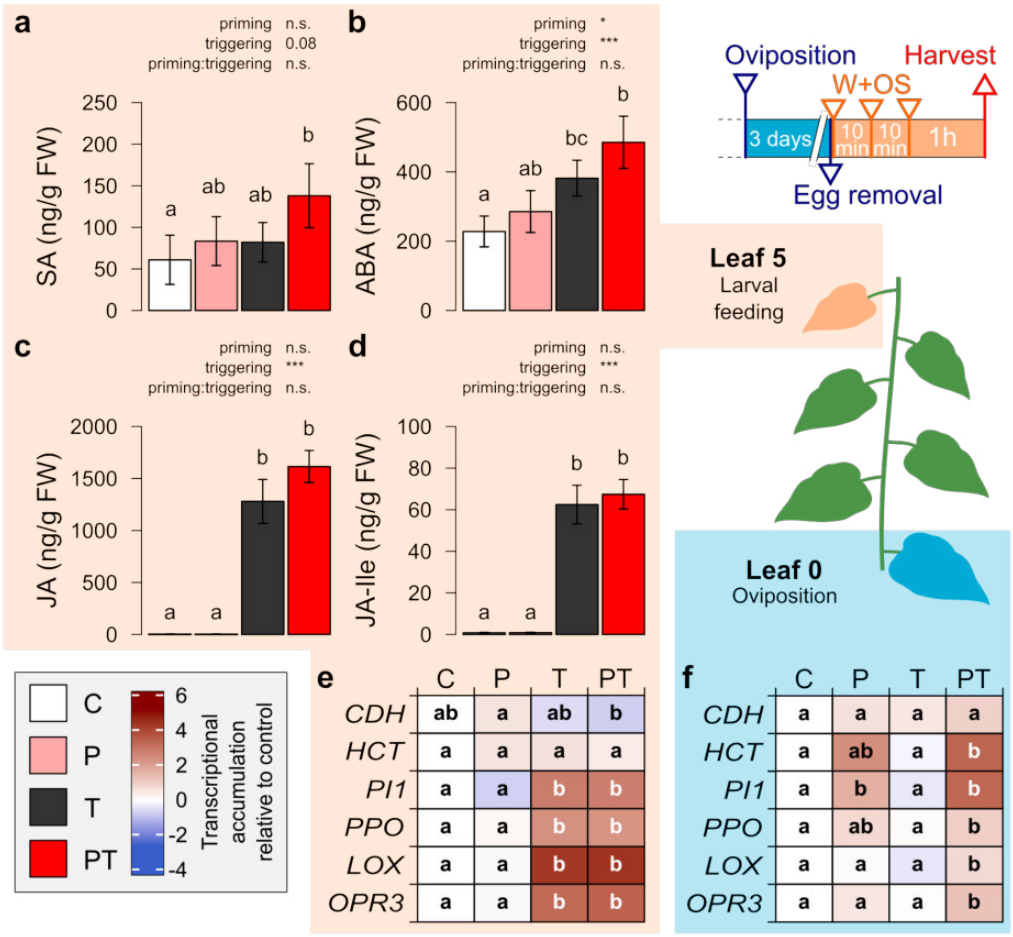

Figure 23: *S. dulcamara* responds on a phytohormonal and transcriptional induction to oviposition by *S. exigua* and simulated herbivory. Plants were either kept untreated (C), exposed to oviposition only (P), simulated herbivory (W+OS treatment) only (T) or a combination of both (PT). Four days after oviposition eggs were removed and three W+OS treatments (simulated herbivory, 10 min time gab between the treatments) were applied to the leaf five leaf positions higher (vascularly fully connected). One hour after the last treatment, leaf material of the oviposition leaf (leaf 0) and the leaf exposed to the triggering stimulus W+OS treatment (leaf 5) was harvested and analysed for phytohormonal and transcriptional contents. Bars represent mean ± SEM levels of (a) salicylic acid (SA), (b) abscisic acid (ABA), (c) jasmonic acid (JA) and (d) jasmonic acid-isoleucine (JA-Ile) of leaf material harvested from the leaf exposed to W+OS treatment (leaf 5). Transcriptional induction of *CDH*, *HCT*, *LOX*3, *PI*1, *PPO*, *PR1* and *OPR3* in leaf material of (e) leaf 0 or (f) leaf 5 are displayed in heatmaps, presenting the $\log_2$ NRQ value relative to control, normalized to average expression of the reference genes CAC, GAPDH and ELF1. $N_{phytohormones/transcripts} = 7/9$. Two technical replicates were conducted for transcriptional measurements. Significant differences according to LMMs (detailed information on the statistics used see Tab. A11): */**/*** ($p < 0.05/0.01/0.001$). Different letters indicate significant differences according to LMMs.

To repeat the previous experiments which aim to exermine responses during the beginning of the attack under more standardized conditions, a full-factorial experiment with simulated herbivory instead of larval feeding was conducted (Exp. 6). As in the previous experiments, four days after oviposition by *S. exigua* (priming stimulus) simulated herbivory, i.e. repeated W+OS treatment (triggering stimulus), was applied. Leaf material of the leaf exposed to oviposition (leaf 0) and the leaf exposed to simulated herbivory (leaf 5) was harvested one hour after the last W+OS treatment.

In the leaf exposed to oviposition, contents of SA or ABA were not affected by any treatment (Fig. A4 a & b), while JA and JA-Ile levels were slightly affected by oviposition (JA: p=0.144; JA-Ile: p=0.135) displaying a low-level induction (2-3 ng / g FW; Fig. A4 c & d). On the transcriptional level, expression of *CDH* was not affected in this leaf position (Fig. 23 f). Accumulation of *HCT* and *PPO* transcripts were significantly induced in plants exposed to oviposition and simulated herbivory compared to control plants or plants exposed to simulated herbivory only (Fig. 23 f). Moreover, oviposition alone caused a by trend higher transcript level of *HCT* (C-P: p=0.067; P-T: p=0.082) and *PPO* (C-P: p=0.063; P-T: p=0.092) than control or feeding-exposed plants, while the difference between oviposited plants and plants exposed to oviposition and simulated herbivory were insignificant (Fig. 23 f). Transcript level of *PI*1 were significant higher induced in plants exposed to oviposition with or without simulated herbivory compared to non-oviposited control plants or plants exposed to simulated herbivory only (Fig. 23 f). Expression of *PI*1 in plants exposed to oviposition and simulated herbivory were even by trend higher than in plants exposed to oviposition only (p=0.091).

In the leaf exposed to simulated herbivory or in the corresponding leaf (leaf 5), contents of SA were by trend affected by oviposition (priming stimulus, p=0.08, Fig. 23 a). However, in treatment-wise comparison SA levels of plants exposed to oviposition and simulated herbivory were higher than those of control plants, while the other treatments exhibit intermediate SA levels (Fig. 23 a). Contents of ABA in this leaf position were induced by the triggering stimulus, but not by the priming stimulus (p=0.244) or the interaction of both (Fig. 23 b). However, the accumulation of ABA exhibits a staircase-shaped induction pattern. Oviposition alone caused only insignificantly higher ABA contents compared to those of control plants. Simulated herbivory caused a significant induction of ABA compared to control plants, while the difference to oviposited plants differed slightly (p=0.083; Fig. 23 b). The combination of oviposition and simulated herbivory

(PT) further increased ABA contents after simulated herbivory about the induced level of the oviposition treatment, while the difference between plants exposed to simulated herbivory with and without prior oviposition was almost significantly higher (p=0.063; Fig. 23 b). JA and JA-Ile contents were clearly induced by simulated herbivory (Fig. 23 c & d). Furthermore, JA contents were almost significantly higher in prior oviposited plants exposed to simulated herbivory compared to non-oviposited plants exposed to simulated herbivory (p=0.054, Fig. 23 c). Oviposition alone or in combination with simulated herbivory had no further effect on JA-Ile accumulation (Fig. 23 d). In this leaf position (leaf 5), transcriptional accumulation was mainly affected by simulated herbivory. Expression of *LOX*, *OPR3* , *PI*1 and *PPO*transcripts were clearly higher in plants exposed to simulated herbivory, without further effects of oviposition (Fig. 23 e). Transcript level of *HCT*were not affected by any treatment in this leaf position (Fig. 23 e). Expression of *CDH*in this leaf position was not altered by any treatment compared to untreated control plants (Fig. 23 e). Expression differed only between oviposited plants and those exposed to oviposition and simulated herbivory.

4.2 Fitness consequences of oviposition priming for *N. attenuata*

4.2.1 Growth and fitness consequences of oviposition and larval feeding by *S. exigua*

To evaluate the fitness consequences of oviposition priming in relation to the consequences of induced defence, the effect of oviposition and larval feeding by the generalist herbivore *S. exigua* on growth and plant fitness of the annual *N. attenuata* was examined (Exp. 7 & 8).

In the first experiment in this context, plants were exposed to oviposition, eggs were removed after three days and subsequently 20 *S. exigua* neonate larvae were applied to each plant for 15 days of herbivory. During the hole experiment fitness parameter were recorded. Larval performance was impaired on prior oviposited plants. After two and six days of feeding larval survival did not differ between oviposited and non-oviposited plants, while after four days (Two Sample t-test, $t(6)$=2.183, p=0.072; Fig. 24 a) and six days (Two Sample t-test, $t(6)$=1.993,

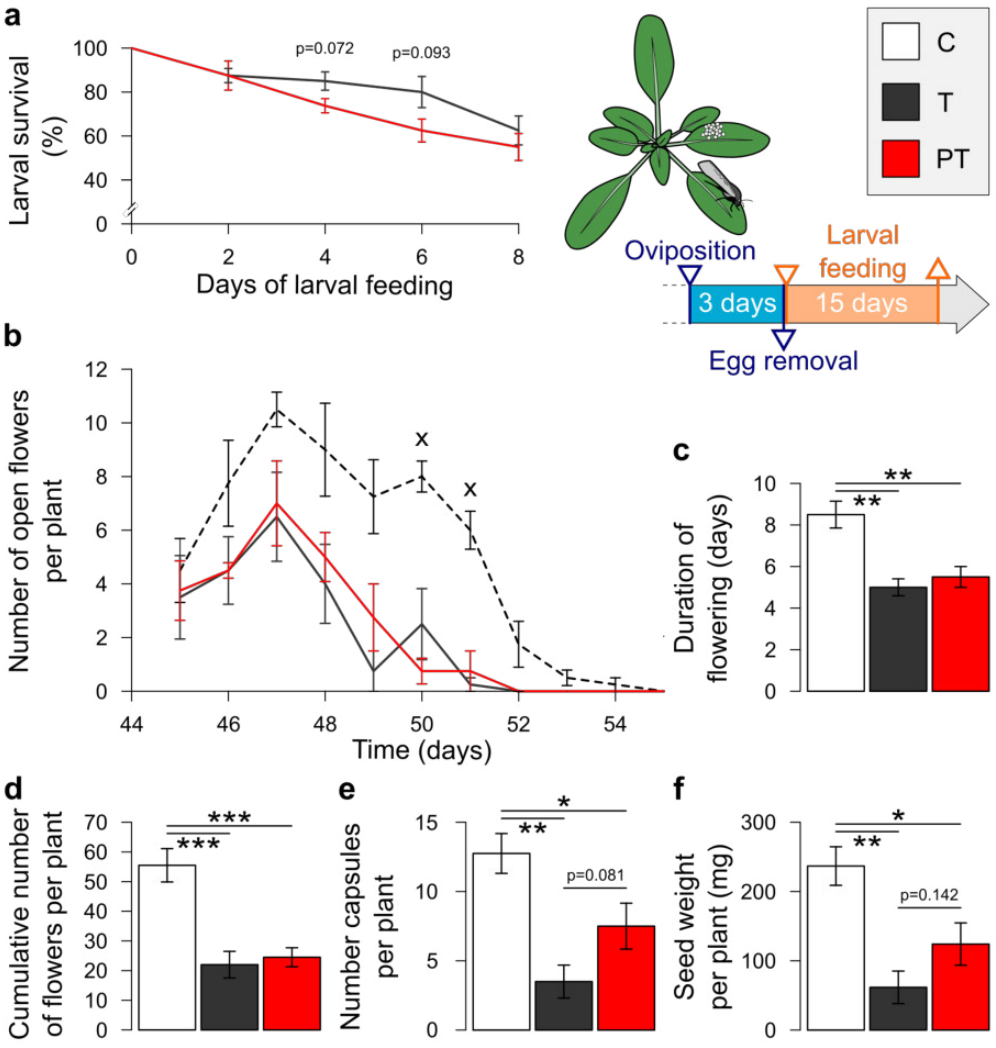

Figure 24: Fitness of *N. attenuata* is affected by *S. exigua* feeding, number of capsules and seed weight are further slightly affected by prior oviposition. *N. attenuata* plants were either kept untreated (C), exposed to larval feeding only (T) or a combination of oviposition followed by larval feeding (PT). Three days after oviposition eggs were removed, 20 neonate larvae were applied and allowed to feed from the plants for 15 days. (a) Larval survival and fitness parameter: (b) number of open flowers per day (cumulatively counted), (c) duration of flowering, (d) number of flowers (individually counted), (e) number of capsules and (f) total seed weight per plant were measured. N = 4. Line graphs and bars represent mean ± SEM. Asterisks indicate significant differences according to two sample t-test (detailed information on the statistics used see Tab. A12): */** ($p< 0.05/0.01$). (b) x indicate significant differences between plants exposed to larval feeding (T & PT) and control (C) plants according to two sample t-test ($p< 0.05$).

p=0.093; Fig. 24 a) larval survival on prior oviposited plants was by trend lower than on non-oviposited plants.

Larval feeding by *S. exigua* clearly impaired flowering. Plants exposed to larval feeding (T and PT) had a shorter duration of flowering (Fig. 24 c). Associated

with that, plants exposed to larval feeding showed a premature ending of flowering. In the beginning of flowering the number of open flowers did not differ, while at the end of the flowering phase (day 50 & 51) untreated control plants exhibit more open flowers than plants exposed to larval feeding (Fig. 24 b). Furthermore, the total number of flowers (cumulatively counted) was lower when plants were exposed to larval feeding (Fig. 24 d). However, flowering was only affected by larval feeding and not affected by oviposition before larval feeding (no differences between T and PT).
Also, parameters assessing the reproductive output were clearly diminished by larval feeding. Both, the number of capsules (Fig. 24 e) and the total seed weight per plant (Fig. 24 f) revealed similar results. Plants exposed to larval feeding (T and PT) had less capsules (Fig. 24 e) and a lower total seed wight (Fig. 24 f) compared to control plants, while the reduction ranged between 70-80 %. Albeit the number of capsules between control plants and those exposed to oviposition and larval feeding differed almost significantly (Two Sample t-test, $t(6)$=2.393, p=0.054; Fig. 24 e). Moreover, oviposition priming seems to minimize the fitness loss due to larval feeding, as for these plants the reduction due to fitness ranged only between 40-50 %. Oviposition before larval feeding caused by trend a higher number of capsules (Two Sample t-test, $t(6)$=-1.960, p=0.098; Fig. 24 e) and seed weight (Two Sample t-test, $t(6)$=-1.622, p=0.156; Fig. 24 f) compared to non-oviposited plants exposed to larval feeding.

Aiming to repeat the previous experiment in a full-factorial setup, another experiment with *S. exigua* was conducted (Exp. 8). In this experiment, eggs remained on the plant for four days until the eggs were removed, 25 neonate larvae were applied and allowed to feed on the plant for 12 days. During the hole experiment fitness parameter were recorded.
In this experiment, larval performance was only slightly affected by larval feeding. Larval survival did not differ on plants with or without prior oviposition at any time point (Fig. 25 a). However, the mean larval weight per plant was by trend lower on oviposited plants after eight (Two Sample t-test, $t(30)$=1.706, p=0.098; Fig. 25 b) and twelve days (Two Sample t-test, $t(30)$=1.565, p=0.128; Fig. 25 c) of larval feeding.
Flowering was clearly affected by larval feeding. Plants exposed to larval feeding hat a lower number of flowers (Fig. 25 d) and a shorter duration of flowering (Fig. 25 e) than plants not exposed to larval feeding. In addition, plants exposed to larval feeding showed a premature ending of flowering. In the beginning of

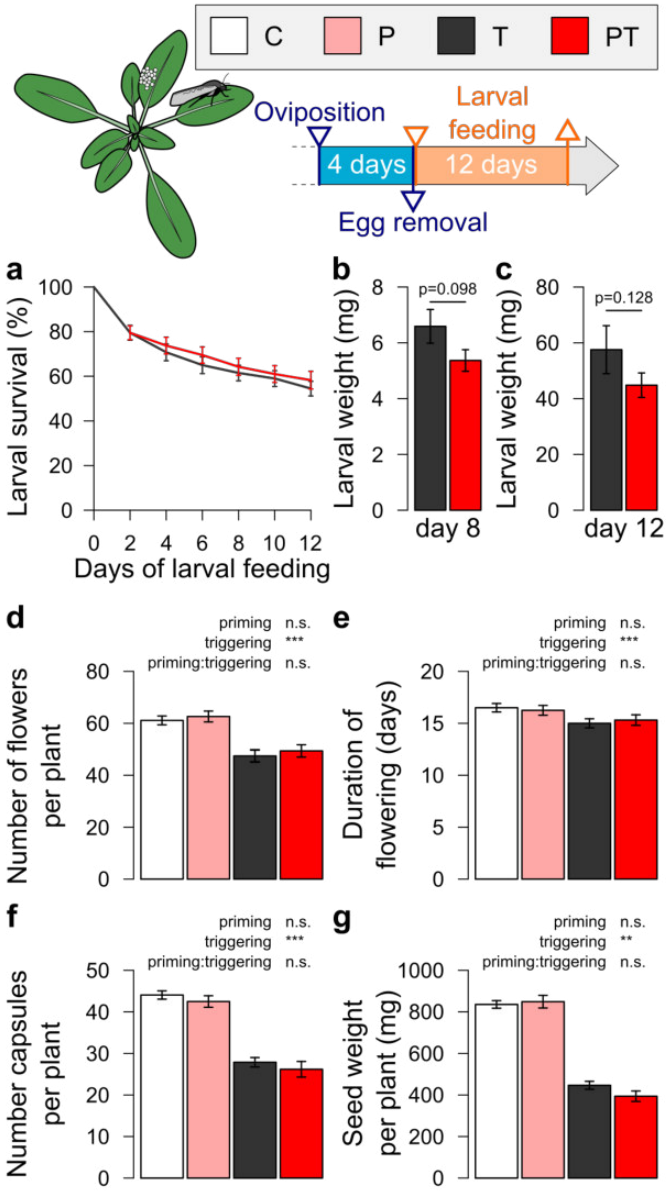

Figure 25: Growth and fitness of *N. attenuata* is affected by *S. exigua* feeding. *N. attenuata* plants were either kept untreated (C), exposed to oviposition only (P), larval feeding only (T) or a combination of both (PT). Four days after oviposition eggs were removed, 25 neonate larvae were applied and allowed to feed from the plants for 12 days. Larval performance was assessed as (a) larval survival and mean larval weight per plant after (b) eight or (c) twelve days of feeding. Plant fitness was measured as (d) number of flowers (individually counted), (e) duration of flowering, (f) number of capsules and (g) total seed weight per plant were measured. N = 16. Line graphs and bars represent mean ± SEM. Asterisks indicate significant differences according to (b & c) two sample t-test or (c – f) two-way ANOVA (detailed information on the statistics used see Tab. A13 & A14): */**/*** ($p< 0.05/0.01/0.001$).

flowering, the number of open flowers did not differ between plants with and without larval feeding (Fig. A6). However, later on (day 45 & 47 - 53) plants which were not exposed to larval feeding had more open flowers than plants exposed to larval feeding (effect sizes according to two-way ANOVA see Tab. A14). Oviposition alone or prior to larval feeding had no effect on the duration of flowering or number of flowers (Fig. 25 d & e).
As flowering, the number of capsules and the total seed weight were affected by larval feeding of *S. exigua*. Plants exposed to larval feeding had a clearly reduced the number of capsules (Fig. 25 f) and a lower total seed weight (Fig. 25 g) than plants not exposed to larval feeding. Oviposition alone or prior to larval feeding had no further effect on the number of capsules or the total seed weight.
In contrast to the missing effect of larval feeding or oviposition on growth, assessed by measuring the stalk length, in experiment seven (Fig. A5 a), growth was impaired by larval feeding of *S. exigua* in experiment eight. Towards the end of elongation stalk length was significantly reduced by larval feeding, as plants exposed to larval feeding exhibit a shorter stalk length (Fig. A6 a). Compared to the plants of the previous experiment (Exp. 7), plants of the second experiment (Exp. 8) had a faster growth. Plants of experiment eight started about five days earlier to elongate than plants of experiment seven (Fig. A6 a). Furthermore, the growth rate (cm stalk length per day), which was calculated based on growth within four days during a comparable growth phase, was higher in experiment eight than in experiment seven (Wilcoxon rank sum test, W=2, p=<0.001; Fig. A6 b).

4.2.2 Growth and fitness consequences of oviposition and larval feeding by *M. sexta*

In order to investigate the fitness consequences of oviposition and larval feeding by the tobacco specialist *M. sexta* for *N. attenuata*, another full-factorial experiment was conducted (Exp. 9). Within this experiment, plants were exposed to oviposition, eggs remained on the plant for three days and two neonate larvae were applied for nine days of larval feeding. During the hole experiment growth and fitness parameter were recorded.
Growth of *N. attenuata* plants were clearly diminished by larval feeding. At the end of elongation, plants exposed to larval feeding had a smaller stalk length than plants which were not exposed to larval feeding (upon day 41, Fig. 26 a). Oviposition alone or prior to larval feeding had no effect on stalk length growth.

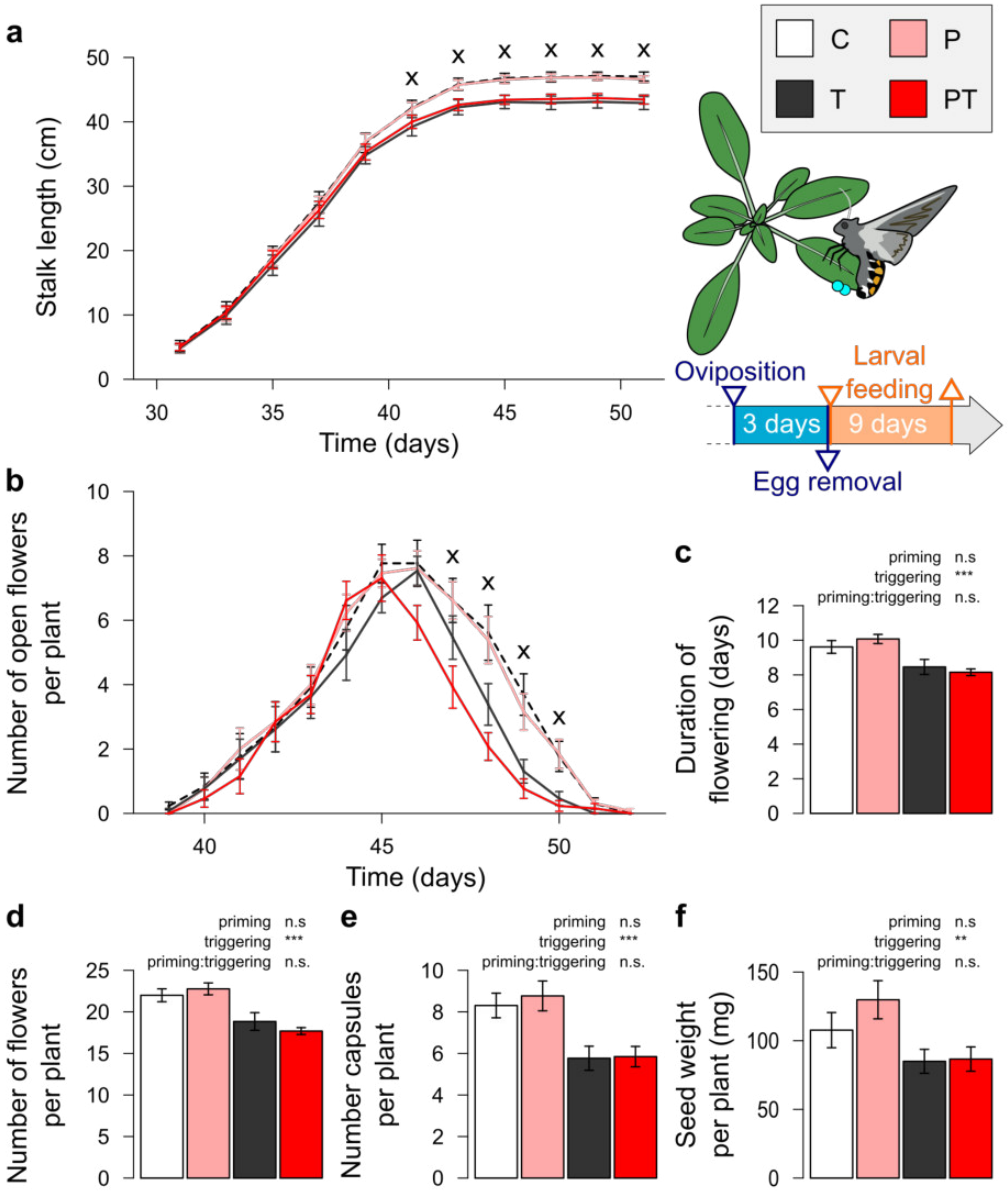

Figure 26: Growth and fitness of *N. attenuata* is affected by *M. sexta* feeding. *N. attenuata* plants were either kept untreated (C), exposed to oviposition only (P), larval feeding only (T) or a combination of both (PT). Three days after oviposition eggs were removed, two neonate larvae were applied and allowed to feed from the plants for nine days. Growth and fitness parameter assessed were (a) stalk length, (b) number of open flowers, (c) duration of flowering, (d) number of flowers (individually counted), (e) number of capsules and (f) total seed weight per plant were measured. N = 13. Line graphs and bars represent mean ± SEM. Asterisks indicate significant differences according to ANOVA (detailed information on the statistics used see Tab. A15): **/*** ($p< 0.01/0.001$). (a & b) x indicate time points with a significant ($p< 0.05$) effect of larval feeding according to two-way ANOVA.

Flowering was also clearly affected by larval feeding. Plants exposed to larval feeding (T and PT) had a shorter duration of flowering (Fig. 26 c) and a lower number of flowers (Fig. 26 d) than plants which were not exposed to larval feeding. Associated with these, plants exposed to larval feeding exhibited a premature ending of flowering, as the number of open flowers per day was lower at the end of the flowering phase, when plants were exposed to larval feeding (day 49 – 52, effect sizes according to two-way ANOVA see Tab. A15; Fig. 26 b). Oviposition alone or prior to larval feeding had no effect on the number of flowers, number of open flowers per day or the duration of flowering (Fig. 26 b - d).
Both parameters assessing the reproductive output were clearly diminished by larval feeding of *M. sexta*. Plants exposed to larval feeding had a lower number of capsules (Fig. 26 e) and a lower total seed weight (Fig. 26 f) than plants not exposed to larval feeding. However, oviposition alone or in combination with larval feeding had no effect on the reproductive output, as control plants and oviposited plants respectively plants exposed to larval feeding with and without prior oviposition had an similar number of capsules and an equal total seed weight.

4.2.3 Growth and fitness consequences of oviposition and simulated herbivory

To discriminate the effects of leaf tissue lost to the herbivores from the effects of the primed defence induction, a full-factorial experiment with was simulated herbivory instead of natural herbivory (larval feeding) as triggering stimulus was conducted (Exp. 10). Four days after oviposition, eggs were removed and simulated herbivory (repeatedly applied W+OS treatment) was applied or three subsequent days repetitively at three different leaves (three treatments per day). During the hole experiment growth and fitness parameter were recorded.
Growth, assessed by measuring the stalk length, was not affected by any treatment (Fig. A7). Triggering by simulated herbivory impaired flowering. Plants exposed to simulated herbivory had a lower number of flowers (triggering: $F(1,27)=13.329$, $p=<0.0001$, Fig. 27 a) than plants which were not exposed to simulated herbivory. The number of capsules (triggering: $F(1,27)=2.043$, $p=0.164$, Fig. 27 b) and the total seed weight (triggering: $F(1,27)=22.296$, $p=0.138$, Fig. 27 c) were only slightly lower when plants were exposed to simulated herbivory. Oviposition alone or in combination with larval feeding had no effect on the number of flowers, the number of capsules and the seed weight (Fig. 27 a - c).

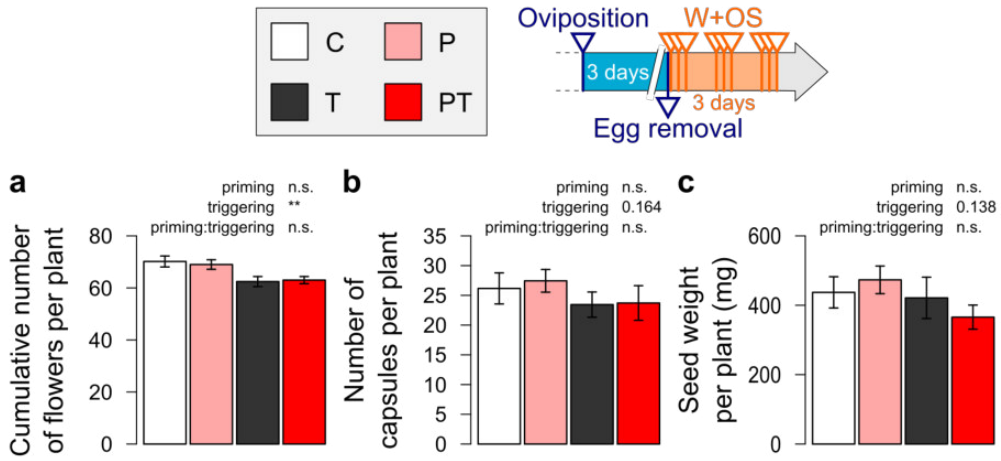

Figure 27: Flowering of *N. attenuata* is affected by simulated herbivory, while number of capsules and seed weight are not affected. *N. attenuata* plants were either kept untreated (C), exposed to *M. sexta* oviposition only (P), simulated herbivory (T) or a combination of both (PT). Four days after oviposition eggs were removed and for three subsequent days, three W+OS treatments per day (time gab three hours) were applied on the oviposited or corresponding leaf and the two next younger leaves (one leaf treated per day). Bars represent mean ± SEM of (a) number of flowers (individually counted), (b) number of capsules and (c) total seed weight per plant. N = 6 - 9. Asterisks indicate significant differences according two-way ANOVA (detailed information on the statistics used see Tab. A16): */**/*** ($p< 0.05/0.01/0.001$).

4.2.4 Effect of oviposition and larval feeding before defoliation on fitness of regrown plants

To investigate if oviposition priming affects the fitness of regrown plants after a total aboveground shoot removal by altering tolerance responses, two independent priming experiments were conducted (Exp. 11 & 12). Similar to the previous experiment with *M. sexta* (Exp. 9), plants were exposed in a full-factorial setup to oviposition by *M. sexta* followed by a phase of herbivory. Right after offset of larvae all remaining aboveground plant parts were removed and plants were allowed to regrow. Subsequently, fitness parameters of regrown plants were recorded.

As growth was differentially affected in both experiments, results were considered seperately (other parameters were affected similarly and were considered condensed). Growth before defoliation was affected by larval feeding in experiment eleven, as the stalk length of control plants was higher than of plants exposed to larval feeding at the time point of defoliation (Fig. A8 a). However, regrown plants which were exposed to oviposition and larval feeding before defoliation had a higher cumulative stalk length than control plants at all measurement time points after defoliation, while larval feeding before defoliation caused intermediate

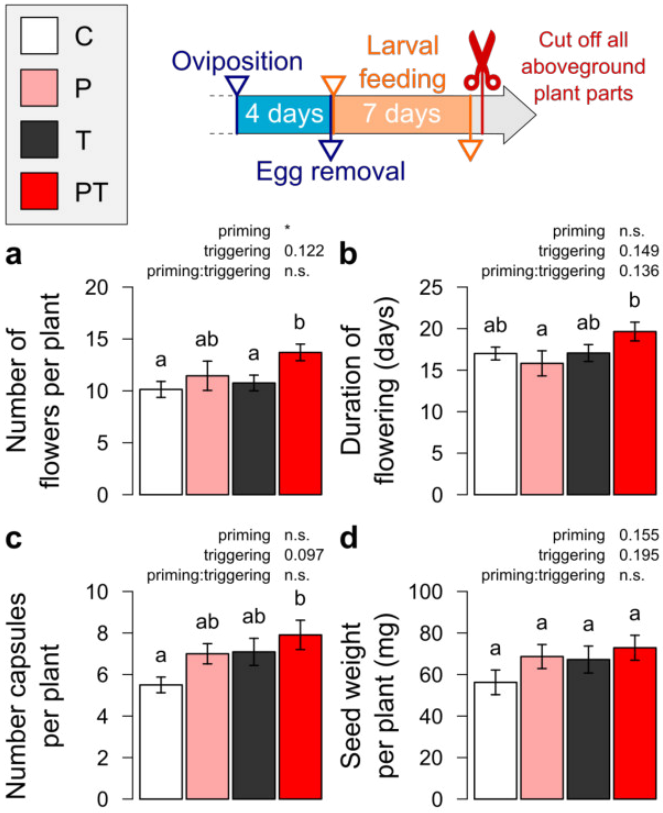

Figure 28: Regrown *N. attenuata* plants show an improved fitness when exposed to oviposition and larval feeding by *M. sexta* before defoliation. *N. attenuata* plants were either kept untreated (C), exposed to oviposition only (P), larval feeding only (T) or a combination of both (PT) before defoliation. Four days after oviposition by *M. sexta* eggs were removed, larvae were applied (first experiment three larvae of the third instar, second experiment: first two neonate larvae, after two days two additional third instar larvae) and allowed to feed from the plants for seven days. Then all aboveground plant parts were removed, and plants were allowed to regrow. Bars represent mean ± SEM of (a) number of cumulative flowers, (b) duration of flowering, (c) number of capsules and (d) total seed weight per plant. N = 11 - 17 (presented results are merged data of two independent experiments). Asterisks indicate significant differences according to (a – c) GLMM or (d) LMM (detailed information on the statistics used see Tab. A17): */**/*** ($p< 0.05/0.01/0.001$). Different letters indicate significant differences according to GLMM ($p< 0.05$).

levels (Fig. A8 c). In experiment twelve, growth was inconsistently affected by oviposition and larval feeding. In this experiment, priming affected the salk length at the first two measurement time points (diminished growth) but not at the subsequent time points before defoliation (Fig. A8 b). The regrown plants were affected by larval feeding before defoliation at the first two measurements, but not at subsequent time points (Fig. A8 d).

Flowering of the regrown plants was affected by oviposition followed by larval feeding before defoliation. The number of flowers was significantly affected

by priming and by trend by triggering (p=0.096, Fig. 28 a). In a treatment wise comparison, plants exposed to oviposition and larval feeding had a higher number of flowers than the other treatments, while the number of flowers between oviposited plants (P) and plants exposed to oviposition and larval feeding (PT) differed only by trend (p=0.0961). Also the duration of flowering was affected by triggering and by trend affected by priming (p=0.078; Fig. 28 b). In a treatment wise comparison plants exposed to oviposition and larval feeding had a longer flowering phase than plants exposed exclusively to oviposition before defoliation (p=0.038; Fig. 28 b). But also compared to control plants or those exposed to larval feeding only, plants which were exposed to oviposition and larval feeding had a by trend longer flowering phase (C-PT: p=0.09; T-PT: p=0.078; Fig. 28 b). The number of capsules (Fig. 28 c) and the total seed weight (Fig. 28 d) were not affected by priming, triggering or the interaction of both. However, in a treatment wise comparison, plants exposed to oviposition and larval feeding before defoliation had a higher number of capsules (p=0.049; Fig. 28 c) and an almost significantly higher seed wight (p=0.0586; Fig. 28 d) than control plants, while the single treatments (P and T plants) exhibit intermediate levels.

4.2.5 Fitness consequences of prior oviposition and larval feeding when defoliation occurs in young rosette or flowering stage

To further dissect a possible effect of oviposition priming on tolerance responses which affect the ability to regrow after defoliation, different developmental stages of *N. attenuata* were examined for their regrowth capacity (Exp. 13). In a full-factorial setup, plants in young rosette stage and plants in flowering stage were exposed to oviposition by *M. sexta* followed by a phase of herbivory before all remaining aboveground plant parts were removed and plants were allowed to regrow. Subsequently, fitness parameters of regrown plants were recorded.
Growth of young rosette plants at the time point of defoliation was affected by larval priming, as oviposited plants had a higher stalk length, while growth of flowering plants at these time points was not affected by any treatment (Fig. A9 a). The cumulative stalk length of regrown rosette plants were at all measurement time points affected by triggering (higher stalk length), while at the first three time points additionally priming had a significant effect (Fig. A9 b). In contrast, the cumulative stalk length of regrown flowering plants was not affected

at any time (Fig. A9 c). Overall, the cumulative stalk length of regrown young rosette plants was ranged on a higher level than the cumulative stalk length of regrown flowering plants.

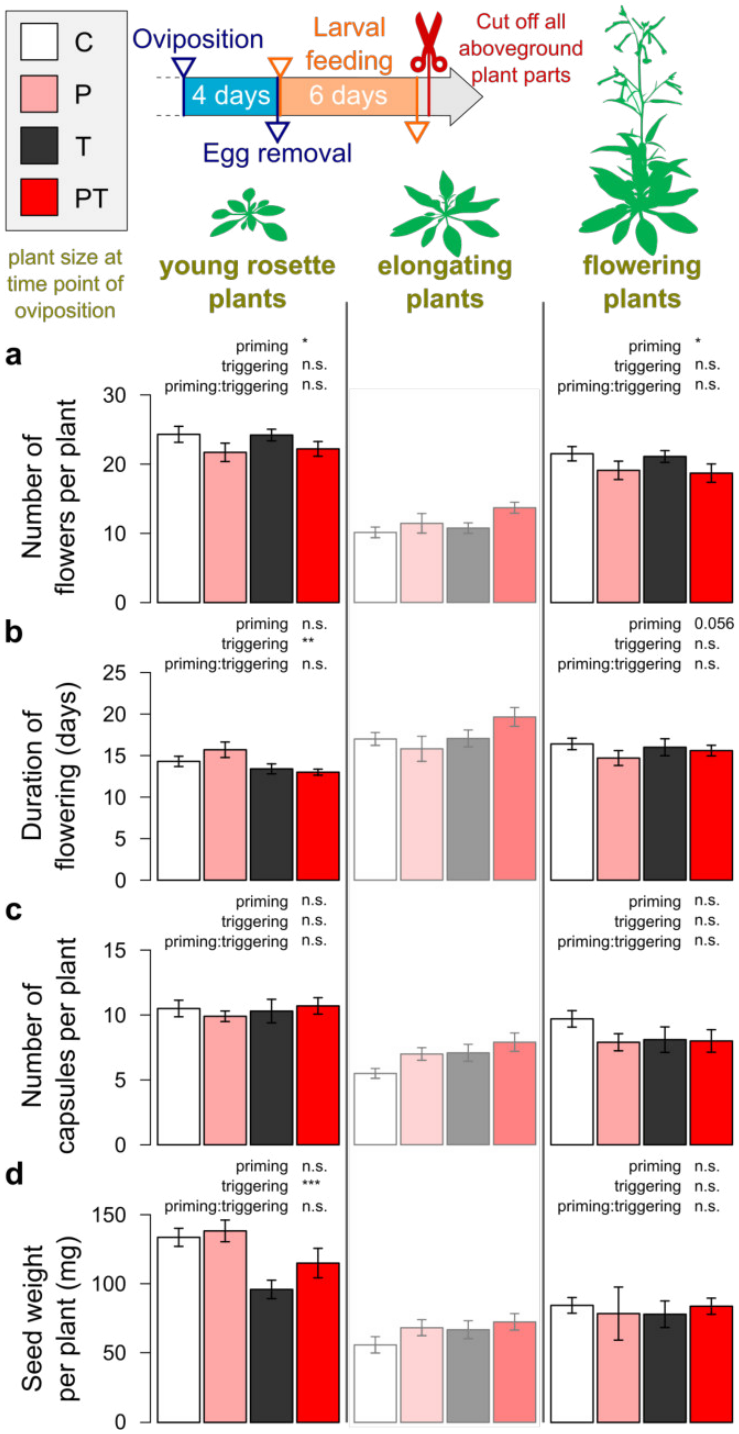

Figure 29: Fitness of regrown plants is not affected by oviposition priming if defoliation occurs in rosette or flowering stage. *N. attenuata* plants were either kept untreated (C), exposed to oviposition only (P), larval feeding only (T) or a combination of both (PT) before defoliation. At the time of oviposition plants were either four-week-old rosette plants or eight-week-old elongated flowering plants (For comparison results of elongating plants are inserted in lighter colours, see Fig. 28). Four days after oviposition by *M. sexta* eggs were removed, larvae were applied (first two neonate larvae, after two days two additional third instar larvae were applied) and allowed to feed from the plants for seven days. Then all aboveground plant parts were removed and plants were allowed to regrow. (a) Number of flowers, (b) duration of flowering, (c) number of capsules and (d) total seed weight per plant were measured. N = 10. Bars represent mean ± SEM. Asterisks indicate significant differences according to two-way ANOVA (detailed information on the statistics used see Tab. A20): */**/*** ($p<$ 0.05/0.01/0.001).

Except the fact that oviposition before defoliation caused a lower number of flowers (Fig. 29 a), regrown flowering plants were not affected in the considered parameters by any treatment (Fig. 29 b - d).

However, the fitness of plants which were in a young rosette stage before defoliation were differentially affected. The number of flowers of regrown young rosette plants was diminished by oviposition, while plants exposed to oviposition had a lower number of flowers (Fig. 29 a). However, the duration of flowering was not affected by oviposition but by larval feeding (Fig. 29 b), as plants exposed to larval feeding before defoliation had a shorter flowering phase than plants not exposed to larval feeding. The number of capsules was not affected by any treatment (Fig. 29 c). The total seed weight per plant was diminished when plants were exposed to larval feeding before defoliation (Fig. 29 d), while oviposition before larval feeding had only a slight effect (priming: $F(1,36)=2.139$, $p=0.152$, Fig. 29 d). In a treatment wise comparison, the seed weights of plants exposed exclusively to larval feeding before defoliation were lower than those of control plants and those exposed exclusively to oviposition. In contrast the seed weights of plants exposed to oviposition and larval feeding before defoliation exhibit a slightly enhanced seed weight compared to non-oviposited plants exposed to larval feeding. Opposite to plants exposed exclusively to larval feeding before defoliation, plants exposed to the combination treatment exhibit no difference to the seed weights of control plants (C-PT: $p=0.377$) or plants exposed to larval feeding only (T-PT: $p=0.358$).

5 Discussion

5.1 Dynamics of the primed state in *S. dulcamara*

5.1.1 Temporal dynamics of phytohormonal and transcriptional responses to oviposition

As several plant species in various plant-herbivore interactions (see 2.4.3, Tab. 1), *S. dulcamara* exhibits an enhanced primed defence after oviposition by *S. exigua* (Geuss et al., 2018) and *A. autumnitella* (Drok et al., unpublished) when conspecific larvae feed from the plant. To facilitate such a primed response, a signal or regulator that is capable of altering defence induction needs to be transduced upon oviposition and transfer the plant in a status, i.e. "primed state", which enable this altered defence response. However, the nature of these signals as well as their aligned induction patterns within the period of egg exposure and thereafter are still poorly understood. To address this gap of knowledge, the first aim of this thesis was to investigate temporal dynamics of the primed state that may contribute to the reinforced anti-herbivore defence in *S. dulcamara*. Therefore, the accumulation of phytohormones and associated transcripts of defence-related genes were analysed within and after the egg incubation time after oviposition by both herbivores, *S. exigua* and *A. autumnitella*. To observe temporal dynamics, responses were examined at different time points: Early time points within the egg incubation time (onset of the primed response), a time point matching the end of the egg incubation time when larvae should hatch (triggering stress should start) and time points after removing the eggs at the end of the egg incubation time (relaxation of the response).

5.1.1.1 Oviposition-induced salicylic acid signalling is limited to the period of egg exposure

In response to oviposition by *S. exigua*, SA was induced at the time points within the egg incubation time, while no induction was detectable at the time points after removing the eggs at the end of the egg incubation time (Fig. 19 a &

b). This is consistent with previous studies in the same plant-insect interaction that found a SA induction three days after oviposition (Geuss et al., 2017) and no SA induction 24 hours after egg removal (Geuss et al., 2018). However, the level of SA induction was variable as in one of the experiments SA levels only tended to be induced three days after oviposition (Fig. 19 a). But the strong upregulation of transcripts of the SA-marker gene *PR1* in these leaves indicated that SA-signalling was also activated in this case (Fig. 20 b). The SA induction after insect oviposition is also known from other plant insect interactions and variation in the induction levels are also apparent between different studies. In *A. thaliana*, SA was induced already one day after *P. brassicae* oviposition and remained constantly induced during the whole egg incubation time (Valsamakis et al., 2020). In contrast to the latter study, Bruessow et al. (2010) found in *A. thaliana* a gradual increase of total SA (including SA-glycosides) over four days in response to either *P. brassicae* oviposition or treatment with egg-extract. Thus, other factors such as the developmental stage or other environmental factors may determine the degree and dynamics of oviposition induced SA-signalling.
In addition to oviposition by the generalist *S. exigua*, also oviposition by the leaf mining specialist *A. autumnitella* induced SA accumulation (Fig. 21 a). However, this induction was relatively low as compared to the SA induction in response to *S. exigua* oviposition (Geuss et al. (2017), Fig. 19 a & b). This may be due to a different mode of oviposition as *A. autumnitella* lays single eggs whereas *S. exigua* lays egg clutches up to a few hundreds of eggs. Consequently, the effect of oviposition by *A. autumnitella* may be more diluted when SA was extracted from whole leaf extracts. This hypothesis is supported by the previous findings, that SA induction in response to *S. exigua* oviposition was highest in the leaf tissue directly under the egg clutches and was weaker in more distant, surrounding tissues of the oviposited leaf (Geuss et al., 2017). In the conducted experiment (Exp. 3), the analysed leaf tissue also included a large area of leaf tissue without direct egg-contact. Six days after oviposition by *A. autumnitella*, which is when the eggs are about to hatch, no SA induction was detectable anymore but again transcripts of the SA marker gene *PR1* were induced (Fig. 21 a & b). Thus, overall the pattern of SA induction and that of SA-regulated transcripts seem to match between oviposition by the generalist and the specialist lepidopteran herbivore, as in response to both, *S. dulcamara* increased SA in the first days after oviposition while SA levels vanished around the time point that the larvae are hatching. This induction pattern may indicate that SA signalling is either only involved in direct responses to the insects eggs and / or establishing the

primed state, but not part of the primed plant response to the feeding larvae nor is SA the signal stored to promote priming.
To sum up, the observed SA induction after oviposition by both herbivores are in line with several studies which also described that oviposition or treatment with egg-extracts result in increased levels of SA in the treated leaf (Little et al., 2007; Bruessow et al., 2010; Gouhier-Darimont et al., 2013; Hilfiker et al., 2014; Bonnet et al., 2017; Geuss et al., 2017; Lortzing et al., 2019). Furthermore, a strong conformable up-regulation of SA related gene sets was found in diverse plant species (*A. thaliana*, *U. minor*, *N. attenuata* and *S. dulcamara*) in response to oviposition by various insect species (Lortzing et al., 2020), indicating that SA related responses in reply to oviposition is quite conformable in different plant-insect interactions. SA signalling in response to eggs could on the one hand be related with defences against the eggs (Geuss et al., 2017). On the other hand, SA could be involved in signalling associated with oviposition priming (discussed later on, see 5.1.2.1).

5.1.1.2 Low-level jasmonate induction in response to oviposition

Remarkably, oviposition by *S. exigua* and *A. autumnitella* induced a low level of jasmonates. Within the egg incubation time, JA and JA-Ile levels were clearly induced one and three days after oviposition by *S. exigua* (Fig. 19 e & g). In the other experiment, they tended to be induced two days after oviposition (JA: p=0.21, JA-Ile: p=0.098) and were still significantly induced up to 6 days after the eggs were removed (6, 8 and 10 days after oviposition; Fig. 19 f & h). In response to the specialist herbivore *A. autumnitella*, only JA levels were slightly but significantly induced at the time point when the larvae are about to hatch from the eggs (Fig. 21 d), while an induction of JA-Ile was not detected (Fig. A2 a). In accordance with increased JA levels, transcript levels of JA-biosynthesis genes were upregulated after oviposition by both herbivores. The transcripts of the lipoxygenase *LOX* was induced one and three days after oviposition by *S. exigua* (Fig. 20 e) as well as three days after oviposition by *A. autumnitella*, for which also induction of another JA-biosynthesis gene *OPR3* was detected (Fig. 21 h & f). Also in experiment six (triggering stimulus is not considered at this point), JA and JA-Ile levels in the oviposited leaf were slightly affected by oviposition on a low level (JA: priming p=0.144, JA-Ile: priming p=0.135; Fig. A5 c & d). The transcriptional induction of jasmonate-related

genes (*LOX*, *OPR3*, *PI*1 and *PPO*) by oviposition (Fig. 23 f) further indicate for an induced jasmonate signalling in these leaf position.
Such a moderate jasmonate induction in response to oviposition by *S. exigua* and *A. autumnitella* was not expected, as oviposition by these lepidopteran moths do not comprise ovipositional wounding, which is most likely the cause of the JA-mediated response to oviposition by the elm leaf beetle (Altmann et al., 2018) and the common pine sawfly (Bittner et al., 2017). As increased JA and JA-Ile levels were detected many days after *S. exigua* eggs were removed (Fig. 19 f & h), also an unintended wounding during egg removal could not explain the observed activation of a low level of JA-signalling. Thus, even without any wounding, insect oviposition can induce jasmonate signalling, which is further consistent with the results of a recent comparative analysis on the transcriptional responses of several plant species to oviposition. This study showed JA-related gene sets to be conformably up-regulated after oviposition in the investigated species, while most of the plants were oviposited without tissue wounding (Lortzing et al., 2020). Also the response of *S. dulcamara* to oviposition by *S. exigua* was included in this comparative analysis, which were further reported to involve an upregulation of JA-responsive genes such as JA-biosynthesis genes like a *LOX*, repressor proteins like *JAZ*, and JA-mediated defence genes such as *PI* and *PPO* (Geuss et al., 2017). Another recently published study, examining phytohormonal responses of *A. thaliana* to oviposition by *P. brassicae* also described an induction of JA within and JA-Ile at the end of the egg incubation time (Valsamakis et al., 2020). This induction ranged similarly on a low level and was also accompanied by a relatively low transcriptional induction of JA biosynthesis and JA-related genes (Valsamakis et al., 2020).
As the jasmonate induction by oviposition in *S. dulcamara* ranges on a low level close to the detection limit and is not comparable to a jasmonate induction in response to wounding or herbivory (discussed later on, see 5.1.2.3), it is unlikely that the enhanced plant defence to feeding larvae after oviposition is solely due to an additive effect of the activation of JA-signalling caused by low quantities of jasmonates before feeding started. But this low-level induction may indicate that the plant prepares JA-mediated responses during the egg incubation time, which may lead to an earlier or faster response to the feeding larvae. Such a mechanisms has been also proposed for the increased defence response of drought stresses *S. dulcamara* plants, which showed a similar low-level induction of JA and JA-Ile (Nguyen et al., 2016). The authors suggest that this low-level induction is not sufficient to induce a full spectrum of defence related gene expression but may

contribute to interactive effects of drought and herbivory (Nguyen et al., 2016). In conclusion, the detected low level induction of JA and JA-Ile after oviposition by *S. exigua* and *A. autumnitella* may indicate for an involvement of jasmonates in the response against the eggs and/or an involvement of jasmonates in signalling associated with oviposition priming. Potential roles of jasmonates during the onset of the primed response after the larvae started to feed will be discussed later on (see 5.1.2.3).

5.1.1.3 Transcriptional response to *S. exigua* oviposition

To identify potential marker genes for the primed state, an untargeted transcriptome analysis (microarray) was performed on the leaf tissue samples of the first experiment four days after *S. exigua* oviposition. The experiment was executed in collaboration with Daniel Geuss, who further analysed the results of the microarray. These analyses are not included here but were the basis for the selection of genes which were further analysed via real-time PCR for their transcriptional accumulation in leaf tissue harvested at the other time points after oviposition. Overall, these analyses revealed that in response to oviposition by *S. exigua* a differential transcriptional induction was detectable up to ten days after oviposition respectively six days after egg removal. All of the analysed genes exhibit highest transcript accumulation four days after oviposition, which may signify that the response to oviposition peaks at the time the larvae are about to hatch from the eggs. However, probably genes with such an activation pattern were selected, as these pattern corresponds to the time point when the microarray analysis was performed.

One of the genes investigated for the transcriptional dynamics in response to oviposition was the peroxidase *PRX2*, which was induced four, six, and eight days after oviposition, but not at the time point of half of the egg incubation time, i.e. two days after oviposition (Fig. 20 a). This is consistent with previous studies investigating the transcriptional induction in *S. dulcamara* after oviposition by *S. exigua* where peroxidases were a prominent group among upregulated genes three days after oviposition (Geuss et al., 2017). In this study, peroxidase activity was suggested to contribute to H_2O_2 accumulation in leaf tissue beneath the eggs, involved in signalling or acting directly as ovicidal agent in the response of the plant to reduce egg survival (Geuss et al., 2017). Also in *A. thaliana*, oviposition of *P. brassicae* induced several peroxidase genes (Little et al., 2007). However, the transcriptional induction of *PRX2* is exceeding far beyond the time point of egg

removal (Fig. 20 a), which may indicate that is not only relevant for the plant's defence to the eggs themselves but also for the increased resistance of oviposited plants to the feeding larvae.

Another gene analysed was the polygalacturonase *PG4* (Fig. 20 c & d) exhibiting a quite similar induction pattern as *PRX2*. In both experiments, *PG4* showed no upregulation at early time points, i.e. one and two days after oviposition, but a strong upregulation when the time point of larval hatching is approaching, i.e. three and four days after oviposition. The upregulation of *PG4* is also maintained up to 4 days after egg removal. Since pectinases, which *PG4* belongs to, are involved in cell-wall structure deconstruction and modification (Gilbert, 2010; Caffall and Mohnen, 2009), the transcriptional induction could indicate for a modification of cell wall structures in response to oviposition. However, an involvement in anti-herbivore defence remains speculative, as polygalacturonase have been mainly investigated in context of fruit ripening (Gilbert, 2010; Caffall and Mohnen, 2009).

The accumulation of *CDH* transcripts displayed an induction pattern that seems limited to the time point of larval hatching. At the time points around larval hatching, i.e. three and four days after oviposition, *CDH* transcripts were induced in oviposited leaves (Fig. 20 i & j), while it only tended to be induced at the next time points before and after (day 2: p=0.069, day 6: p=0.057). Even though *CDH* transcript levels in oviposited leaves dropped to control levels, eight days after oviposition, an unexpected induction was again determined two day later. Such a transcript accumulation ten days after oviposition is difficult to explain but could eventually be associated with the also unexpected increased ABA contents at this time point (Fig. 19 d). Levels of ABA were not induced during the phase of egg exposure (Fig. 19 c & d). However, six days after oviposition (two days after egg removal) ABA contents tended to be higher in oviposited plants (p=0.11), not altered eight days after oviposition, but significantly induced ten days after oviposition. Whether these late inductions of ABA and *CDH* are actually associated with oviposition by *S. exigua* or maybe due to an interacting abiotic factor needs to be examined in further experiments. However, since the induction of *CDH* transcripts seem to be temporally focussed on the phase at the end of the egg incubation time when larvae should hatch, the induction pattern may indicate that *CDH* is involved in the response to the feeding larvae respectively part of the preparation for the upcoming herbivory. The induction of *CDH* transcripts, which encode for enzymes responsible for most cytokinin catabolism and inactivation (Schmülling et al., 2003; Cortleven et al., 2019),

could indicate for a promoted catabolism of cytokinins, associated with a reduced cytokinin signalling. In response of inducting transcription of genes involved in cytokinin degradation, the plants could reduce cytokinin-mediated functions in regulating growth and developmental processes (Werner and Schmülling, 2009; Albrecht and Argueso, 2017; Kieber and Schaller, 2018). In contrast, cytokinin signalling could also be enhanced as expression of several *CDH* genes are induced by cytokinins, providing a feedback mechanism to dampen cytokinin signalling (Kieber and Schaller, 2018). This would match with the assumed involvement of cytokinins in regulating plant defence responses against herbivores (Giron et al., 2013; Schäfer et al., 2015). Unfortunately, cytokinin levels were not measured within the conducted experiments. Nevertheless, these transcriptional results indicate for a potential involvement of cytokinins in signalling in response to oviposition.

5.1.1.4 Persistent transcriptional induction of phenylpropanoid metabolism in response to oviposition

Other than the induction patterns of *PRX2* , *PG4* or *CDH*, transcripts of *HCT*, encoding for an enzyme involved at numerous steps of the defence-related phenylpropanoid pathway (see 2.3.4, Fig. 6) was constantly induced in plants oviposited by both herbivore species at all investigated time points (Fig. 20 f & g, 21 g; one day after *S. exigua* oviposition transcript level were just by trend higher: p=0.076, else significant). This constant transcriptional induction of *HCT* after oviposition suggests an involvement of the phenylpropanoid metabolism in the enhanced defence response of oviposited plants to the feeding larvae.
This is in agreement with previous studies that suggested the importance of phenylpropanoids for oviposition-mediated priming. In *S. dulcamara*, Geuss et al. (2018) found that numerous genes involved in phenylpropanoid metabolism were more strongly expressed in previously oviposited plants exposed to larval feeding compared to plants exposed to larval feeding without a previous exposure to insect eggs. Also in other plant species (*A. thaliana*, *U. minor*, *N. attenuata*, and *S. dulcamara*), a strong conformable up-regulation of gene sets related to phenylpropanoid biosynthesis was found in response to oviposition (Lortzing et al., 2020). In the same plant species this transcriptional up-regulation is accompanied by an enhanced feeding-induced accumulation of different phenylpropanoid derivatives in response to oviposition within different plant insect interactions (Bandoly

et al., 2015, 2016; Austel et al., 2016; Lortzing et al., 2019). Moreover, the oviposition-mediated increased resistance of *N. attenuata* against *S. exigua* larvae was shown to depend on the inducibility of phenylpropanoid-polyamine conjugates (Bandoly et al., 2015). As *HCT* transcripts are induced by oviposition of both herbivores in the conducted experiments, which holds in the interaction with *S. exigua* for at least ten days, this induction further underlines the suggested relevance of the phenylpropanoid pathway in context of oviposition priming.

5.1.1.5 Temporal expression patterns of responses to oviposition

To mount an efficient response against the eggs respectively the larvae hatching thereof, the temporal coordination of distinct plant responses is crucial. Results indicate that the different phytohormonal and transcriptional changes in response to oviposition by *S. exigua* and *A. autumnitella* show distinct patterns during egg incubation time (onset of the response). A recently published study examined the changes in expression of defence related genes and phytohormone levels in *A. thaliana* during the period of exposure to *P. brassicae* eggs and suggested three

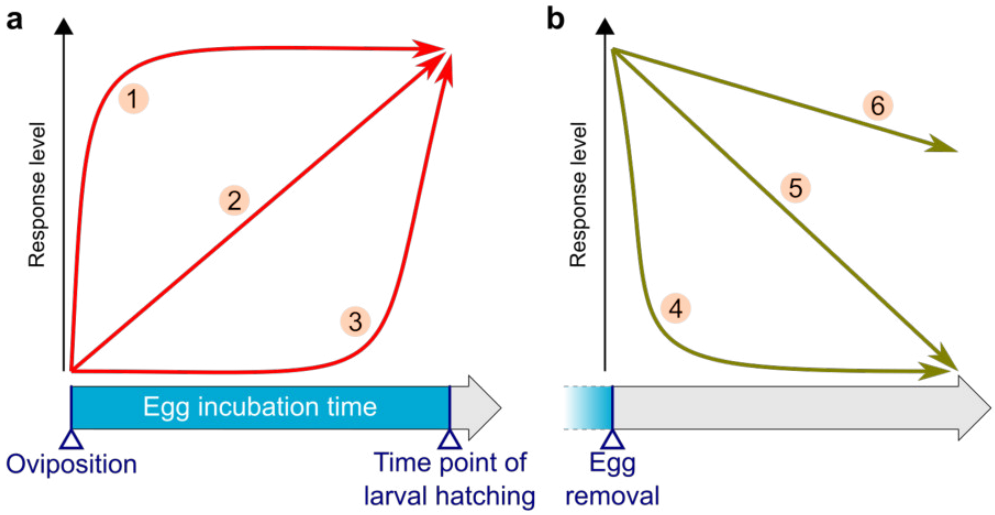

Figure 30: Schematic overview of temporal expression of responses to oviposition (a) within the egg incubation time and (b) after egg removal. (a) Within the egg incubation time (onset of the response), responses might be induced (1) early after oviposition and maintained during the egg incubation time, (2) gradually increased within the egg incubation time or (3) induced late after oviposition. (b) After egg removal (offset of the response), responses may fall back to control level (4) relatively quickly after removal of the priming stimulus or (5) decrease gradually respectively within a certain time frame. Furthermore, (6) responses could stay induced for a longer period after egg removal. (a) adapted from Valsamakis et al. (2020).

different temporal response patterns (Valsamakis et al., 2020). The first pattern consists of early induced responses, activated shortly after oviposition and maintained during egg incubation time (Fig. 30 a). Valsamakis et al. (2020) assigned the induction of SA, JA and JA-related genes to this pattern. Conformably, the measured induction of SA, JA, JA-Ile and JA-biosynthesis genes as well as the accumulation of *HCT* transcripts in oviposited *S. dulcamara* leaves (Fig. 19 & 20) would fall in this response pattern. The second response pattern comprises a gradual increase over the egg exposure time and the third pattern describes responses activated late within the egg exposure time (Fig. 30 a). Valsamakis et al. (2020) suggested that these responses are not only relevant in the response to the eggs but also in responses targeting the larvae. As the experiments here covered just two time points within the egg incubation period, it cannot be separated between these two response patterns for *S. dulcamara*'s response to *S. exigua* oviposition. However, the transcriptional induction of *PG4*, *PRX2* and *CDH* should belong to either of these two response patterns (Fig. 20).
Moreover, the first experiment includes time points exceeding the egg incubation time of *S. exigua* up to almost a week but without allowing the larvae to feed as eggs were removed shortly before larval hatching. This may allow to follow the offset of the response and / or to reveal signals associated with the primed state if it persists for a longer period. Plants can induce direct defences like for example the formation of ovicidal plant responses (Geuss et al., 2017) or indirect defences like the attraction egg parasitoids (Pashalidou et al., 2015b,c) which target the eggs and prevent larval hatching so that feeding larvae (i.e. the triggering stress) are not always following upon oviposition by an herbivorous insect (i.e. the priming stimulus). In general, if the triggering stress does not occur, at some point, plants are expected to forget the oviposition stimulus and lose the primed state, i.e. the reset back from a primed to a naïve (inexperienced) state (Bandoly et al., 2016). Paralleling to the response patterns during the period of egg exposure (Fig. 30 a), plant responses to insect oviposition exceeding this period, i.e. the time point when larvae should have hatched, could also show different patterns. Responses induced at the end of the egg incubation time could quickly fall back to control level (Fig. 30 b, response pattern 4), gradually decrease over a relatively short (Fig. 30 b, response pattern 5) or a longer period of time (Fig. 30 b, response pattern 6) after egg removal. The lack of increased SA levels in oviposited plants in the period after egg removal suggests that SA levels follow the response pattern four. A previous study showed that even 24 h after egg removal no SA induction was detected anymore in prior oviposited

S. dulcamara plants (Geuss et al., 2018). The expression of *CDH* could also indicate for a quick decrease of the induction, although the expression was still upregulated by trend two days after egg removal. This pattern of a fast response offset is might be related to responses associated with defence against the eggs themselves or to transient responses that are involved in the onset of priming but not the memory. Quickly decreasing these responses after the stimulus vanished could be cost saving. Transcripts of *PG4* and *PRX2* are still induced four days after but not six days after removing the eggs, suggesting a gradually decrease or a decreasing over a relatively short period of time after the discontinuation of the priming stimulus (Fig. 30 b, response pattern 5). Furthermore, responses induced after oviposition may be more persistent and still be induced for a longer period after egg removal (Fig. 30 b, response pattern 6). Contents of JA and JA-Ile as well as the induction of the *HCT* gene indicate that the information of oviposition is preserved for a longer period, at least up to ten days after oviposition respectively six days after egg removal. Thus, *S. dulcamara* seems to maintain some internal signals in response to oviposition beyond natural egg incubation time, however, whether these persistently induced responses after egg removal are still sufficient to induce a primed-defence response respectively facilitate a primed defence induction affecting the larvae at later time points needs to be elucidated. In some studies, changes associated with defence priming can even be transmitted from one generation to the next, providing a significant advantage to the primed offspring (Luna et al., 2012; Luna and Ton, 2012; Slaughter et al., 2012; Rasmann et al., 2012).

5.1.1.6 Response of *S. dulcamara* to *A. autumnitella* oviposition

Heretofore, phytohormonal and transcriptional responses of *S. dulcamara* to oviposition by the specialist leaf miner *A. autumnitella* have not been described. Compared to non-mining herbivores, little is known about the induction of defences against mining lepidopteran insects, as well as about plant responses against insect eggs of leaf-mining herbivore species. As discussed above responses of *S. dulcamara* to oviposition by *A. autumnitella* revealed several similarities with responses to oviposition by *S. exigua*, although partly with different intensities and dynamics. Oviposition by both herbivores caused an induction of SA, expression of the SA-marker gene *PR1*, a low-level induction of JA-biosynthesis and JA-signalling as well as transcript levels of *CDH* and *HCT*. The latter may

indicate that phenylpropanoids also play a role in the interaction with the specialist leaf-miner *A. autumnitella* (see 5.1.1.4). These common responses signify a considerable overlap in the response of *S. dulcamara* to oviposition the generalist and a monophagous specialist, which may differentiate *S. dulcamara* from other plant species that show different responses to generalist and specialist herbivores. For example, *N. attenuata* plants have been shown to differentiate between the oviposition by a specialist and a generalist lepidopteran insect, as oviposition shaped the specificity of the plant response to a later larval feeding (Drok et al., 2018). Different studies investigating the effects of oviposition on herbivore defence with more than one herbivore species described the effects as species specific, as distinct herbivore species performed differently (Bruessow et al., 2010; Pashalidou et al., 2013; Bandoly et al., 2016). However, such a species specificity was not detected in the response of *S. dulcamara* to *S. exigua* and *A. autumnitella*, which is in line with the fact that oviposition by both species diminished performance of subsequently feeding larvae (Geuss et al. (2018), Drok et al., unpublished).

To learn more about *S. dulcamara*'s response to oviposition by *A. autumnitella*, also the transcriptional induction of JA-mediated defence proteins such as *PI* and *PPO* were examined. In oviposited leaves, these transcripts were induced at both analysed time points after oviposition (Fig. 21 h & i). PIs and PPOs are general defence measures in various plant species that have been shown to confer or at least to correlate with plant resistance against different herbivore species (see 2.3.4). In accordance with that, also *S. dulcamara* has been shown to induce transcription of *PI* and *PPO* related genes and their protein activities in response to different generalist herbivores such as *S. exigua* larvae (Nguyen et al., 2016; Geuss et al., 2017; Lortzing et al., 2017) but also in response to non-arthropod herbivory by slugs (Calf et al., 2020). Such increased *PI* and *PPO* levels correlated with increased resistance and reduced egg hatching (Nguyen et al., 2016; Geuss et al., 2017). The presented transcriptional induction in response to oviposition by *A. autumnitella* indicates that PI and PPO are also involved in the interaction of *S. dulcamara* with a leaf-mining specialist herbivore. Although actual PI and PPO activities have not been determined in this experiment, it is very likely that these increased transcription rates are also conferred to protein activity as this has been shown in the interaction of *S. dulcamara* with different herbivores before (Nguyen et al., 2016; Geuss et al., 2017; Calf et al., 2020).

However, little is known about plant response to mining herbivores. The few studies on plant defences against leaf-mining herbivores also suggest an involvement

of jasmonates in defence against leaf miners. For example, the leaf miner *Folra absoluta* grew better on JA-deficient tomato plants (Campos et al., 2009). In apple (*Malus domestica*) herbivory by the leaf miner *Phyllonorycter blancardella* upregulated JA, JA-pathway and GO terms related to JA-responsive defences (Zhang et al., 2016a). Remarkably, the leaf-mining herbivore in this interaction further manipulates CK levels in the mined leaf tissue, increasing cytokinin levels locally to form a green patch that will remain green even after the leaf is dissected (Zhang et al., 2016a). In *S. dulcamara* already the eggs of *A. autumnitella* seem to cause cytokinin signalling to some extent, as *CDH* transcripts were induced three days after oviposition (Fig. 21 c). However, if these different transcriptional induction in response to oviposition also causes differences in CK-signalling during the defence against the feeding larvae remains to be studied.

5.1.2 Phytohormonal and transcriptional induction during onset of oviposition primed response to herbivory

Although, oviposition by *S. exigua* causes an impaired herbivore performance of subsequently feeding larvae (Geuss et al., 2018), the underling mechanism or mediators facilitating such an oviposition-mediated priming effect remain largely unknown. The low level jasmonate induction in response to oviposition associated with an induced transcription of JA-responsive genes (Fig. 19 e - h, 20 e - g, 21 d - g) may indicate that jasmonates are involved in this mechanism. Also previous findings suggest an involvement of jasmonates in mediating the primed defence, as for example jasmonate-mediated phenylpropanoid metabolism is assumed to play a major role in oviposition priming (Geuss et al., 2018; Lortzing et al., 2020). However, after 24 hours of *S. exigua* herbivory, phytohormonal levels, i.e. jasmonate levels, between previously oviposited and non-oviposited plants exposed to larval feeding displayed no differences (Geuss et al., 2018). As jasmonate levels change rapidly in a strong time-dependent manner within minutes after an injury (Koo and Howe, 2009; Wasternack and Hause, 2013), this calls into question whether an earlier or faster primed response could be responsible for the enhanced defence after oviposition. In tomato plants, oviposition by *Helicoverpa zea* indeed caused a higher induction of JA upon subsequent simulated herbivory immediately after triggering (Kim et al., 2012). Consequently, the next aim was to examine how the possible oviposition-mediated responses to larval feeding are mediated in the beginning of the larval attack. Therefore, phytohormonal

and transcriptional responses of ovipositioned and non-ovipositioned plants were compared after initial four hours of larval feeding (Exp. 4 & 5) or one hour after exposure to simulated herbivory (repeated W+OS treatment; Exp. 6). To separate the severe local reactions of *S. dulcamara* underneath the eggs (Geuss et al., 2017) from the responses associated with the priming of defence responses to the feeding larvae, oviposition and larval feeding were spatially separated and applied to two different but vascularly fully connected leaf positions (see 2.5.1, Fig. 9, Viswanathan and Thaler (2004)).

5.1.2.1 Salicylic acid induction during onset of oviposition-primed response to herbivory

Contents of SA were induced in plants exposed exclusively to oviposition compared to untreated control plants in all three considered leaf positions in the first full factorial experiment (Fig. 22 a, b & d). This matches with the SA induction observed within the egg incubation time (Geuss et al. (2017), Fig. 19 a & b) and further indicates a systemic induction of SA in response to oviposition. The comparatively high SA contents in leaf eight could be explained by higher basic SA levels in younger leaves, accompanied with a higher metabolite density most likely due to the higher cell density in younger leaves (Ceulemans et al., 1995). In response to larval feeding alone, SA levels were not induced in any leaf position (Fig. 22 a, b & d). Opposite to for example brassicaceous plant species (Lortzing et al., 2019; Valsamakis et al., 2020) or other solanaceous plant species as for example *N. attenuata* (Diezel et al., 2009; Drok et al., 2018), a SA induction upon feeding is not reported in *S. dulcamara* (Geuss et al., 2018). If oviposition precedes larval feeding, SA levels in all three considered leaf positions were not induced compared to control plants (Fig. 22 a, b & d). But in the triggered leaf (leaf 5) and in the systemic leaf (leaf 8) of oviposited plants, SA levels were reduced when the plants were exposed to larval feeding compared to those that were not. Maybe, the prior induced SA levels (which increased within the period of egg exposure) are rapidly dismantled during the onset of the feeding-induced response. One potential explanation could be that the feeding-induced JA and JA-Ile levels (Fig. 22 e & h) antagonise with the previously accumulated SA levels, as it has been classically assumed (see 2.3.3.3). However oviposition-induced SA levels are relatively low (e.g. in comparison to pathogen-induced SA) and when both phytohormones were transiently applied

at moderate levels JA and SA-signalling can even interact synergistically (Mur et al., 2006). In *A. thaliana*, mutants impaired in SA accumulation fail to cause a diminished herbivore performance after oviposition, indicating that SA signalling is required to establish a primed defence (Lortzing et al., 2019; Valsamakis et al., 2020). Although *S. dulcamara* did not exhibit a primed a feeding-induced SA accumulation like brassicaceous plant species (Lortzing et al., 2019; Valsamakis et al., 2020), SA signalling may also is involved to reinforce the plant defence against insect larvae by prior oviposition as it is similarly induced by oviposition. Contrary to the results of the first full-factorial experiment (Exp. 4), SA level in the repetition experiment with larval feeding were not altered by any treatment in the leaf position local to larval feeding (Fig. A4 a). Also in the experiment with simulated herbivory (Exp. 6), SA induction differed from those in the first experiment in this context. SA levels were not induced in the oviposited leaf (Fig. A5 a) which could indicate that oviposition treatment failed to evoke plant responses. However, this seems unlikely since oviposition induced a transcriptional induction (Fig. 23 f) in the same leaves. Alternatively, methodical or technical difficulties could have caused the missing detection of an SA induction in response to oviposition. Also in the triggered leaf position, the SA accumulation in experiment six exhibits a different induction pattern from the first experiment, as simulated herbivory affected by trend the accumulation of SA (p=0.080), while highest SA levels were detected in leaves exposed to oviposition and larval feeding (Fig. 23 a). Such a higher SA induction after oviposition and simulated herbivory was not detected in prior experiments. Maybe the changed triggering stimulus, i.e. simulated herbivory instead of larval feeding, affected the accumulation of SA differently than natural herbivory. For example, the transcriptional responses of *S. dulcamara* to simulated herbivory were shown to not fully imitate responses to natural herbivory, although there was no indication that the regulation of genes involved in phytohormonal signalling were differentially affected by natural and simulated herbivory (Lortzing et al., 2017). Another potential explanation could lie in the measurement time points that differed between the experiments (Exp. 4 & 5: larvae continuously but uncontrolled feed from the plant i.e. applied wounding stimuli; Exp. 6: simulated herbivory applied three times and leaf material was harvested after one hour).

In summary, SA level in the first experiment were consistent with all previous studies, which is that they are locally and systemically induced in oviposited plants, while in plants exposed to oviposition and larval feeding, they were not induced (Fig. 22 a, b & d). This could indicate for an involvement of SA in acquir-

ing a primed defence response. However, as the other experiments fail to repeat these results, probably though methodically problems, further experiments are required to determine the exact role of SA during the onset of the primed response.

5.1.2.2 Additive effect of oviposition and larval feeding on abscisic acid during the onset of oviposition primed response to herbivory

The staircase-shaped induction pattern of ABA (rising levels from C- over P- and T- to PT-plants) consistently observed after four hours of larval feeding (Fig. 22 c) or one hour after simulated herbivory treatment (Fig. 23 b) suggests an additive effect of oviposition and larval feeding in the leaf position local to triggering. After 24 hours of herbivory, ABA contents in *S. dulcamara* were only affected by larval feeding, while oviposition alone or in combination with larval feeding had no effect (Geuss et al., 2018). Thus, the effect of oviposition on ABA accumulation may be limited to the onset of the defence response. Possibly the rather weak effect of oviposition (priming) on ABA accumulation that was only indicated by trend in both conducted experiments (Exp. 4: p=0.101; Exp. 6: p=0.244), may have be more pronounced at an even earlier time point. Interestingly, during the beginning of the larval attack by *P. brassicae* in oviposited and non-oviposited *A. thaliana* plants, a similar induction pattern of ABA was found after 12 hours of larval feeding (Valsamakis et al., 2020). After three hours of larval feeding these pattern was not detectable, indicating a different temporal dynamic of ABA accumulation in *A. thaliana* as it indicates that the accumulation of ABA in this interaction takes longer (Valsamakis et al., 2020). But also in this interaction ABA contents after 24 hours of herbivory was only affected by feeding, while oviposition alone or in combination with larval feeding had no effect (Lortzing et al., 2019). Although *S. dulcamara* exhibits an earlier ABA accumulation than *A. thaliana* in the context of oviposition priming, the similar induction pattern and the similarity that this induction is restricted to the onset of larval feeding, might indicate for a common response in plants of two different plant families. Moreover, these results indicate that during the onset of the response to herbivory ABA signalling could be involved in facilitating a primed defence induction, while later on, ABA level of the oviposited and non-oviposited plants seem to converge and reach similar levels.

Heretofore the role or contribution of ABA in facilitating an enhanced defence responses in context of oviposition priming received little attention, although ABA

is suggested to interact for example with JA by modulating JA-mediated defence responses (see 2.3.3.2). Both, results of Valsamakis et al. (2020) and the conducted experiments, indicate for an involvement of ABA in oviposition-mediated priming of anti-herbivore defence. ABA is assumed to contribute plant defence against chewing herbivores, highlighted for example by the observation that plants deficient in ABA accumulation or signalling fail to induce a full anti-herbivore defence and are more susceptible to herbivores (Bodenhausen and Reymond, 2007; Dinh et al., 2013; Vos et al., 2013b; Thaler and Bostock, 2004). Furthermore, it is assumed that an interplay of several phytohormones, among them also ABA, is required for an improved defence against herbivore larvae after oviposition (Lortzing et al., 2020). ABA is tightly interconnected with JA and it is assumed that ABA can modulate JA-mediated defence responses (Chen and Yu, 2014). For example, in context of larval feeding under drought stress, JA and ABA signalling interact synergistically to enhance resistance to feeding insects (Nguyen et al., 2016). But also in absence of drought, ABA signalling seem to influence JA-mediated responses. The finding that the expression of chloroplast-localized glycerolipid A1 lipases *PLIP2* and *PLIP3* are induced by ABA and lead to JA accumulation provides an attractive mechanistic link between ABA accumulation and downstream JA-defence responses (Wang et al., 2018). Interestingly, the onset of herbivore-induced jasmonate-dependent defences in systemic leaves of primed (i.e. systemically pre-induced by herbivory) *Arabidopsis* plants is activated by ABA, as enhancement of ABA levels in these systemic leaves facilitates a potentiated expression of MYC-mediated JA responses (Vos et al., 2013b). The fact that contents of ABA were not altered in the oviposited (Fig. A3 a) or systemic leaf (leaf 8; Fig. A3 d), but only in the leaf exposed to larval feeding and further that in this leaf oviposition on the vascularly fully connected leaf below affected ABA accumulation (Fig. 22 c), also indicate for a systemic aspect of ABA accumulation in *S. dulcamara*. ABA could serve as activator in this context facilitating an enhanced signalling or enforce other aspects of the primed defence induction in response to oviposition. Moreover, the interaction effect of ABA and JA signalling could be required to facilitate a primed defence induction. However, if this systemic induction of ABA, accompanied with the local induction in response to larval feeding, is involved in signalling in context of oviposition priming needs to be further investigated.

5.1.2.3 Primed jasmonate induction during onset of herbivory

The accumulation of jasmonates on a low-level with an associated transcriptional induction within the period of egg exposure, indicated for a potential preparation of the jasmonate-mediated anti-herbivore defence response, as discussed above (see 5.1.1.2). In experiment six, the expression of jasmonate-related genes (*LOX*, *OPR3* and *PPO*) in the oviposited leaf were only significantly induced compared to the control when plants were exposed to oviposition and simulated herbivory (Fig. 23 f), indicating for an altered signalling in these plants although the triggering was applied to the leaf five positions above. In the local leaf exposed to natural or simulated herbivory, JA and JA-Ile levels were clearly induced (Fig. 22 e & h, A4 c & d, 23 c & d), similar to the results after 24h of larval feeding (Geuss et al., 2018). However, in contrast to the results of Geuss et al. (2018), oviposition further enhanced the induction of JA and JA-Ile during the onset of the induced response, i.e. after four hours of larval feeding, in the first full-factorial experiment (Fig. 22 e & h). This pattern was repeated in the experiment with simulated herbivory (Exp. 6), since contents of JA were almost significantly higher (p=0.054) in oviposited compared to non-oviposited plants exposed to simulated herbivory (Fig. 23 c). However, in this experiment oviposition had no further effect on JA-Ile induction and the accumulation of JA and JA-Ile were approximately three-times higher induced (Fig. 23 c & d), probably caused by the altered triggering stimulus and harvesting time point. Although this not fully resembles the primed jasmonate induction of the first full-factorial priming experiment, these slightly higher JA induction could again indicate for a primed jasmonate induction.

A similar induction pattern was also described in tomato, where the induction of JA upon simulated herbivory was enhanced during the onset of the response when oviposition by *H. zea* preceded triggering (Kim et al., 2012). Also in this interaction, the enhanced induction was only detectable 30 and 60 minutes after applying the triggering stimulus, while it was no longer measurable after three hours. But opposite to the latter study, which further found a stronger wounding-induced transcriptional accumulation of a gene encoding protease inhibitor in oviposited plants at later time points (Kim et al., 2012), the transcriptional induction of JA-related genes (*LOX*, *OPR3*, *PI*1 and *PPO*) in the leaf position local to triggering were not further altered by prior oviposition (Fig. 22 g, 23 e). The transcriptional induction consequently not indicate for an enhanced jasmonate

signalling on the transcriptional level at these time points. However, in tomato the altered transcriptional induction due to prior oviposition occurred at later time points than the altered induction of JA (Kim et al., 2012). Probably, an altered transcriptional induction in *S. dulcamara* caused by prior oviposition occurs with a different temporal dynamic than effects on the phytohormonal induction. Also in *A. thaliana* plants a primed jasmonate induction during the beginning of the larval attack by *P. brassicae* was recently described (Valsamakis et al., 2020). After three hours of herbivory, feeding-induced contents of JA-Ile in oviposited plants were higher than in non-oviposited plants exposed to larval feeding only (Valsamakis et al., 2020). However, similar to the results attained here with *S. dulcamara* also in this interaction the primed induction is lost later on as after 12 and 24 hours of herbivory jasmonates were only feeding induced while oviposition had no further effect (Valsamakis et al., 2020; Lortzing et al., 2019).

Contrary to the primed jasmonate induction detected in the other experiments, JA and JA-Ile contents in experiment five were merely feeding-induced while prior oviposition had no further effect (Fig. A4 c & d). Interestingly, in this experiment, in which oviposition failed to induce SA, also ABA accumulation was only feeding-induced and not further elevated by prior oviposition as in the other experiments (Fig. A4 b). This might indicate for a connection between oviposition induced SA induction, ABA levels increased additively by oviposition and larval feeding and the primed induction of JA / JA-Ile. Although the exact interactions are unknown, it is assumed that an interplay of several phytohormones mediate the improved anti-herbivore defence of oviposited and feeding-exposed plants (Lortzing et al., 2020). However, the effect of an interaction can enormously depends on the quantities and ratios of the interacting phytohormones. For example, in *A. thaliana* a synergistic enhancement in the expression of genes involved in JA and SA-mediated defence was observed when both phytohormones were transiently applied at moderate levels, whereas the phytohormones antagonize each other when applied for a longer time or at high concentrations (Mur et al., 2006). Consequently, the missing reproducibility could be based on a mismatched interplay of different phytohormones which facilitate a primed defence induction. For example, in the experiment (Exp. 5) with a divergent phytohormonal induction pattern, JA and JA-Ile induction exceeded those in the previous experiment about three-fold, which might be due to a higher feeding damage (due to shortage in young larvae, slightly bigger larvae were used in this experiment). Yet under natural conditions, hatching neonate larvae only cause minor damage

in the beginning. But, also in the experiment with simulated herbivory as triggering stimulus, JA and JA-Ile contents were approximately three-times higher induced (Fig. 23 c & d) than in first full-factorial experiment (Exp. 4, Fig. 22 e & h), although in this experiment the feeding-induced accumulation of JA exhibit a primed induction (Fig. 23 c). However, a more gradually induction of jasmonates, i.e. a slight increase upon onset of larval feeding, probably reflects the natural conditions more accurately and could support a primed induction upon oviposition. However, it seems more realistic that the lacking impact of oviposition on ABA and JA induction is rather related to the lack of the typical SA-induction upon oviposition, but further studies are required to investigate the complex interplay of phytohormones in this context. In conclusion these results indicate that the accumulation of JA and JA-Ile in response to larval feeding is subjected to an elevation due to prior oviposition that is restricted to the onset of herbivory and likely depends on the interplay with other hormones like SA and ABA which are also induced by oviposition. The higher feeding-induced jasmonate level in oviposited plants (Fig. 22 e & h, Fig. 23 c) may indicate for an earlier or faster jasmonate induction of primed plants, while the difference between oviposited and non-oviposited plants is lost later on. Consequently, the induction or the achievement of an effective level of JA-related defence responses, e.g. the induction of phenylpropanoids or PIs (see 2.3.4), could occur earlier or faster.

5.1.2.4 Primed induction of marker genes for phytohormonal and phenylpropanoid pathways during the onset of herbivory

Also some of the genes considered at different time points after oviposition (see 5.1.1.3), were investigated for their transcriptional induction during the onset of the response to herbivory. Opposite to the induction within the first experiment at the time point matching with the time of larval hatching (Fig. 20), transcripts of *PG4*, *PR1*, *HCT*and *PRX2* were not altered in the oviposited leaf or the leaf exposed to larval feeding in the first full-factorial experiment (Fig. 22 f). Consequently, these genes are rather unsuitable as markers for the primed state. However, the expression of *HCT* in the experiment with simulated herbivory was affected by oviposition (Fig. 23 f), which again match with the induction in the first experiments (Fig. 20 f & g). In the leaf position local to triggering, expression of *HCT* was clearly induced by larval feeding (Fig. 22 g), while

simulated herbivory did not cause an induction (Fig. 23 e). This might indicate that simulated herbivory is not capable of inducing *HCT* expression. Potentially *HCT* transcripts, associated with phenylpropanoid metabolism, further follow other temporal dynamics than for example jasmonates, maybe a primed induction of these transcripts occurs at a different time point than after four hours of larval feeding or one hour after simulated herbivory. In contrast, transcript level of *CDH*, which encodes for enzymes responsible for most cytokinin catabolism and inactivation, revealed a primed induction in experiment four. In the leaf position local to oviposition (leaf 0), *CDH* expression in both experiments were affected by oviposition (Fig. 22 f, Fig. 23 f), matching with results of the first experiments (Fig. 20 i & j). In the leaf position exposed to larval feeding (leaf 5) transcript level were feeding-induced and the induction was further increased when oviposition precedes larval feeding (Fig. 22 g). This higher expression of *CDH* further indicates that CK signalling might be involved in mediating or facilitating a primed defence response. However, expression of *CDH* in the leaf position local to simulated herbivory remained largely unaffected related to the control (Fig. 23 f). Maybe simulated herbivory is not able to induce *CDH* expression in the same was as natural herbivory, as transcriptional responses of *S. dulcamara* to simulated herbivory not fully imitate responses to natural herbivory (Lortzing et al., 2017). However, the higher induction of *CDH* in response to larval feeding and oviposition, may indicate for a pronounced CK signalling (see 5.1.1.3) for the discussion the potential role of CK-signalling in context of oviposition priming). Whether CK signalling is also required for oviposition-mediated enhancement of anti-herbivore defence needs to be further examined. Together with the results of the first experiments, the induction upon natural herbivory indicates *CDH* as an auspicious marker gene for oviposition priming in *S. dulcamara*. Transcriptional levels seem to be induced in a temporally limited window around the time of larval hatching (Fig. 23 i & j) and during the onset of larval feeding transcripts of *CDH* showed a similar primed induction parallel to the induction of jasmonates (Fig. 22 g).

5.1.2.5 Earlier or faster feeding-induced defence

Differences between feeding-induced plants with and without prior oviposition on the phytohormonal and transcriptional level (Fig. 22, 23) during the beginning of the defence induction, combined with the absence of these differences after 24 hours of feeding (Geuss et al., 2018), suggest an earlier or faster feeding-induced defence

response upon oviposition. Similar results in the interaction of *A. thaliana* and *P. brassicae* (Lortzing et al., 2019; Valsamakis et al., 2020) further support this suggestion as well as the enhanced JA accumulation at early time points upon simulated herbivory of prior oviposited tomato (Kim et al., 2012).
Such an earlier or faster feeding-induced defence initiation if oviposition precedes larval feeding would probably also cause an earlier achievement of an effective defence against the herbivores. In doing so, oviposition priming would minimize the window of vulnerability, i.e. the time without effective defence immanent for induced defences, a major drawback of induced defence (Cipollini et al., 2003; Karban, 2011). In addition, an earlier defence against the feeding larvae could be beneficial for the plant as neonates are more vulnerable and easier to affect with relatively low amounts of secondary metabolites than larvae of later instars. An earlier or faster defence response upon herbivory would further be in accordance with the observation that the effect of prior oviposition on larval performance of *S. exigua* feeding from oviposited and non-oviposited *N. attenuata* (Bandoly et al., 2015) and hole *S. dulcamara* plants (Geuss et al., 2018) was detectable within the first days of feeding and persisted constantly diminished throughout development until pupation. A similar pattern was observed in interaction of *S. dulcamara* with the leaf miner *A. autumnitella* (Drok et al., unpublished). Approving to this hypothesis, the negative effect of prior oviposition by *P. brassicae* on the subsequently feeding larvae on *A. thaliana* was also suggested to result from a developmental retardation that the larvae experienced as neonates, that they can hardly overcome as elder larvae (Oberländer et al., 2019). Consequently, the beneficial effect of oviposition priming for the plant is probably among other factors based on an early negative impact on the larvae, an earlier or faster onset of the defence response could contribute to this effect. Often it is assumed that slower growing animals are at increased risk of predation and parasitization, as accompanied with a longer developmental time the window of vulnerably to natural enemies in higher trophic levels is extended (Loader and Damman, 1991; Benrey and Denno, 1997; Bukovinszky et al., 2009; Harvey and Gols, 2011). Maybe connected with an earlier or faster defence in response to oviposition priming and an associated early retarded development, oviposition priming renders larvae more susceptibility to higher trophic levels. Under natural conditions, i.e. in presence of native predators, the plant-mediated effects of *B. nigra* induced by oviposition and larval feeding of *P. brassicae* cascaded up to parasitoids of the herbivore at the third and fourth trophic level by affecting parasitation rate and parasitoid performance (Pashalidou et al., 2015b).

5.2 Fitness consequences of oviposition priming for *N. attenuata*

5.2.1 Larval feeding reduces fitness of *N. attenuata*

The aim in the second part of this thesis is to examine the growth and fitness consequences of oviposition priming for the annual plant *N. attenuata* in interaction with the generalist herbivore *S. exigua* and the tobacco specialist *M. sexta*. However, to investigate effects of oviposition priming on growth or fitness in relation to the consequences associated with larval feeding, one first needs to consider the growth and fitness consequences of the defence induced by herbivory alone. In all three experiments, larval feeding by either of the two herbivores diminished growth, which is displayed by a reduced stalk length of plants exposed to larval feeding compared to plants not exposed to larval feeding (Fig. A6 a, 26 a). Furthermore, plants exposed to larval feeding were impaired in flowering and reproduction. These plants had a shorter flowering phase (Fig. 24 c, 25 e, 26 c), a lower number of flowers (Fig. 24 d, 25 d, 26 d) and a premature termination of the flowering phase (Fig. 24 b, A7, 26 b) compared to untreated control plants. As a consequence, these fed plants exhibited a lower number of capsules (Fig. 24 e, 25 f, 26 e) and a lower seed weight (Fig. 24 f, 25 g, 26 f) than plants not exposed to larval feeding. Such a diminished growth, flowering, and reproduction of plants exposed to larval feeding, i.e. fitness costs of larval feeding, was reported before in several studies examining different plant insect interactions (Kessler and Baldwin, 2004; Strauss et al., 2002; Züst et al., 2015; Bustos-Segura et al., 2020).

But which factors entail such a reduced fitness? Focusing on the plant physiology (excluding ecological or evolutionary costs at this point), the impact of larval feeding on plant fitness probably mainly results from the leaf tissue loss suffered due to herbivory, which reduces the photosynthetic active leaf area and lowers the level of assimilates produced by the plant (Agrawal et al., 1999; Agrawal, 2005). The amount of leaf tissue loss due to hornworm herbivory is negatively correlated with lifetime seed capsule production, as was shown under field conditions in different *N. attenuata* plant populations (Kessler and Baldwin, 2004). In addition to the direct loss of photosynthetic active leaf area just by simply loosing canopy area, the photosynthetic capacity of plants can be further reduced due to a physiological down-regulation of the photosynthetic machinery in response to larval feeding, which has been frequently reported (Zangerl et al., 2002; Nabity

et al., 2009; Halitschke et al., 2011). In addition, fitness costs that may result from allocation of fitness-limiting resources to defence responses, such resource-based trade-offs between growth and defence have long been discussed (van der Meijden et al., 1988; Herms and Mattson, 1992; Steppuhn and Baldwin, 2008; Schwachtje and Baldwin, 2008).

5.2.2 Simulated herbivory affects number of flowers but has no effect on fitness

In addition to experiments with natural herbivory, experiments with simulated herbivory were conducted, in order to induce the defences of *N. attenuata* in a standardized manner while minimizing the effects of tissue loss.
Opposite to natural herbivory, simulated herbivory had no effect plant growth (Fig. A8) but reduced flower number (Fig. 27 a) compared to plants not exposed to simulated herbivory. However, reproduction parameters were only sligthly reduced, as the number of capsules (p=0.164, Fig. 27 b) or the seed weight (p=0.138, Fig. 27 c) only tended to be affected by simulated herbivory, not comparable to the fitness loss caused by natural herbivory (Fig. 24 - 26). This indicates that simulated herbivory, which induces defence induction but causes only a minor leaf tissue loss compared to natural herbivory, had only a small effect on the fitness of *N. attenuata* under these experimental conditions. A possible explanation for this missing impact of simulated herbivory on plant fitness could be that the applied amount of triggering stress (i.e. quantity of damage) was insufficient to cause fitness consequences. However, the treatment applied in a similar experimental setup was shown to be able to induce a defence induction (Bandoly et al., 2016). As described above, a major difference between natural and simulated herbivory is the amount of leaf damage the plants experience. The damaged area of leaves to which puncture wounds were applied (pattern wheel wounding) is neglectable compared to the extensive leaf tissue lost to the feeding larvae, which can comprise the loss of whole leaves. In association with the clearly detectable negative impact of larval feeding by both insect species on plant fitness of *N. attenuata* (Fig. 24 - 26), the minor effect of simulated herbivory on *N. attenuata* (Fig. 27) indicates that the impact on plant fitness associated with induced defence is way smaller than the impact associated with leaf tissue loss due to larval feeding.

5.2.3 Oviposition without larval feeding has no effect on plant fitness

To investigate fitness consequences associated with oviposition priming, also the fitness consequences of responses to oviposition *per se*, i.e. experiencing oviposition (priming stimulus) without subsequent larval feeding (triggering stimulus), must be taken into account. Onset and maintaining the primed state in response to oviposition is expected to be associated with some costs. However, oviposition by neither of the two investigated herbivore species on *N. attenuata* is associated with any tissue loss and was not found to induce any defence response (Bandoly et al., 2015, 2016). Therefore, it can be expected, that the impact of oviposition on plant fitness should be relatively small. This assumption was confirmed, as in interaction with both herbivores, oviposition had no effect on growth or plant fitness of *N. attenuata*. Unlike plants with feeding larvae, plants exposed exclusively to oviposition by *S. exigua* or *M. sexta* displayed a similar fitness as untreated control plants (Fig. 25, 26, 27). Consequently, under these experimental conditions onset and maintenance of the primed state after oviposition seem to be associated with no or very low costs (e.g. due to changes in the regulatory network), which were not detectable at the level of plant fitness. Priming of anti-herbivore defence is assumed to be an advantageous mechanism for the plant by which costs of defence induction are avoided on all occasions the triggering stress does not occur. Such a mechanism can only work if the costs associated with the setup and maintenance of the primed state are clearly below that of defence induction. Yet, studies investigating fitness consequences of defence priming often methodically fail to examine responses to the priming stimulus without the following triggering stimulus, for example due to the fact that they conduced field studies were herbivores cannot be excluded (Karban and Maron, 2002; Karban et al., 2012; Kost and Heil, 2006). Therefore, knowledge on the fitness consequences of defence priming in occasions were the actual triggering stress (i.e. costs of priming) does not occur is limited. However, in nature it can actually happen that no larval feeding occurs after oviposition, for example when eggs are killed through plant defences against the eggs or through predators respectively parasitoids (see 2.4.2). Two of the few studies examining fitness consequences of defence priming, found that priming of pathogen defences had no negative impact on the plant fitness of *A. thaliana* or barley (*Hordeum vulgare*) when no attack occurred (van Hulten et al., 2006; Walters et al., 2008). Opposite to the fitness consequences for *N. attenuata* of the response to oviposition, *B.*

nigra plants exposed to eggs by *P. brassicae* were found to have a higher number of flowers and seeds but less leaves than non-exposed plants, although larval feeding did not cause effects on these parameters (Pashalidou et al., 2020). In this scenario, it is difficult to explain why plants exhibit a lower fitness when not oviposited by insects, however, these results are also in line with the assumption that plants exposed to oviposition suffer no fitness incurs compared to non-oviposited plants. Consequently, the adaptive value of oviposition priming over directly inducing anti-herbivore defence in response to oviposition may lie in the avoidance of fitness costs in all occasions in which the herbivores do not hatch (due to direct or indirect defences against the eggs) and no triggering stress occurs.

5.2.4 Oviposition priming can be beneficial if the defence priming is effective against the herbivore

But how can prior oviposition affect the fitness of attacked plants? Due to an enhanced defence in response to the triggering stress, primed plants are expected to perform better and have an improved plant fitness compared to non-primed plants (Hilker et al. (2016); Martinez-Medina et al. (2016), see 2.2.4). However, adequate proof for such an improved plant fitness of oviposition primed plants is missing. It is assumed that oviposition priming is an adaptive strategy by which the plant optimizes fitness trade-offs of induced defence. In the course of that, oviposition priming is expected to increase the beneficial effects of induced defence more than the costs associated with an improved defence response. On the one hand, the leaf tissue loss caused by the herbivore is potentially minimized if larval performance is impaired by priming. *S. exigua* larvae on prior oviposited *N. attenuata* plants, which suffer a higher mortality, less weight gain and a retargeted development, also cause a lower feeding damage and leaf tissue loss compared to larvae on non-oviposited plants (Bandoly et al., 2015). On the other hand, potential costs associated with defence induction are presumably higher, as the primed defence induction causes higher amounts of secondary metabolites or defensive proteins in prior oviposited plants than in non-oviposited plants (Bandoly et al., 2015, 2016). If priming is an adaptive strategy, the benefits due to a lower leaf tissue loss should exceed any higher fitness costs that might be associated with the primed increase in defence induction resulting in a net-benefit for oviposition-primed plants.

To assess these assumptions, the fitness of *N. attenuata* plants exposed to larval feeding by either of the two herbivores was considered in comparison to the fitness

of oviposited plants exposed to larval feeding. As only one of the two herbivores, *S. exigua*, is affected by the primed defence response (Bandoly et al., 2016), it would be expected that plant fitness in this interaction can reveal net-benefits of oviposition priming, while the interaction with the specialist, *M. sexta*, that is not affected, could reveal fitness costs of defence priming.

In interaction with *M. sexta*, no differences between of oviposited and non-oviposited plants exposed to larval feeding were detectable in any of the considered fitness parameters (Fig. 26). Similarly, plants exposed to oviposition by *M. sexta* and simulated herbivory exhibited no altered fitness compared to non-oviposited plants exposed to simulated herbivory (Fig. 27). Consequently, oviposition priming in interaction with *M. sexta* did not indicate effects on the fitness of *N. attenuata*, although oviposited plants were shown to induce higher amounts of PPCs in response to larval feeding than non-oviposited plants exposed to larval feeding (Bandoly et al., 2016). Thus, any fitness costs associated with this primed increase in defence induction, falls far below the fitness costs of *M. sexta* herbivory and also below costs of the triggered defence induction, which was detectable in form of a significantly reduced flower number of plants induced by simulated herbivory. Unfortunately, it was not possible to validate the successful onset of the primed state (e.g. through physiological measurements of plant responses to oviposition) as tissue removal would have triggered the plants and therefore it cannot be excluded that oviposition were not able to induce the response leading to a primed state.

Opposite to *M. sexta*, larvae of the generalist herbivore *S. exigua* are strongly affected in their performance by oviposition priming (Bandoly et al., 2015, 2016). Therefore, in this interaction oviposition priming could have a fitness benefit for *N. attenuata*. However, results of the experiments with *S. exigua* investigating the fitness consequences of oviposition priming gave two different outcomes. In the first experiment with *S. exigua* examining the fitness of *N. attenuata* (Exp. 7), oviposited and feeding-induced plants tended to produce more capsules (p=0.097) and seed weight (p=0.142) compared to non-oviposited plants exposed to larval feeding (Fig. 24 e & f). All plants exposed to the more than two weeks of herbivory suffered high fitness losses obvious from all assessed fitness parameters. Compared to control plants, non-oviposited plants suffered a 70 - 80 % reduction in terms of capsule numbers and seed mass from this herbivory, while oviposited plants lost 40 - 50% of capsule numbers and seed mass. This corresponds to a reduction of the fitness loss in oviposited plants in terms of capsule number by a half and in terms of seed weight by a third. These effects on the plant

fitness likely reflect that there were less larvae feeding on oviposited than on non-oviposited plants, as oviposition priming reduced larval survival, which was indicated in this experiment (likely due to the low replication only) by trend for day four and six (p=0.072 and p=0.093, Fig. 24 a). Consequently, these results indicate that through oviposition-priming plants can minimize the fitness loss due to herbivory. Because the first experiment fell short in numbers of available plants, which strongly limited the number of replicates and treatments (lacking P-treatment), it was repeated with more replicates and in a full-factorial setup (Exp. 8). Unfortunately, in this experiment failed to reproduce the results of the first experiment. No differences were detected between the fitness of oviposited and non-oviposited plants (Fig. 25 d - g). However, also the larvae were affected differently in this experiment. Opposite to the previous experiment (Exp. 7) and preceding studies (Bandoly et al., 2015, 2016), larvae on oviposited plants were not affected in their survival and showed only a tendency of a diminished body weight (Fig. 25 a & c). This rather small effect of oviposition priming on larval performance was probably insufficient to diminish the fitness loss caused by the larval feeding.

A possible explanation for the different outcomes of both experiments with *S. exigua* could be the fact that the second experiment (Exp. 8) was executed during summer with high temperatures in the greenhouse, while the first experiment (Exp. 7) was executed in spring. Associated with these high temperatures, plants (Exp. 8) started about five days earlier to elongate and had a higher growth rate (Fig. A6 a & b) than plants in the first experiment (Exp. 7). This faster development may enable the plants to better compensate for the tissue loss to herbivory and alter its investments in defences. Furthermore, larvae also exhibit faster development at higher temperatures (Kingsolver and Woods, 1997; Lee and Roh, 2010; Du Plessis et al., 2020), which may further affect their ability to cope with induced plant defences. Faster developing larvae could for instance outgrow phases in which the developmental retardation and impaired performance due to oviposition priming is mainly settled, which is mainly the initial phase of larval development (Bandoly et al. (2015); Lortzing et al. (2019); Geuss et al. (2018); Drok et al., unpublished). Thus, the rather small effect of oviposition priming on larval performance may be explained by the faster larval development.

Nevertheless, the results of the second experiment with *S. exigua* (Exp. 8) resemble the outcome of the experiments with *M. sexta* and altogether they indicate that the fitness of *N. attenuata* is not measurably affected beyond incurs due to tissue lost caused by larval feeding in all occasions the larvae are, for whatever reasons,

not or only minimally affected by priming. Overall, the results suggest that while the fitness costs of priming are rather low in relation to the fitness consequences of defence responses elicited by feeding herbivores, the beneficial effect of oviposition priming on plant fitness (i.e. minimization of fitness losses due to herbivory) may be high, when the herbivore is strongly affected by the primed defence.

As described before, consequences of oviposition priming for the plant fitness have hardly been investigated before with very few exceptions focussing on *B. nigra* (see 2.4.3, Tab. 1). After exposure to oviposition and larval feeding by *P. brassicae*, *B. nigra* plants were shown to grow higher, flower earlier, produced earlier seeds in higher numbers compared to control plants and plants exposed exclusively to larval feeding (Pashalidou et al., 2015b, 2013; Lucas-Barbosa et al., 2013). Contrary to these results, another recent study with the same plant (Pashalidou et al., 2020), found an increased number of flowers and seeds in response to oviposition (without larval feeding), which was lost when plants were exposed to larval feeding (no difference between control and oviposited plants exposed to larval feeding). Moreover these results are difficult to reconcile with the fact that opposite to *N. attenuata*, *B. nigra* did not show a diminished fitness in response to larval feeding (Pashalidou et al. (2015b); the only of the mentioned studies that included a comparison between control and feeding-exposed plants). Hence, fitness consequences of oviposition priming for *B. nigra* are not comparable to those observed for *N. attenuata*.

However, some of the few studies that examined fitness consequences of defence priming to cues other than oviposition show similarities to the observed fitness consequences in *N. attenuata*. In presence of the triggering stress, i.e. herbivory or a pathogen attack, primed plants often performed better than non-primed plants suggesting that if priming was associated with costs, they were outweighed by the benefits (Vos et al., 2013a). For example, primed *A. thaliana* and barley plants which are not affected in their fitness in the absence of an attack, gain a higher fitness compared to non-primed plants in response to pathogen attack or high disease pressure (van Hulten et al., 2006; Walters et al., 2008). Within ten years of observation, sagebrush (*Artemisia tridentata*) plants primed by volatiles of experimentally clipped neighbours exhibited between the years varying fitness benefits of this volatile priming, such as higher seedling survivorship, branch growth and flower production, while in none of the ten years costs of this volatile priming were detected (Karban et al., 2012). Furthermore, *N. attenuata* plants primed by volatiles of clipped sagebrush neighbours produced more flowers and capsules than plants with unclipped neighbours (Karban and Maron, 2002).

Interestingly, the pattern of fitness consequences observed in the first experiment with *S. exigua* (Fig. 24), were highly similar to those observed in the same plant species in response to another priming stimulus of anti-herbivore defence, namely the pre-infestation with a small sucking insect (mirid bug, *Tupiocoris notatus*) that alone does neither elicit a full defence response nor a reduce plant fitness (Kessler and Baldwin, 2004). In this study, hornworm herbivory also strongly reduced lifetime seed production of *N. attenuata*, but if the plants were pre-infested by the mirid bug plants realize significant fitness benefits in environments with both herbivores (Kessler and Baldwin, 2004).
Afterall, it needs to be considered that the greenhouse experiments conducted here are not able to assess ecological trade-offs associated with the primed and induced responses which occur in the natural environment of the plant (Yip et al., 2019). For example, *N. attenuata* plants primed by volatiles of clipped sagebrush neighbours, which minimizes leaf damage, suffered more frost damage than controls in one year (Karban and Maron, 2002). Also the benefits of oviposition priming could be more pronounced in *N. attenuata*'s natural habitat, where it grows in strong intraspecific competition (Baldwin, 1999). Under such conditions, the more efficient defence of oviposition primed plants might force especially larvae of the higher instars to move to another non-primed neighbouring plant, which would further benefit the primed plant.

5.2.5 Oviposition in combination with larval feeding can increase the fitness of regrown plants

In native North American populations of *N. attenuata*, hornworm larvae typically stay on the plant they were oviposited on until they reached the fourth larval instar (Kessler and Baldwin, 2002b; van Dam et al., 2001). Generally, herbivory of specialized hornworms, like for example *M. sexta*, can cause vast defoliation of aboveground plant parts as especially larvae of the later instars consume large leaf areas respectively several plants (Kessler and Baldwin, 2002b; van Dam et al., 2001). Larvae of the tobacco specialist *M. sexta* are highly adapted to defence responses of *N. attenuata*, as these larvae for example rapidly dispose nicotine (Appel and Martin, 1992; Wink and Theile, 2002). But beside defence responses, plants can furthermore deploy tolerance responses in order to cope with herbivory (see 2.2.1). From a study with 38 species of milkweed, the authors assumed that in environments where plants are mainly consumed by specialist herbivores, regrowth (or tolerance) may be favoured over defence traits during

the diversification process over macro-evolutionary time (Agrawal and Fishbein, 2008). For the same reasoning and as *N. attenuata* is phenotypically plastic in both defence and tolerance traits, it may also in the interaction with its specialist herbivore *M. sexta*, favour tolerance above defence responses. As other plant species, *N. attenuata* can overcome high defoliation due to its ability to regrow, which is facilitated by tolerance responses (Strauss and Agrawal, 1999; Machado et al., 2017). This challenges the question, whether such tolerance responses and the associated ability to regrow from roots after a vast defoliation is affected or enhanced by oviposition priming.

To test this question, two further experiments (Exp. 11 & 12) were conducted, in which all aboveground plant parts of *N. attenuata* plants of all treatments all were removed after a feeding phase of *M. sexta* larvae on the T- and TP-plants. The plants were allowed to regrow and subsequently, the fitness of regrown plants was investigated. Growth was differentially affected in both experiments before and after defoliation, why effects are difficult to interpret. Plants in one experiment were affected by feeding before defoliation and had a higher cumulative stalk length after regrowth when oviposition preceded larval feeding, while in the other experiment effects on growth occured only occasionally (Fig. A8). Interestingly, plants exposed to oviposition and larval feeding before defoliation developed more flowers than untreated control plants, and plants exposed to the larval feeding only (Fig. 28 a). A GLMM on the data set of both experiments revealed a significant effect of oviposition on flower number, while an effect of larval feeding was only indicated by trend (p=0.122) similar as the difference between flower numbers of oviposited plants with and without larval feeding (p=0.096). Furthermore plants exposed to oviposition and larval feeding before defoliation had a longer flowering period than plants exposed exclusively to oviposition and also tended to have a longer flowering period than of plants exposed to the other treatments (C vs. PT: p=0.090; T vs. PT: p=0.078; Fig. 28 b). Effects of oviposition and larval feeding were also carried through to reproductive units as they were detected in capsule numbers and by trend in the seed weight produced. Plants exposed to both, oviposition and larval feeding, produced significantly more capsules and also a seed weight that tended to be higher compared to control plants (p=0.059, Fig. 28 c & d), while the single treatments caused intermediate level of both parameters suggesting that both factors may have affected plant fitness additively. These results suggests that experiencing oviposition in combination with subsequent larval feeding before defoliation can enhance a plant's tolerance traits. This contrasts previous priming experiments with *M. sexta* examining the

fitness consequences for *N. attenuata* that did not involve complete defoliation, in which larval feeding drastically reduced fitness, while oviposition alone or in combination with larval feeding had no effect (Fig. 27). As this effect on fitness is unlikely associated with the altered defence response in oviposited plants, these results indicate that plant responses to early warning cues associated with herbivory can extend to plant traits not directly related to defence but to tolerance.

But how is such an enhanced fitness of regrown plants after oviposition and larval feeding established? As mentioned above, one potential explanation may lie in tolerance responses such as an increased allocation of photoassimilates to the roots, a mechanism by which plants may "bunker" these assimilates inaccessible for the folivore herbivore but available for a future regrowth after the herbivore is gone. Indeed, many plant species have be shown to increase in carbon transport from both damaged and undamaged tissues to the roots (Dyer et al., 1991; Briske et al., 1996; Holland et al., 1996; Babst et al., 2008; Schwachtje et al., 2006; Bazot et al., 2005; Kaplan et al., 2008). In *N. attenuata* plants challenged with simulated herbivory, C11 photosynthate labelling revealed an increased allocation of sugars to the roots, which is regulated independently of jasmonate signalling by the β-subunit of an SnRK1 (SNF1 related kinase) protein kinase, GAL83 (Schwachtje et al., 2006). Herbivore attack during early stages of development increases root reserves, a less vulnerable location within the plant during an attack by folivores, which in turn delay senescence and prolongs flowering (Schwachtje et al., 2006). This observed phenomenon could partly explain the observed results. After oviposition, such a tolerance response in reaction to larval feeding could be more pronounced and cause a greater fitness of the plant after regrowth, which should be addressed in further experiments.

However, there are also inconsistent results from different studies on plant regrowth abilities with respect to herbivory. Another study with *N. attenuata* could confirm on the one hand that regrowth in response to herbivory is improved by the down-regulation of *GAL83*, but on the other hand reported that *M. sexta* attack constrained regrowth from rootstock and consequently plant fitness (Machado et al., 2013). These findings are in contradiction to results of the experiments described above (Fig. 28) as well as to the studies by Schwachtje et al. (2006), but may be explained by several differences.

One difference between the studies are the parameters assessed as fitness proxies. As such, Machado et al. (2013) recorded flower number at three time points, cumulative branch length, and rosette diameter but no direct reproduction

parameter such as seed production. In the experiments of this thesis assessing the fitness of *N. attenuata*, direct reproduction parameter such capsule number and seed weight were measured and flower number were assessed daily. Such a close monitoring could be crucial as Schwachtje et al. (2006) showed that herbivore attack during early stages of development delays senescence and prolongs flowering, which can easily be overseen when flowers are assessed at only few time points. Therefore, the choice of less informative parameter meassured in the study by Machado et al. (2013) may explain the discrepancy between the outcomes of that study and the experiment in this thesis as well as those in the study by Schwachtje et al. (2006).
Another important factor that could explain different outcomes in such regrowth experiments could be differences in the plant stage exposed to herbivory and defoliation. The plant traits which facilitate the ability to regrowth follow a developmental pattern as source-sink relationships change over ontogeny and therewith photoassimilate partitioning (Boege and Marquis, 2005; Barton and Koricheva, 2010). However, the plant stage used in the study of Machado et al. (2013) is not explicitly mentioned. To resolve how the herbivore-induced augmentation of *N. attenuata*'s regrowth ability relates to plant development, experiments with different developmental stages were conducted (described in the following).

5.2.6 Fitness of regrown plants is not affected by oviposition priming if defoliation occurs in rosette or flowering stage

The ability of plants to reallocate resources to different processes such as defence or tolerance depends on the developmental stage of the plant, as these plants are situated in different physiological conditions with distinct deployment, distribution, and turnover of assimilates between diverse tissues of the plant (Boege and Marquis, 2005; Barton and Koricheva, 2010). In all previously described experiments with *N. attenuata* (Exp. 7 - 12), the used plants were fully established rosette plants that started to elongate when treatments were applied (beginning usually with oviposition or the corresponding mock treatment). To test whether herbivory-induced tolerance traits as well as the effects of oviposition on them is affected by the developmental stage of the plant, the experiments including defoliation (Exp. 11 & 12) were repeated with (a) very young rosette plants (juvenile/establishing phase) and (b) plants that were already flowering

(matured/reproductive phase) and compared to the results of the two experiments with early elongating plants (Fig. 28). The results of this experiment (Exp. 13) revealed that neither juvenile rosette plants nor mature plants that were fully elongated and flowering before defoliation showed an elevated fitness in response to prior herbivory and/or oviposition by *M. sexta* (Fig. 29). In contrast to early elongating plants, the number of flowers was even reduced in response to prior oviposition on juvenile and reproductively active plants similarly (Fig. 29 a). Neither this effect of oviposition nor the larval herbivory did affect the fitness of plants that had to regrow from rootstocks of mature plants. Juvenile rosette plants, on the other side, were more strongly affected by the week of herbivory than established rosette plants starting to elongate and mature plants in the flowering stage. Plants regrown from the roots of young rosette plants after they were exposed to *M. sexta* larvae, regrew smaller (Fig. A9 b), flowered for a shorter period of time and produced a significantly lower seed mass than plants not exposed to herbivory before defoliation (Fig. 29).

That even a week of herbivory did not negatively impact the fitness of plants regrown from rootstocks of established plants (either early elongating or plants in the reproductive phase) suggests that these plants had already build up root storages before the phase larval feeding constrained the photosynthetically active tissues. Other than early elongating plants (Exp. 11 & 12), plants in the reproductive phase did not respond to herbivory with an increase of their regrowth capacity either. In line with the optimal defence hypothesis (McKey, 1974; Meldau et al., 2012), plants in the reproductive phase likely prioritise the production and development of flowers and seed-bearing capsules over vegetative tissues. Even under herbivore attack, plants in this developmental stage are expected to devote resources reallocation to reproductive tissues rather than to defence and tolerance traits, like for example increasing resource storage in vegetative organs as roots (Schwachtje and Baldwin, 2008; Boege and Marquis, 2005). Moreover, plants in the reproductive phase could employ other strategies to ensure a high reproductive output under unfavourable conditions. For example, tomato plants are suggested to have plastic control over reproduction and speed up fruit- and seed production when infested by spider mites (Liu et al., 2017a).

Juvenile plants, on the other hand, are generally more vulnerable to the tissue loss suffered from herbivory as they still have to establish and consequently loose larger portions of their photosynthetic active leaf area compared to further developed plant stages. For example, *Raphanus sativus* plants in a juvenile stage are more vulnerable to clipping (i.e. removing 50 % of the leaf area) than plants

in the reproductive phase, indicated by a lower seeds per flowers ratio of juvenile plants (Boege et al., 2007). This higher vulnerability is likely also reflected in the diminished fitness of regrown plants exposed to larval feeding as very young rosettes before defoliation. In an early phase of development, in which assimilates are still a limited resource, an herbivore attack is especially challenging. On the one hand, they build up relatively high levels of defences in the vegetative tissues, which demands resources (Kaur et al., 2010; Van Dam et al., 2001) and on the other hand, the leaf area is still limited and every loss of it to the herbivore constrains assimilate production further. In consequence, attacked plants in this early stage suffer a larger loss of assimilates than elongating or flowering plants, so that no assimilates can be afforded to be stored in the roots. In line with this argumentation, *N. attenuata* seedings induced by simulated herbivory were shown to decrease partitioning of recent photoassimilates to roots (Schmidt et al., 2015), in contrast to the reported increased allocation of sugars to roots in older *N. attenuata* plants described before (Schwachtje et al., 2006). This distinct portioning may further indicate that plants in early developmental phases depict a different assimilate allocation than older plants.

Furthermore, the reduced fitness of plants exposed to larval feeding before defoliation parallels the impaired regrowth of feeding-induced *N. attenuata* plants in the study of Machado et al. (2013), which gives a different outcome than the experiments with early elongated *N. attenuata* plants (Exp. 11 & 12, Fig. 28) in this thesis (discussed above). Maybe Machado et al. (2013) used plants in a similar early developmental stage (age of the plants at the beginning of the experimental procedure is not explicitly mentioned).

Interestingly, the mean seed weight of young rosette plants after the different treatments (Fig. 29 d) revealed a slightly similar induction pattern to the results of the experiment with *S. exigua* without defoliation (Fig. 24 f). Seed weights were diminished when plants were exposed to larval feeding, while oviposition before larval feeding slightly enhanced the seed weight compared to plants only exposed to larval feeding before defoliation (Fig. 29 d). In a treatment wise comparison, the seed weights of plants exposed exclusively to larval feeding before defoliation were lower than the seed weights of untreated control plants, while the seed weights plants exposed to oviposition and larval feeding before defoliation exhibit no difference to the seed weights of untreated control plants (p=0.377). However, the difference between plants exposed to larval feeding with and without prior oviposition were not significant (p=0.358). This pattern might indicate that plants exposed to oviposition and larval feeding before defoliation

were affected by oviposition priming, as the fitness loss (i.e. lower seed weight) was minimized respectively not detectable when plants perceived oviposition before larval feeding. Hence, oviposition priming might have caused an improved fitness also in interaction with the specialist herbivore in an experimental setup including defoliation and regrowth.
Overall, plants that were exposed to defoliation in the juvenile phase, subjected to the herbivore or not, were able to regrow to larger plants producing more flowers and capsules with seeds than plants that were defoliated after they matured (Fig. 28 & 29). This could be caused by a larger amount of nutrients available for regrowth of these plants, as plants before defoliation probably did not dissipate many nutrients from the soil before defoliation. In contrast, plants in later stages before defoliation already incorporated larger fractions of nutrients from the soil for example in their aboveground tissues, which were then removed with the shoots. Thus, the larger fitness of plants regrown from plants cut back in a juvenile stage may reflect the impact of resource availability for regrowth, which is likely an important factor to consider when investigating tolerance responses.
In conclusion, the varying effect of larval feeding and prior oviposition on the plant fitness of plants in distinctive developmental stages within the experiments including defoliation (Exp. 11 - 13, Fig. 28 & 29) suggests that the ability to regrow and the enhancing effect of prior oviposition follow a developmental pattern. Especially plants that passed the establishing phase but did not yet enter the reproductive phase are most responsive with respect to tolerance traits. This would correspond to the pattern of tolerance as well as defence expression during plant ontogeny previously suggested, explicitly that plants exhibit a phase of improved expression of tolerance and defence mechanisms with increasing development after a phase of establishment and before reproduction (Boege and Marquis, 2005; Barton and Koricheva, 2010).

5.3 Conclusion and outlook

5.3.1 *S. exigua* oviposition: A potentially interfering factor that may cause experimental inconsistencies

Before summarising the major conclusions from the data attained in this thesis, it needs to be discussed that during the experiments with *S. exigua*, several difficulties with a standardised oviposition treatment were encountered, that may in part explain the divergent outcomes of repeated experiments.

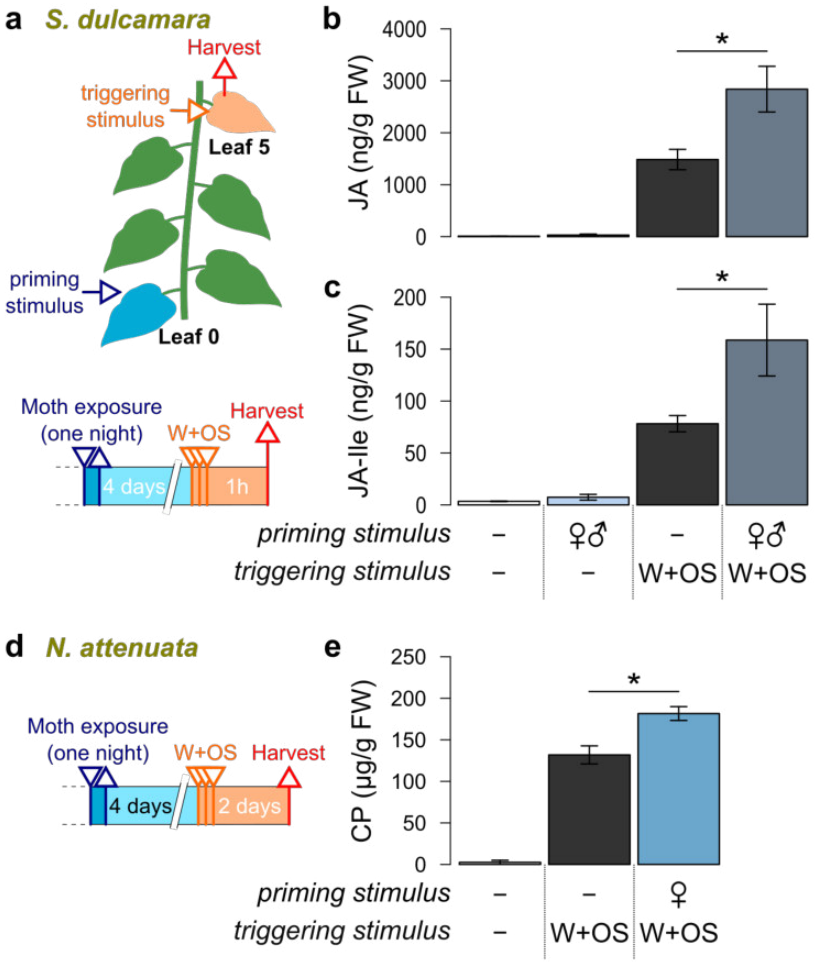

Figure 31: *S. dulcamara* and *N. attenuata* respond to the presence of *S. exigua* moths even without egg deposition. (a) In the experiment with *S. dulcamara* plants were exposed to a priming (presence of male and female moths for one night but without egg deposition) and a triggering (repeated W+OS treatment, three times, 10 min time gab) stimulus in a full-factorial setup. The priming stimulus was applied on leaf 0, while the triggering stimulus was applied to the leaf five positions higher (leaf 5) which was harvested harvest one hour after the last treatment. Level of (b) jasmonic acid (JA) and (c) jasmonic acid-isoleucine (JA-Ile) were quantified. (d) *N. attenuata* plants were either left untreated, exposed to W+OS treatment (triggering stimulus) or a combination of the presence of unmated female *S. exigua* moths (priming stimulus) and W+OS treatment. Four days after moth exposure for one night plants were exposed to three W+OS treatments (3 h time gab) and leaf material of the treated leaf was harvested after two more days for quantification of (e) caffeoylputrescine (CP). $N_{S.\ dulcamara/N.\ attenuata}$ = 5-6 / 4-5. Bars represent mean ± SEM. Asterisks indicate significant differences according to two sample t-test ($p < 0.05$).

The nocturnal moths of *S. exigua* lay their eggs in varying numbers and degree of attachment to the leaf surface (sometimes eggs or egg clutches are only slackly attached to the leaf surface causing that eggs fall off) during one night with consequently varying time points of oviposition (up to 14 h difference possible). Furthermore, *S. exigua* oviposit not exclusively on leaves but on almost every surface (also the netting-bag which keeps the moths on a certain leaf position), thus in some oviposition treatments not the sufficient number of oviposited plants was achieved. Contrary to this rather unstandardized procedure with *S. exigua*, oviposition by *M. sexta* is achieved in a more standardized setup (see 3.4.1, Bandoly and Steppuhn (2016)), where eggs are oviposited within a certain time frame, in similar loads and attachment.

Another distinction to previous experiments with oviposition by *S. exigua* were differences within the oviposition treatment. Bandoly et al. (2015) and Bandoly et al. (2016) exposed hole *N. attenuata* plants to a flight cage with *S. exigua* moths (procedure described in Bandoly and Steppuhn (2016)). In contrast, within the experiments of this thesis, single leaves were exposed utilizing a fine netting bag to moths in order to achieve oviposition on a defined leaf (see 3.4.1) to account for the dependency of induction of defensive compounds and leaf ontogeny (Van Dam et al., 2001). In order to achieve the largest possible number of replicates, all available plants were exposed to *S. exigua* moths for oviposition. However, partly leaves were not oviposited after one night of exposure to the moths. In a some of the conducted experiments, these non-oviposited but moth exposed plants were partly further used in the experiment as non-oviposited plants, i.e. as control plants or plants exposed to larval feeding only. This was done to increase the number of replicates with ontogenetically matched plants dealing with a limited number of available plants. Considering that potential priming cues can directly be associated with the presence of the herbivores (e.g. insect footsteps or broken trichomes, Hall et al. (2004); Peiffer et al. (2009)), this close contact of *S. exigua* moths and the encaged leaf raises the question if already this contact caused effects in the plant. The yet unknown elicitor of the primed defence response could be associated with the moths.

To assess whether already the presence of *S. exigua* moths respectively female moths can induce responses in *N. attenuata* or *S. dulcamara*, first promising preliminary tests were conducted during the final phase of this doctoral thesis work. In the first preliminary experiment, *S. dulcamara* plants were exposed in a full-factorial setup either to male and female *S. exigua* moths (priming stimulus) for one night without oviposition and / or simulated herbivory (trig-

gering stimulus, W+OS treatment applied in a similar temporal pattern as in Exp. 6) applied on the leaf five positions higher as in previous experiments (Fig. 31 a). Interestingly, in response to simulated herbivory presence of male and female moths on the plant four days before (and also at a different leaf position, Fig. 31 a) caused a higher induction of JA (two sample t-test W+OS vs. W+OS & moths: $t(10)$=-2.8459, p=0.01737) and JA-Ile (two sample t-test W+OS vs. W+OS & moths: $t(10)$=-2.5227, p=0.03024) than simulated herbivory alone (Fig. 31 b & c). In the second preliminary experiment, *N. attenuata* plants were either kept untreated or exposed to simulated herbivory (triggering stimulus, W+OS treatment applied in a similar temporal pattern as in Exp. 10) without or with prior exposure to unmated female *S. exigua* moths for one night (priming stimulus; exposure four days before triggering stimulus was applied). Remarkably, simulated herbivory induced levels of CP, but presence of female moths further increased this induction (two sample t-test W+OS vs. W+OS & female moths: $t(7)$=-3,477, p=0.01031). To claim profound conclusions, experiments need to be repeated with a sophisticated experimental setup, including comparisons between different combinations of male and female moths respectively and an actual oviposition treatment.

Both experiments may indicate that the presence of *S. exigua* moths without oviposition can cause similar modifications of responses to simulated herbivory on the phytohormonal and metabolic level as the oviposition-primed defence induction upon herbivory. Thus in those experiments, in which non-oviposited leaves exposed to moths for one night were used as non-oviposited plants (for C or T treatment), such responses to the presence of the moths could have increased the variability of the respective treatments and therewith confound the analyses. However, not all non-oviposited plants were exposed to moths in the respective experiments and the identity of the moths-exposed plants was not documented. Consequently, the size of the effect resulting from the presence of moths on the leaves for one night within the conduced experiments cannot be determined. Furthermore, in the other experiments involving *S. exigua* oviposition only plants exposed to empty netting bags were destined for treatments without oviposition. Nevertheless, results indicate that potential cues or elicitors of priming could be associated with the moths or even with the female moths, as for example oviposition or the attachment of eggs on the leaf surface was not included. A major drawback for research in context of oviposition priming, is caused by the fact that the cues or elicitors of priming which cause an enhanced defence response after perceiving oviposition are mostly unknown. In interaction of *A. thaliana*

and *P. brassicae*, a recent study found that application of an egg-associated glandular secretion which attaches the eggs to the surface of the leaf elicited the enhancing effect on plant defence normally induced after oviposition, suggesting that the elicitor of priming in this interaction is associated or part of this secretion (Paniagua Voirol et al., 2020). However, also sex-pheromones could be potential cues or elicitors of priming, which in case of *S. exigua* are emitted by females to attract males (Tumlinson et al., 1990). In the interaction of tall goldenrod (*Solidago altissima*) plants with the gall-inducing fly *Eurosta solidaginis* plants are able to respond to putative sex attractants of male flies and prime their defence response to various feeding herbivores, including an enhanced JA induction in exposed plants after herbivory (Helms et al., 2013, 2017; Yip et al., 2017). Furthermore, *Pinus sylvestris* responds to the male attracting sex pheromones of sawfly (*Diprion pini*) and strengthens its defences against the insect eggs resulting in a reduced egg survival (Bittner et al., 2019). Similarly, both solanaceous plant species utilized in this thesis may respond to the sex pheromone of *S. exigua* to prime their anti-herbivore defence. With a known elicitor experiments could be performed in a standardized way with high numbers of replicates. Nevertheless, further investigations are needed in this context.

5.3.2 Major conclusions and prospects

Altogether results obtained in the first part of this doctoral thesis add further knowledge to the understanding of the signalling which facilitates an enhanced anti-herbivore defence in oviposited *S. dulcamara* plants. Results suggest an involvement of JA, ABA and JA signalling in establishing a primed defence induction. In first place, the persistent low-level induction of JA and JA-Ile, as well as the induction of associated transcripts, in response to oviposition may indicate for a prepared JA signalling during the period of egg exposure. Interestingly this pattern was not only found in interaction with *S. exigua*, but also found in interaction with the specialist leaf miner *A. autumnitella*. Secondly, the higher jasmonate induction in oviposited compared to non-oviposited plants in response to natural herbivory or simulated herbivory suggests an earlier or faster induction of the jasmonate related signalling cascade which could entail a more effective defence against the larvae. Furthermore, the results could point to an important role of the phytohormonal interplay to attain a primed defence induction. Probably SA, ABA, and jasmonates (JA & JA-Ile) need to be concerted and induced in certain quantities. The transcriptional induction of *CDH*,

further suggests an involvement or additional interaction of cytokinins. After oviposition, signalling associated with a rather low SA induction in combination with a low-level jasmonate induction could be required to prepare the plant for a primed defence induction (primed state). When larvae then start to feed, ABA, JA and JA-Ile are probably coordinately induced in certain quantities and ratios in order to facilitate a primed defence induction.
The observed persistent induction of jasmonates and transcripts after egg removal causes the question if these primed plants are still capable of mounting a primed-defence induction and cause an associated impaired larval performance at these time points after egg removal. Consequently, future experiments should test varying time gaps between egg the end of egg exposure and the onset of larval feeding, to assess how long the persistent induction of basal JA levels can mount a primed-defence induction and affect larval performance. Additional studies are further needed to investigate the so far undescribed signalling in responses to larval feeding caused by *A. autumnitella*. As larvae of these leaf miner are also impaired in their performance when feeding on oviposited *S. dulcamara* plants, investigations during the onset of the primed response to feeding would give insights if oviposition-mediated priming in this interaction with a specialized herbivore of a distinct feeding guild is facilitated by similar processes as for example in the interaction with the generalist leaf-chewer *S. exigua*. Future experimental investigations should furthermore consider a higher temporal resolution of analyses during the onset of the feeding-induced response in oviposited and non-oviposited *S. dulcamara* plants, to reflect the dynamics and effects of oviposition priming more precisely on the different layers of signalling (transcriptional, phytohormonal and metabolic level).

The second part of this doctoral thesis consists of an initial characterisation of the fitness consequences of oviposition-mediated priming of anti-herbivore defences for *N. attenuata* in interaction with generalist and specialist lepidopteran herbivores. Larval herbivory by *S. exigua* and *M. sexta* and associated induced defence caused a clearly diminished growth and fitness of *N. attenuata*. Oviposition by both herbivores without subsequent larval feeding had no effect on growth or plant fitness, indicating that fitness incurs as a consequence of the onset and maintenance of the primed state are compared to those of the induced defence relatively low. Such low fitness costs associated with oviposition priming could be especially beneficial for the plant in occasions when herbivory does not follow upon oviposition, e.g. due to parasitation or predation. Consistent with the

diminished performance of *S. exigua* larvae on oviposited *N. attenuata* plants, the fitness reduction caused by larval feeding was slightly smaller for oviposited plants in terms of capsule and seed production. Thus, benefits of oviposition priming may lie in the decline of fitness losses due to herbivory. In contrast, the fitness incurs due to herbivory by *M. sexta* were not affected by prior oviposition while in this interaction also the larval performance is not impaired. The induced defence, triggered by simulated herbivory without leaf tissue loss, had only an effect on flowering but did not affect the reproductive output, suggesting that the fitness incurs due to herbivory rather result from the leaf tissue loss. Oviposition in combination with simulated herbivory, i.e. a higher primed defence induction, had no further negative effect on plant fitness, indicating relatively low costs of the primed defence induction. Consequently, fitness consequences of oviposition priming are largely influenced by the effect of the primed defence induction on the herbivore and associated lower leaf tissue loss caused by the impaired herbivore than due to physiological costs caused by a higher defence induction. Interestingly, the fitness of regrown plants after a complete removal of aboveground plant parts was enhanced if plants were exposed to oviposition in combination with subsequent larval feeding before defoliation. These results suggest that oviposition priming may not just affect defence but also increase tolerance responses to larval feeding, such as a transient carbon allocation to the roots that could enhance the ability to regrow after the herbivore threat is gone. As the effects of larval feeding and prior oviposition on fitness of regrown plants varied between plants in distinctive developmental stages, the ability to regrow and the enhancing effect of prior oviposition probably follow a developmental pattern.

Future experiments investigating the fitness consequences of oviposition priming should further validate the hypothesis that fitness consequences of oviposition priming are largely influenced by the effect of the primed defence induction on the herbivore. As *N. attenuata* naturally grows under strong intraspecific competition, additional experiments should also involve interactions with other plants to better reflect natural conditions under which ecological interactions likely affect benefits and costs of oviposition priming, which were not considered in this doctoral thesis. For example, this could be realized through experiments including intraspecific competition by exposing primed and non-primed plants together to herbivory, while larvae can freely move between the plants. Furthermore, (semi-)field experiments including the natural abiotic and biotic environmental conditions would give a comprehensive understanding of the fitness consequences

caused by herbivory and oviposition priming. Future work examining the effect of oviposition priming on tolerance responses and the ability to regrow, should focus on investigating the molecular mechanisms facilitating such an enhancing effect. For instance, carbon labelling could reveal if photoassimilate partitioning to the roots is more pronounced in oviposited and feeding-induced plants.

Moreover, future work should further investigate the promising and highly interesting results of the preliminary experiments, which indicate that potential cues or elicitors of the primed response in both utilized plant species could be associated with the moths. These initial results should be validated by a larger sample size and the effect of moth exposure should be further investigated, e.g. by testing different combinations of male and female moths for their effects on the plant and by examining the volatile profiles of the moths. A characterized elicitor of the primed defence would be an important milestone for future research in this context, as it would enable experiments with a standardize primed induction with high numbers of replicates.

Part II

Appendix and References

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B Appendix

B.1 Additional figures

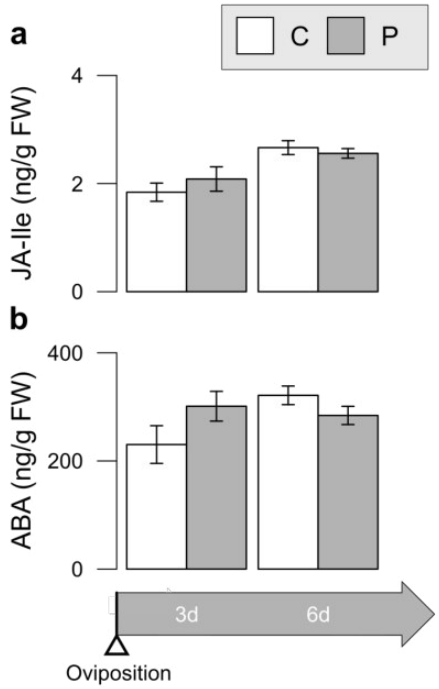

Figure A1: Temporal pattern of phytohormonal induction in *S. dulcamara* leaves after oviposition by *A. autumnitella* (Exp. 3). Levels of (a) jasmonic acid-isoleucine (JA-Ile) and (b) abscisic acid (ABA) in leaves local to oviposition (eggs, black bars) or corresponding leaves of non-oviposited plants (control, white bars) harvested either three or six days after oviposition from individual plants. N = 5-9. Bars represent means ± SEM.

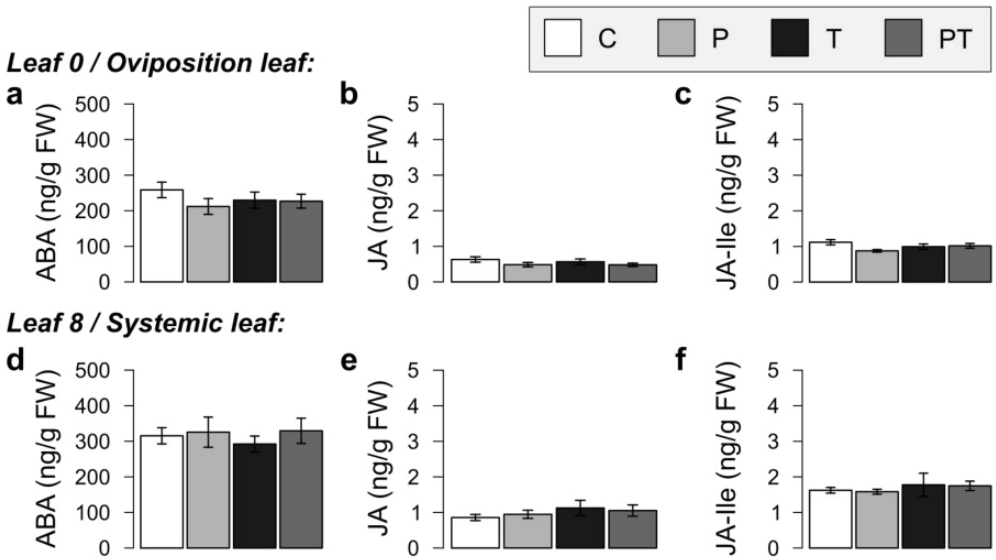

Figure A2: Phytohormonal induction of *S. dulcamara* to oviposition and larval feeding by *S. exigua* in the oviposited leaf (a - c) and a young systemic leaf (d – f; Exp. 4). Plants were either kept untreated (C), exposed to oviposition only (P), larval feeding only (T) or a combination of both (PT). Four days after oviposition eggs were removed and 20 neonate larvae were applied to the leaf five leaf positions higher (vascularly fully connected to the oviposition leaf). After four hours of larval feeding, leaf material of the oviposited leaf (leaf 0) and a young systemic leaf (leaf 8) was harvested and analysed for phytohormonal contents (other results of leaf 5 see 22). Bars represent mean ± SEM levels of (a & d) abscisic acid (ABA), (b & e) jasmonic acid (JA) and (c & f) jasmonic acid-isoleucine (JA-Ile). N = 6-8.

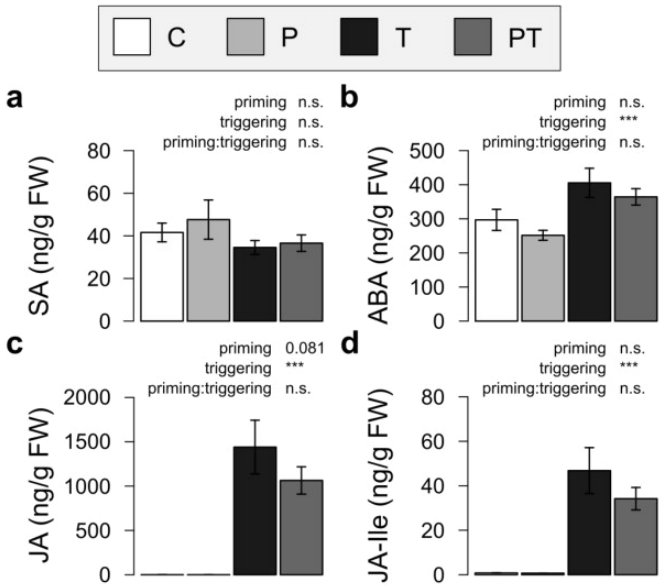

Figure A3: Phytohormonal response of *S. dulcamara* to oviposition and larval feeding by *S. exigua* in the triggered leaf (Exp. 5). Plants were either kept untreated (C), exposed to oviposition only (P), larval feeding only (T) or a combination of both (PT). Four days after oviposition eggs were removed and 20 neonate larvae were applied to the leaf five leaf positions higher (vascularly fully connected to the oviposition leaf). After four hours of larval feeding, leaf material of the leaf exposed to larval feeding was harvested and analysed for phytohormonal and transcriptional contents. Bars represent mean ± SEM levels of (a) salicylic acid (SA), (b) abscisic acid (ABA), (c) jasmonic acid (JA) and (d) jasmonic acid-isoleucine (JA-Ile). N = 10. Asterisks indicate significant differences according to LMMs (detailed information on the statistics used see Tab. A10): *** ($p < 0.001$).

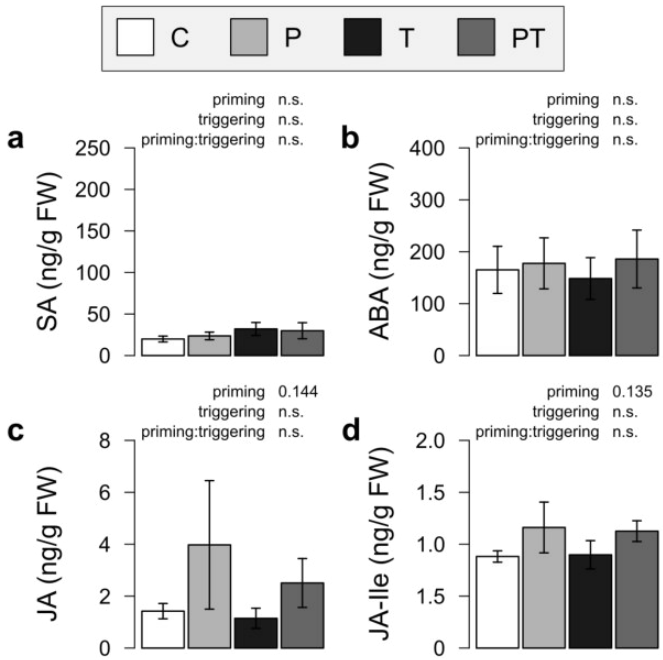

Figure A4: Phytohormonal response of *S. dulcamara* to oviposition by *S. exigua* and simulated herbivory in the oviposition leaf (leaf 0; Exp. 6). Plants were either kept untreated (C), exposed to oviposition only (P), simulated herbivory (W+OS treatment) only (T) or a combination of both (PT). Four days after oviposition eggs were removed and three W+OS treatments (simulated herbivory, 10 min time gab between the treatments) were applied to the leaf five leaf positions higher (vascularly fully connected to the oviposition leaf). One hour after the last treatment, leaf material of the oviposition leaf (leaf 0) was harvested and analysed for phytohormonal contents (phytohormonal and transcriptional levels of leaf 5 see Fig. 23). Bars represent mean ± SEM levels of (a) salicylic acid (SA), (b) abscisic acid (ABA), (c) jasmonic acid (JA) and (d) jasmonic acid-isoleucine (JA-Ile). N = 9. Significant differences according to LMMs (detailed information on the statistics used see Tab. A11).

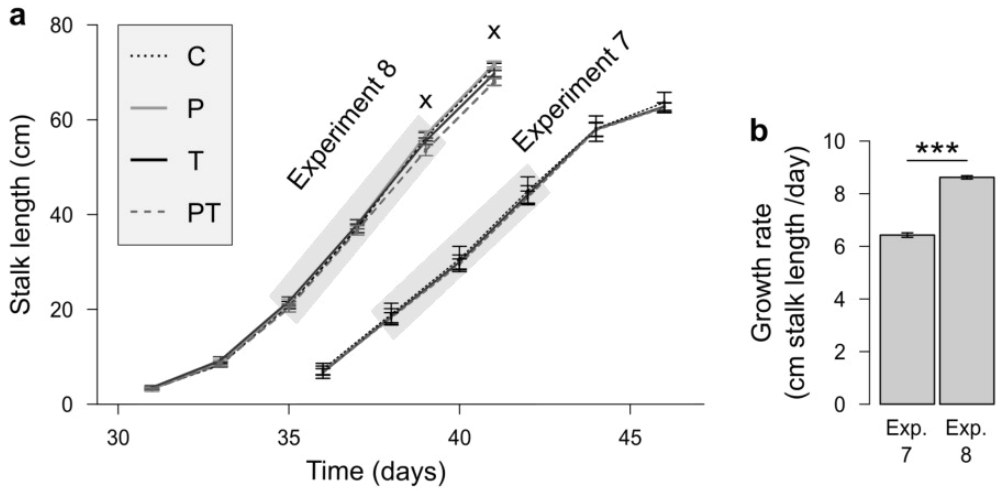

Figure A5: Differences in (a) stalk length and (b) growth rate of the sixth and seventh experiment. *N. attenuata* plants were either kept untreated (C), exposed to oviposition only (P), larval feeding only (T) or a combination of both (PT). Four days after oviposition eggs were removed, 20 (Exp. 7) / 25 (Exp. 8) neonate larvae were applied and allowed to feed from the plants for 15 days (Exp. 7) / 12 days (Exp. 8). (a) Salk length of two fitness experiments with *S. exigua* were combined in one graph. Grey boxes illustrate the three stalk length measurements of each experiment used for calculation of the (b) growth rate (cm stalk length per day). Line graphs and bars represent mean ± SEM. $N_{\text{Exp. 7 /Exp. 8}} = 4/16$. (a) x indicate time points with a significant ($p < 0.05$) effect of larval feeding according to ANOVA (detailed information on the statistics used see Tab. A12 and A14). (b) Asterisks indicate significant differences according to Wilcoxon rank sum test: *** ($p< 0.001$).

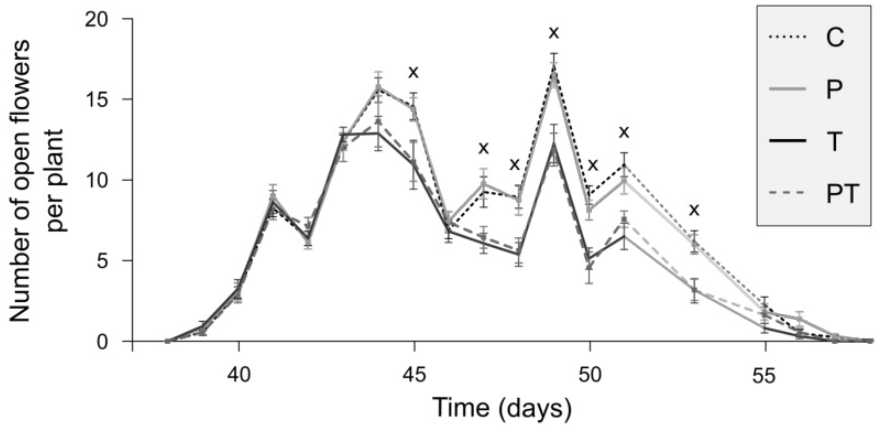

Figure A6: Flowering of *N. attenuata* is affected by *S. exigua* larval feeding (Exp. 8). *N. attenuata* plants were either kept untreated (C), exposed to oviposition only (P), larval feeding only (T) or a combination of both (PT). Four days after oviposition eggs were removed, 25 neonate larvae were applied and allowed to feed from the plants for 12 days. Line graphs represent mean number of open flowers ± SEM. N= 16. x indicate time points with a significant ($p< 0.05$) effect of triggering according to two-way ANOVA (detailed information on the statistics used see Tab. A14).

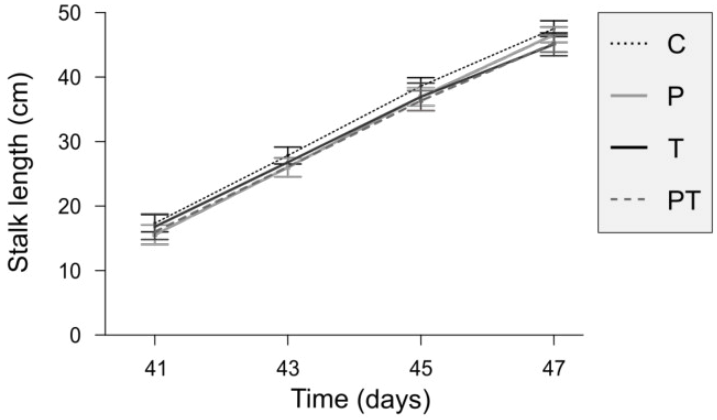

Figure A7: Stalk length of plants from experiment ten (detailed information on the statistics used see Tab. A17). *N. attenuata* plants were either kept untreated (C), exposed to *M. sexta* oviposition only (P), simulated herbivory (T) or a combination of both (PT). Four days after oviposition eggs were removed and for three subsequent days, three W + OS treatments per day (time gab three hours) were applied on the oviposited or corresponding leaf and the two next younger leaves (one leaf treated per day). Line graphs represent mean ± SEM. No differences according to two-way ANOVA (detailed information on the statistics used see Tab. A16).

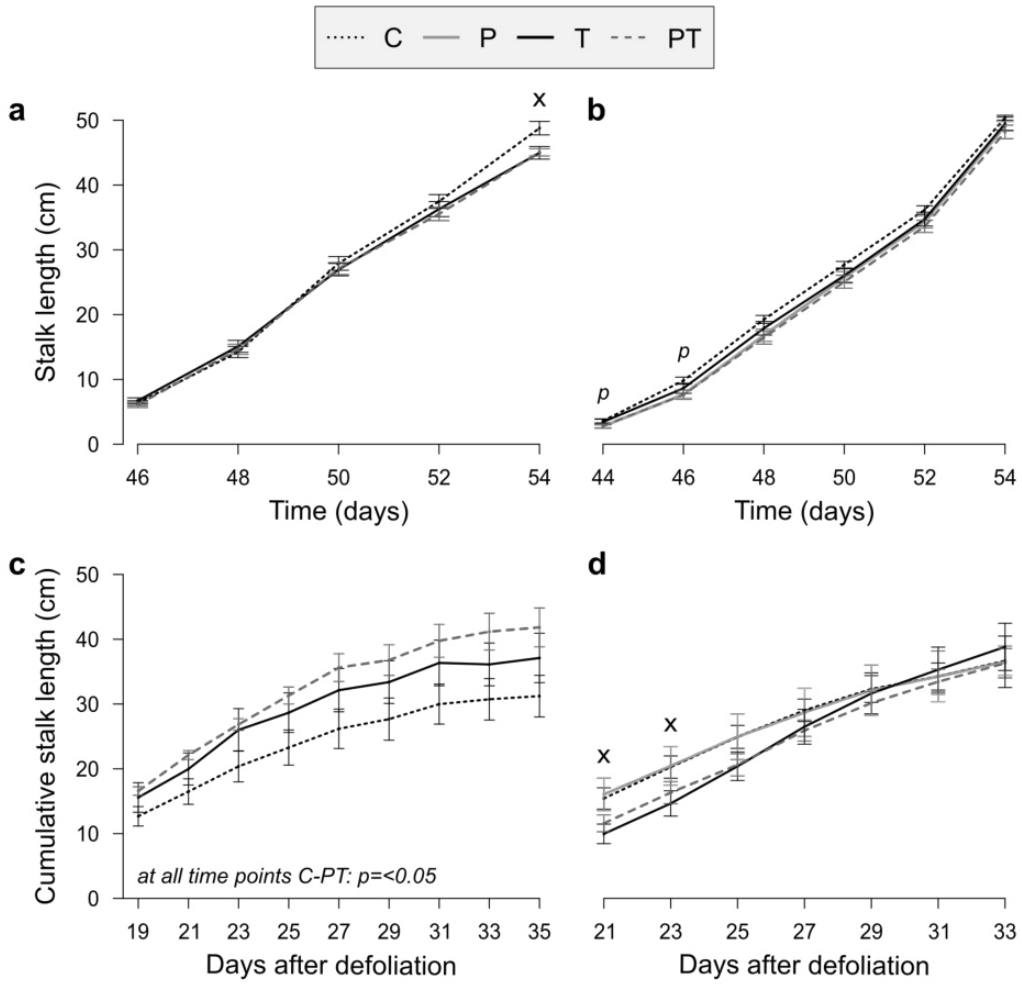

Figure A8: Stalk length of plants from experiment eleven and twelve (a & b) before and (c & d) after defoliation. *N. attenuata* plants were either kept untreated (C), exposed to oviposition only (P), larval feeding only (T) or a combination of both (PT) before defoliation. Four days after oviposition by *M. sexta* eggs were removed, larvae were applied (first experiment three larvae of the third instar, second experiment: first two neonate larvae, after two days two additional third instar larvae) and allowed to feed from the plants for seven days. Then all aboveground plant parts were removed, and plants were allowed to regrow. Line graphs represent mean (± SEM) (a & b) stalk length before defoliation and (c & d) cumulative stalk length (sum of all stalks of a plant) after regrowth. (a & c) Exp. 11 N = 6 (b & d) Exp. 12 N = 8 - 11. (a) x indicate significant ($p< 0.05$) difference between c-T and T-PT (b & d) x indicate time points with a significant ($p< 0.05$) effect of triggering, while p indicate a significant ($p< 0.05$) effect of priming according to two-way ANOVA (detailed information on the statistics used see Tab. A18 and A19)

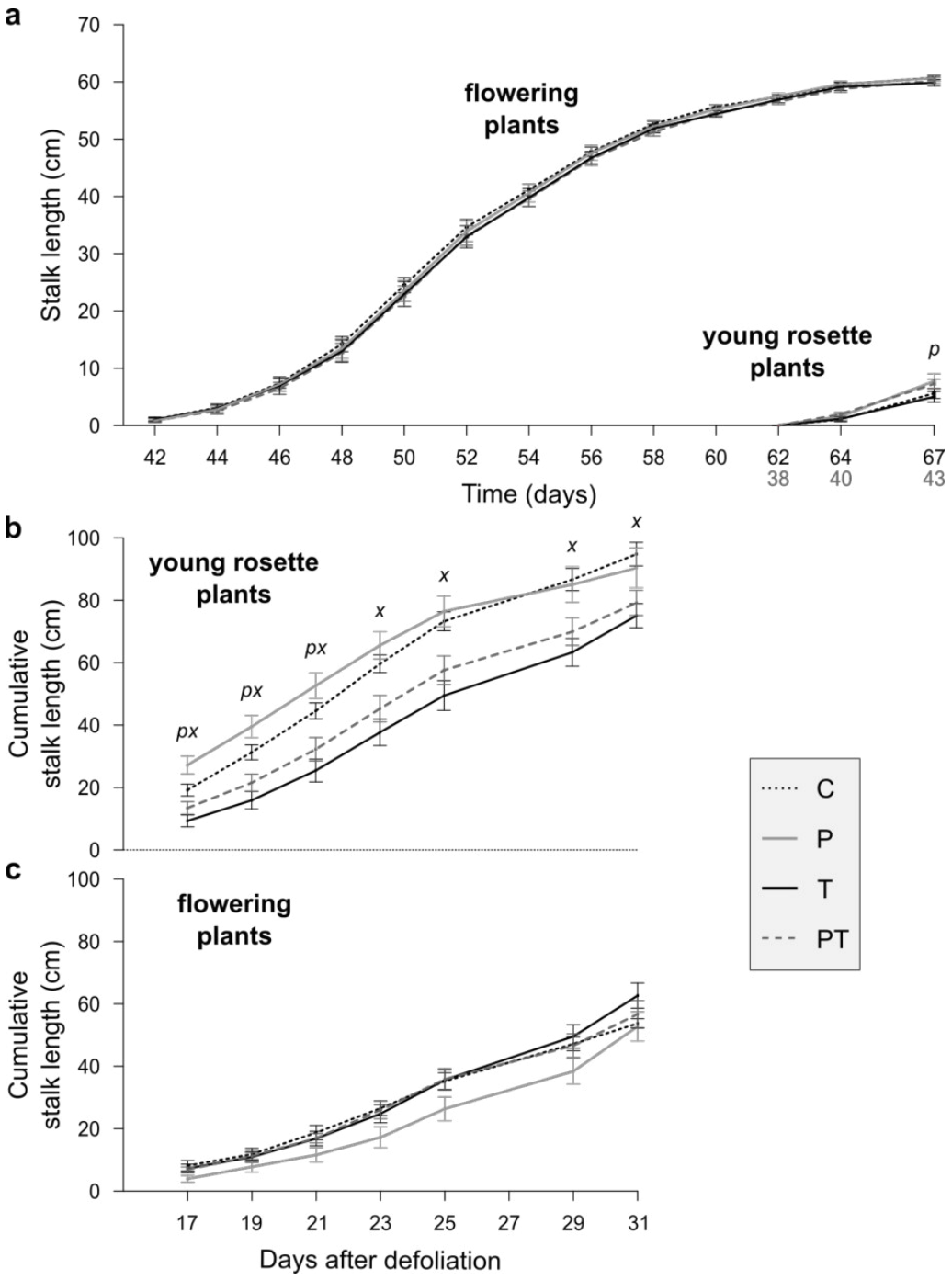

Figure A9: Stalk length of plants from Exp. 13. Stalk length of plants from experiment thirteen (a) before and (b & c) after defoliation. *N. attenuata* plants were either kept untreated (C), exposed to *M. sexta* oviposition only (P), simulated herbivory (T) or a combination of both (PT). At the time of oviposition plants were either four-week-old rosette plants or eight-week-old elongated flowering plants. Four days after oviposition by *M. sexta* eggs were removed, larvae were applied (first two neonate larvae, after two days two additional third instar larvae were applied) and allowed to feed from the plants for seven days. Then all aboveground plant parts were removed and plants were allowed to regrow. Line graphs represent mean (± SEM) (a & b) stalk length before defoliation and (c & d) cumulative stalk length (sum of all stalks of a plant) after regrowth. N = 10. x indicate time points with a significant ($p < 0.001$) effect of triggering, while p indicate a significant ($p < 0.05$) effect of priming according to two-way ANOVA (detailed information on the statistics used see Tab. A21).

B.2 Supporting information

B.2.1 Supplementary material and methods

Experimental setup of preliminary tests

Experiment with *S. dulcamara* To assess wether already the presence of *S. exigua* moths can induce responses on the phytohormonal level in *S. dulcamara* and serve as a priming stimulus, a preliminary experiment was performed. Therefore, *S. dulcamara* plants were exposed in a full-factorial setup to male and female *S. exigua* moths for one night (priming stimulus) without oviposition and/or simulated herbivory (triggering stimulus, W+OS treatment applied in a similar temporal pattern as in Exp. 6). Four-week-old *S. dulcamara* plants (Siethen population; grown as described, see 3.1.1) were compiled in replicates according to plant height. Plants were exposed at the 5th fully developed leaf to male and female moths for one night (14 h) without oviposition (conducted as described before for oviposition, see 3.4.1). At the next day bags with moths were removed and after four days simulated herbivory was applied to the vascularly fully connected leaf five positions higher than the leaf exposed to moths (or corresponding leaves). Simulated herbivory consisted of W+OS treatment (conducted as described, see 3.4.2), i.e. two rows (one on each side) of wounding per leaf in parallel to the mid vein. The treatment was repeated three times in intervals of ten minutes and one hour after the last treatment, leaf material of the triggered leaf was harvested into screw cap tubes containing matrix, flash-frozen in liquid nitrogen and stored at -80 °Cuntil phytohormonal analysis.

Experiment with *N. attenuata* To investigate if the presence of *S. exigua* moths can induce responses on the metabolic level in *N. attenuata* and serve as a priming stimulus, another preliminary experiment was performed. *N. attenuata* plants were either left untreated, exposed to repeated W+OS treatment (triggering stimulus) or a combination of the presence of unmated female *S. exigua* moths (priming stimulus) and W+OS treatment. Two days before the experiment, the sex of *S. exigua* pupae was determined, separated from male pupae, and allowed to hatch. Five-week-old *N. attenuata* plants (grown as described, see 3.1.2) were matched in replicates according to their ontogeny (by size and elongation state) and the second youngest source leaf received for one night either an empty fine netting bag or was engaged in a bag with selected unmated female *S. exigua* moths (conducted as described before for oviposition, see 3.4.1).

At the next day, bags were removed and after four days simulated herbivory was applied to the exposed leaf. The W+OS treatment was repeated two times with a time gab of 3 h between the treatments (conducted as described, see 3.4.2). After two days leaf material was then harvested into aluminium bags, flash-frozen in liquid nitrogen and stored at -80 ℃until analysis of secondary metabolites, i.e. CP.

Quantification of secondary metabolites

Extraction of secondary metabolites to quantify caffeoylputrescine (CP) was implemented according to Keinänen et al. (2001) with modifications. Harvested leaf material was grinded with mortar and pestle in liquid nitrogen and 100 mg powdered leaf material was filled in screw cap tubes (2 ml) containing matrix. Samples were additionally homogenized (two times 20 seconds at 4.5 m/sec) in a FastPrep homogenizer. Then 1000 µl extraction buffer (40 % methanol (ROTISOLV® GC Ultra Grade, Carl Roth GmbH + Co. KG, Karlsruhe Germany), 0.5 % acetic acid (Carl Roth GmbH + Co. KG, Karlsruhe Germany)) was added (if the amount of leaf material was lower, the amount of extraction buffer was added in a ratio of 1:10 according to the amount of leaf material) and the samples were shaken on the homogenizer (three times 20 seconds at 4.5 m/sec) followed by shaking for 15 min on a vortex. After centrifugation (24 ℃/ 10 min / 16.2 g) the supernatant was transfered to a new tube (2 ml). Following another centrifugation (24 ℃/ 10 min / 16.2 g) the supernatant was then transferred without any particles to 1.5 ml HPLC glass vials. All steps were performed at room temperature. To control for purity of solvents and technical malfunctions, a blank was included which did not contain leaf material but went trough all extraction steps handled equally to the samples. Before measurement, samples were stored overnight at 4 °C.

Samples were then analysed in a HPLC system (controller (CBM-20A), two degasser (DGU-20A3), two pumps (LC-20AD XR), auto sampler (SIL-20AC XR), rack changer (Rack Changer II, Nexera), column oven (CTO-20AC), UV/VIS detector (SPD-20A), diode array detector (SPD-M20A); detection range: 190-400 nm; Shimadzu Corporation, Japan) equipped with an Inertsil column (Inertsil ODS-3, 3 um, 4.3 x 150 mm, GL Science, Japan). As eluents served A: 94.875 % water (ROTISOLV® HPLC Gradient Grade, Carl Roth GmbH + Co. KG, Karlsruhe Germany), 0.25 % phosphoric acid (Carl Roth GmbH + Co. KG, Karlsruhe Germany) and 4.875 % acetonitrile (ROTISOLV® HPLC Gradient Grade, Carl Roth GmbH + Co. KG, Karlsruhe Germany) and B: 94.875 %

acetonitrile and 4.875 % water with 0.25 % phosphoric acid. Samples (injection volume 20 µl) were separated on the column for 30 min, starting with 100 % eluent A (for 2:30 min), followed by a phase with increasing concentration of eluent B (for 15:30 min (0-26 % eluent B) and finally 100 % eluent B (1:50 min 26-100 % eluent B, 3 min 100 % eluent B). Finally, concentration of eluent B was again decreased to 0 % (1.10 min 100-0 % eluent B, 6 min 0 % eluent B). Additionally to the samples, external standards were included in the measurement. Standards were derived in a serial dilution (Standard 1 (S1): 200 ng µl^{-} chlorogenic acid in HPLC extraction buffer, S2: 100 ng µl^{-}, S3: 50 ng µl^{-},S4: 25 ng µl^{-}, S5: 12.5 ng µl^{-}, S6: 6.25 ng µl^{-}, S7:3.125 ng µl^{-}).
Data analysis was conducted with the LabSolutions software (version 5.92, Shimadzu Corporation). Peaks of certain compounds were identified via their spectra and retention times. When the peaks occurred in several samples, separation was clear and they showed meaningful measurable amounts, peaks were included in further calculations. The concentration of CP within the samples was calculated by comparing the peak area to the peak area of the chlorogenic acid standard. Values were log2-transformed for calculation.

Supplementary material and methods: Tables

Table A1: Coordinates of *S. dulcamara* populations

Population	Coordinates
Henningsdorf	52°38’49.6"N 13°13’37.5"E
Langes Luch	52°27’39.1"N 13°15’06.5"E
Siethen	52°16’53.65"N 13°11’18.65"E

Table A2: Fertilizer declaration

Content	Nutrients	
16 %	N	total nitrogen, 5.0 % ammonium nitrogen, 11.0 % CDU nitrogen
8 %	P_2O_5	neutral ammon citrate and water soluble phosphate 6 % water soluble phosphate
12 %	K_2O	water soluble potassium oxide
4 %	MgO	total magnesium oxide 3 % water soluble magnesium oxide
9 %	S	total sulfur, 7.2 % water soluble sulfur
0.02 %	B	boron
0.04 %	Cu	copper
0.1 %	Fe	iron
0.1 %	Mn	manganese
0.02 %	Mo	molybdenum
0.01 %	Zn	zinc

Table A3: Ingredients of the bean flour-based artificial diet. Supplier either declared in brackets or specified by suspended numbers. [1] = Carl Roth GmbH + Co. KG, Karlsruhe Germany

Ingredient	Quantity
Water	1.5 l
Agar-agar[1]	35 g
4- Hydroxybenzoic acid methyl- ester[1]	4 g
Wesson salt mix (MP Biomedicals, Illkirch France)	1 g
L-(+)-Ascorbic acid[1]	6 g
Sorbic acid[1]	6 g
L-Leucine[1]	1 g
Brewer‘s Yeast[1]	64 g
Alfalfa flour pellet	23 g
Bean flour (Bauk GmbH, Rosche Germany)	213 g
Maize germ oil (Mazola ®)	213 g
Vitamin mix	
Water	20 ml
Nicotine acid[1]	20 mg
Riboflavin[1]	10 mg
Thiamin[1]	4.7 mg
Pyroxidine[1]	4.7 mg
Folic acid[1]	4.7 mg
Biotin[1]	0.4 mg

Table A4: Ingredients of the wheat germ-based artificial diet. Supplier either declared in brackets or specified by suspended numbers. [1] = Carl Roth GmbH + Co. KG, Karlsruhe Germany

Ingredient	**Quantity**
Water	1.2 l
Wheat germ (Herrnmühle Harald Feick OHG, Reichelsheim Germany)	240 g
Casein (MySupps GmbH, Ellerbek Germany)	50 g
Agar-agar[1]	35 g
Wesson salt mix (MP Biomedicals, Illkirch France)	16 g
Ascorbic acid[1]	8 g
Sorbic acid[1]	2 g
Methyl-p-hydroxybenzoate (Sigma-Aldrich Chemie GmbH, Steinheim Germany)	2 g
Vitamin mix	
Water	20 ml
Nicotine acid[1]	20 mg
Riboflavin[1]	10 mg
Thiamin[1]	4.7 mg
Pyroxidine[1]	4.7 mg
Folic acid[1]	4.7 mg
Biotin[1]	0.4 mg

Table A5: List of primer sequences.

Primer		Sequence 5´ -> 3´
ELF	forward	AAGGTAAGGGAGGTAAGAACAGGAAGAGA
	reverse	ACAACGACCATTACCAAGCATACGAA
CAC	forward	GGTAGTGTGCTCCGTTGCGATG
	reverse	GCGGGATTTAAGCTGCGACTCT
GAPDH	forward	ATTGGTGGCTCGGGTTGCTCTC
	reverse	ATGATGCTTCCACTGGCCGTGT
LOX	forward	TGTAGGCAGCAGCAGTGATCTC
	reverse	CTCGCCAGAGCTTACTCAATGC
CDH	forward	GCTGCTGTTTTTGTGGACATC
	reverse	CTGCTGACATCCTATCATCCC
HCT	forward	GGGTGAAACTGCCAGAGGTA
	reverse	CTCAGCGCCAAAACAGAAGG
OPR3	forward	CTGTGACGACTGCTTGAACCAC
	reverse	AGCTCACGGGTACTTGATCGAC
PG4	forward	AAAGACCAGCACCTTGACCA
	reverse	GCACTCTGTTAGCTCCCTCT
PR1	forward	GCTGGTGCCGTGAAGATGT
	reverse	ATAAAATACCACCCGTTGTTGC
*PI*1	forward	CCACTGATGGCAAGCGAATT
	reverse	TCTCCCCAACAAGTTCAGGC
PPO	forward	GCTTCGTCGAGGACTCAACA
	reverse	GAATAGGTCCCCGCGAACTC
PRX2	forward	CAACTGAACGCCACGAGATG
	reverse	CGGACCAAGTGTTGATGGGA

B.2.2 Summaries of the applied statistics

Table A6: Statistical comparisons to assess the accumulation of phytohormones in *S. dulcamara* leaves at different time points after oviposition by *S. exigua* (Exp. 1 & 2). At each time point (Exp. 1: 2 d, 4 d, 6 d, 8 d and 10 d (eggs were removed after 4 d); Exp. 2: 1 d and 3 d) level of salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA) and jasmonic acid-isoleucine (JA-Ile) of oviposited leaves (P) were compared with untreated control leaves (C) from individual plants. In case the data did not meet the requirements of the specified parametric tests, the data was transformed (TF) as listed. Significant *p*-values are highlighted in bold.

Comparison	***TF***	***Test***	***t***	***df***	***p***
SA					
C-P 2d (Exp. 1)	1/sqrt	Welch Two Sample t-test	3.7749	11.83	**0.0027**
C-P 4d (Exp. 1)	sqrt	Welch Two Sample t-test	-2.6842	12.182	**0.0197**
C-P 6d (Exp. 1)	sqrt	Welch Two Sample t-test	-0.8458	11.999	0.4142
C-P 8d (Exp. 1)	sqrt	Welch Two Sample t-test	-0.5701	12.577	0.5786
C-P 10d (Exp. 1)	sqrt	Welch Two Sample t-test	-1.4216	12.198	0.1802
C-P 1d (Exp. 2)	log	Two Sample t-test	-2.4703	18	**0.0237**
C-P 3d (Exp. 2)	log	Two Sample t-test	-1.5034	14	0.155
ABA					
C-P 2d (Exp. 1)	1/sqrt	Welch Two Sample t-test	-1.4089	2.163	0.1839
C-P 4d (Exp. 1)	sqrt	Welch Two Sample t-test	0.4933	10.89	0.6316
C-P 6d (Exp. 1)	sqrt	Welch Two Sample t-test	-1.6667	13.972	0.1178
C-P 8d (Exp. 1)	sqrt	Welch Two Sample t-test	-0.2444	12.385	0.8109
C-P 10d (Exp. 1)	sqrt	Welch Two Sample t-test	-3.0251	14	**0.0091**
C-P 1d (Exp. 2)	log	Two Sample t-test	-0.44446	17.879	0.662
C-P 3d (Exp. 2)		Two Sample t-test	-0.30597	14	0.7641
JA					
C-P 2d (Exp. 1)	log	Welch Two Sample t-test	-1.3051	11.685	0.217
C-P 6d (Exp. 1)	log	Welch Two Sample t-test	-3.4263	10.517	**0.0060**
C-P 8d (Exp. 1)	log	Welch Two Sample t-test	-3.5231	8.1246	**0.0076**
C-P 10d (Exp. 1)	log	Welch Two Sample t-test	-2.9231	11.721	**0.0130**
C-P 1d (Exp. 2)	1/sqrt	Two Sample t-test	2.1701	18	**0.0436**
C-P 3d (Exp. 2)	1/sqrt	Two Sample t-test	4.0693	9.6916	**0.0024**
JA-Ile					
C-P 2d (Exp. 1)	log	Two Sample t-test	-1.7771	13	0.0989
C-P 6d (Exp. 1)	log	Two Sample t-test	-3.3596	14	**0.0047**
C-P 8d (Exp. 1)	log	Two Sample t-test	-4.4948	14	**0.0005**
C-P 10d (Exp. 1)	log	Two Sample t-test	-4.4072	14	**0.0006**
C-P 1d (Exp. 2)	1/sqrt	Two Sample t-test	2.7511	18	**0.0131**
C-P 3d (Exp. 2)	1/sqrt	Two Sample t-test	2.762	14	**0.0153**

Table A7: Statistical comparisons to assess the transcriptional accumulation of several defence related genes in *S. dulcamara* leaves at different time points after oviposition by *S. exigua* (Exp. 1 & 2). At each time point (Exp. 1: 2 d, 4 d, 6 d, 8 d and 10 d (eggs were removed after 4 d); Exp. 2: 1 d and 3 d) transcript level of the specified genes in oviposited leaves (P) were compared with those in untreated control leaves (C) from individual plants. In case the data did not meet the requirements of the specified parametric tests, the data was transformed (TF) as listed. Significant p-values are highlighted in bold.

Comparison	***TF***	***Test***	***t***	***df***	***p***
PRX2 (peroxidase 2)					
C-P 2d (Exp. 1)	1/x	Welch Two Sample t-test	1.2526	8.298	0.2445
C-P 4d (Exp. 1)	1/x	Welch Two Sample t-test	6.6639	7.978	**0.0002**
C-P 6d (Exp. 1)	1/x	Welch Two Sample t-test	2.8080	9.130	**0.0202**
C-P 8d (Exp. 1)	1/x	Welch Two Sample t-test	5.2760	11.035	**0.0003**
C-P 10d (Exp. 1)	1/x	Welch Two Sample t-test	1.3102	8.535	0.2243
PR1 (pathogenesis-related gene 1)					
C-P 1d (Exp. 2)		Two Sample t-test	-1.1637	17	0.2606
C-P 3d (Exp. 2)		Two Sample t-test	-4.1981	12	**0.0012**
PG4 (polygalacturonase 4)					
C-P 2d (Exp. 1)	sqrt	Welch Two Sample t-test	-0.6580	13.250	0.5218
C-P 4d (Exp. 1)	sqrt	Welch Two Sample t-test	-7.7116	11.021	**<0.0001**
C-P 6d (Exp. 1)	sqrt	Welch Two Sample t-test	-2.5759	9.073	**0.0297**
C-P 8d (Exp. 1)	sqrt	Welch Two Sample t-test	-4.3779	13.993	**0.0006**
C-P 10d (Exp. 1)	sqrt	Welch Two Sample t-test	-1.5301	10.715	0.1550
C-P 1d (Exp. 2)		Two Sample t-test	2.2039	17	**0.0416**
C-P 3d (Exp. 2)		Two Sample t-test	-4.0573	12	**0.0016**
LOX (lipoxygenase)					
C-P 1d (Exp. 2)		Two Sample t-test	-4.9550	18	**0.0001**
C-P 3d (Exp. 2)		Two Sample t-test	-4.6094	12	**0.0006**
OPR3 (12-oxophytodienoate reductase 3)					
C-P 1d (Exp. 2)		Two Sample t-test	-0.1064	18	0.9164
C-P 3d (Exp. 2)		Two Sample t-test	-1.3561	12	0.2000
HCT (hydroxycinnamoyl-CoA shikimate/quinate-hydroxycinnamoyl transferase)					
C-P 2d (Exp. 1)		Two Sample t-test	-2.2997	14	**0.0374**
C-P 4d (Exp. 1)		Two Sample t-test	-11.5680	14	**<0.0001**
C-P 6d (Exp. 1)		Two Sample t-test	-3.9386	14	**0.0015**
C-P 8d (Exp. 1)		Two Sample t-test	-7.9055	14	**<0.0001**
C-P 10d (Exp. 1)		Two Sample t-test	-4.1317	14	**0.0010**
C-P 1d (Exp. 2)		Two Sample t-test	-1.8814	18	0.0762
C-P 3d (Exp. 2)		Two Sample t-test	-3.1909	12	**0.0078**
CDH (cytokinin oxidase/dehydrogenase)					
C-P 2d (Exp. 1)		Two Sample t-test	-1.9712	14	0.0688
C-P 4d (Exp. 1)	log	Two Sample t-test	-6.2366	4	**<0.0001**
C-P 6d (Exp. 1)	log	Two Sample t-test	-2.0780	14	0.0566
C-P 8d (Exp. 1)	log	Two Sample t-test	0.0771	14	0.9396
C-P 10d (Exp. 1)	log	Two Sample t-test	-2.5288	14	**0.0241**
C-P 1d (Exp. 2)		Two Sample t-test	-1.6382	18	0.1187
C-P 3d (Exp. 2)		Two Sample t-test	-2.8551	12	**0.0145**

Table A8: Statistical comparisons to assess the phytohormonal and transcriptional accumulation of several defence related genes in *S. dulcamara* leaves at different time points after oviposition by *A. autumnitella* (Exp. 3). At each time point (3 d and 6 d) level of salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA) and jasmonic acid-isoleucine (JA-Ile) respectively the transcriptional accumulation of the specified genes in oviposited leaves (P) were compared with those in untreated control leaves (C) from individual plants. In case the data did not meet the requirements of the specified parametric tests, the data was transformed (TF) as listed. Significant p-values are highlighted in bold.

Comparison	***TF***	***Test***	***t***	***df***	***p***
SA					
C-P 3d		Two Sample t-test	-4.0454	8	**0.0037**
C-P 6d		Two Sample t-test	-0.5029	10	0.6259
ABA					
C-P 3d		Two Sample t-test	-1.5970	8	0.1489
C-P 6d		Two Sample t-test	1.5429	10	0.1539
JA					
C-P 3d	1/sqrt	Two Sample t-test	1.2870	8	0.2341
C-P 6d	1/sqrt	Two Sample t-test	2.3032	10	**0.0440**
JA-Ile					
C-P 3d		Two Sample t-test	-0.8682	8	0.4106
C-P 6d		Two Sample t-test	0.6825	10	0.5104
HCT (hydroxycinnamoyl-CoA shikimate/quinate-hydroxycinnamoyl transferase)					
C-P 3d		Two Sample t-test	-2.7710	11	**0.0182**
C-P 6d		Two Sample t-test	-6.0278	10	**0.0001**
PR1 (pathogenesis-related gene 1)					
C-P 3d		Two Sample t-test	-2.9032	9	**0.0175**
C-P 6d		Two Sample t-test	-2.6402	11	**0.0230**
LOX (lipoxygenase)					
C-P 3d		Two Sample t-test	4.0466	14	**0.0012**
C-P 6d		Two Sample t-test	-1.6060	12	0.1343
PI1 (proteinase inhibitor 1)					
C-P 3d		Two Sample t-test	-4.2967	14	**0.0007**
C-P 6d		Two Sample t-test	-3.7281	12	**0.0029**
CDH (cytokinin oxidase/dehydrogenase)					
C-P 3d		Two Sample t-test	-4.3613	13	**0.0008**
C-P 6d		Two Sample t-test	-0.8680	12	0.4024
OPR3 (12-oxophytodienoate reductase 3)					
C-P 3d		Welch Two Sample t-test	-4.6162	11.315	**0.0007**
C-P 6d		Welch Two Sample t-test	-1.4896	7.1425	0.1791
PPO (polyphenol oxidase)					
C-P 3d		Two Sample t-test	-3.3214	14	**0.0050**
C-P 6d		Two Sample t-test	-3.5029	12	**0.0044**

Table A9: Statistical models assessing the effect of oviposition and larval feeding by *S. exigua* on phytohormonal and transcriptional accumulation in *S. dulcamara* leaves (Exp. 4). Summaries of linear mixed models (LMMs) testing the effect of priming (oviposition) and triggering (larval feeding) and their interaction on the accumulation of salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA) and jasmonic acid-isoleucine (JA-Ile) respectively the transcriptional accumulation of cytokinin oxidase/dehydrogenase (*CDH*), polygalacturonase 4 (*PG4*), hydroxycinnamoyl-CoA shikimate/quinate-hydroxycinnamoyl transferase (*HCT*), pathogenesis-related gene 1 (*PR1*) peroxidase 2 (*PRX2*) and lipoxygenase (LOX) within three different leaf positions. Leaf material of the oviposited leaf (leaf 0), the leaf exposed to larval feeding (leaf 5) and a young systemic leaf (leaf 8) was analysed (respectively corresponding leaf positions). LMMs included priming (oviposition), triggering (larval feeding) and their interaction as fixed factors and the replicate block as random factor (blocked experimental design). Significant *p*-values are highlighted in bold.

SA Leaf 0					
Random effect	*variance*	*SD*			
replicate	79.15	8.897			
residuals	876.38	29.604			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	81.133	10.929	7.424	**<0.0001**	
priming	-5.155	15.358	-0.336	0.7370	
triggering	24.686	16.069	1.536	0.1240	
priming:triggering	-42.445	22.21	-1.911	0.0560	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	47.6	17.5	26.0	2.719	0.0115
C-T	17.76	16.6	25.1	1.067	0.2959
C-PT	22.91	16.0	24.4	1.436	0.1636
P-T	-29.84	18.2	27.0	-1.639	0.1128
P-PT	-24.69	17.5	26.0	-1.410	0.1704
T-PT	5.16	16.6	25.1	0.310	0.7592
SA Leaf 8					
Random effect	*variance*	*SD*			
replicate	17879	133.71			
residuals	8269	90.93			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	180.05	56.34	3.195	**0.0014**	
priming	72.20	44.42	1.625	0.1041	
triggering	197.88	53.10	3.727	**0.0002**	
priming:triggering	-220.86	69.90	-3.159	**0.0016**	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	148.7	57.7	25.9	2.576	**0.016**
C-T	23	48.9	25.4	0.47	0.6424
C-PT	-49.2	47.8	25.5	-1.029	0.313
P-T	-125.7	57.7	25.9	-2.178	**0.0387**
P-PT	-197.9	57.4	26.1	-3.449	**0.0019**
T-PT	-72.2	47.8	25.5	-1.51	0.1433
SA Leaf 5					
Random effect	*variance*	*SD*			
replicate	634.8	25.20			
residuals	775.8	27.85			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	52.420	13.279	3.948	**0.0001**	
priming	3.947	13.927	0.283	0.7768	
triggering	65.056	15.295	4.253	**<0.0001**	
priming:triggering	-52.299	20.685	-2.528	**0.0115**	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	48.35	16.5	26.1	2.926	**0.0070**
C-T	-12.76	15	25.4	-0.852	0.4023
C-PT	-16.70	15	25.4	-1.115	0.2752
P-T	-61.11	16.5	26.1	-3.698	**0.0010**
P-PT	-65.06	16.5	26.1	-3.937	**0.0005**
T-PT	-3.95	15	25.4	-0.264	0.7943

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ABA Leaf 5					
Random effect	*variance*	*SD*			
replicate	3108	55.75			
residuals	1310	36.20			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	322.651	23.501	13.729	**<0.0001**	
priming	-29.693	18.099	-1.641	0.1009	
triggering	-47.806	19.953	-2.396	**0.0166**	
priming:triggering	-4.897	26.938	-0.182	0.8558	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	34.6	21.5	25.7	1.608	0.1200
C-T	2.7	19.5	25.4	2.707	**0.0120**
C-PT	82.4	19.5	25.4	4.232	**0.0003**
P-T	18.1	21.5	25.7	0.842	0.4075
P-PT	47.8	21.5	25.7	2.222	**0.0353**
T-PT	29.7	19.5	25.4	1.525	0.1396
JA Leaf 5					
Random effect	*variance*	*SD*			
replicate	759.2	27.55			
residuals	9787.6	98.93			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	401.45	36.31	11.057	**<0.0001**	
priming	-157.76	49.47	-3.189	**0.0014**	
triggering	-398.75	53.67	-7.430	**<0.0001**	
priming:triggering	155.71	72.99	2.133	**0.0329**	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	2.04	58.3	27.1	0.035	0.9723
C-T	243.03	53.2	25.4	4.572	**0.0001**
C-PT	400.79	53.2	25.4	7.540	**<0.0001**
P-T	240.99	58.3	27.1	4.135	**0.0003**
P-PT	398.75	58.3	27.1	6.842	**<0.0001**
T-PT	157.76	53.2	25.4	2.968	**0.0065**
JA-Ile Leaf 5					
Random effect	*variance*	*SD*			
replicate	0.548	0.740			
residuals	15.184	3.897			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	16.191	1.402	11.546	**<0.0001**	
priming	-6.172	1.948	-3.168	**0.0015**	
triggering	-14.962	2.109	-7.093	**<0.0001**	
priming:triggering	6.086	2.871	2.120	**0.0340**	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.086	2.29	27.2	0.037	0.9704
C-T	8.876	2.09	25.4	4.240	**0.0003**
C-PT	15.048	2.09	25.4	7.189	**<0.0001**
P-T	8.790	2.29	27.2	3.836	**0.0007**
P-PT	14.962	2.29	27.2	6.529	**<0.0001**
T-PT	6.172	2.09	25.4	2.949	**0.0068**
CDH Leaf 0					
Random effect	*variance*	*SD*			
replicate	2.090	1.446			
residuals	2.633	1.623			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	0.900	0.768	1.172	0.2412	
priming	-0.637	0.811	-0.785	0.4327	
triggering	1.661	0.954	1.742	0.0816	
priming:triggering	-2.250	1.282	-1.755	0.0793	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	2.887	1.084	22.9	2.664	0.0139
C-T	0.589	0.981	23.5	0.6	0.5540
C-PT	1.226	0.981	23.5	1.249	0.2239
P-T	-2.298	1.048	23.8	-2.192	0.0384
P-PT	-1.661	1.048	23.8	-1.585	0.1263
T-PT	0.637	0.882	22.5	0.721	0.4781

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CDH Leaf 5					
Random effect	*variance*	*SD*			
replicate	0.847	0.921			
residuals	0.282	0.531			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	2.871	0.384	7.468	**<0.0001**	
priming	-1.098	0.309	-3.556	0.0004	
triggering	-2.522	0.329	-7.657	**<0.0001**	
priming:triggering	1.019	0.467	2.185	0.0289	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.079	0.392	20.6	0.201	0.8423
C-T	1.503	0.352	20.0	4.271	0.0004
C-PT	2.602	0.343	20.0	7.595	**<0.0001**
P-T	1.424	0.377	20.2	3.774	0.0012
P-PT	2.522	0.367	20.2	6.867	**<0.0001**
T-PT	1.098	0.344	20.1	3.193	0.0045
PG4 Leaf 0					
Random effect	*variance*	*SD*			
replicate	2.49E-22	**<0.0001**			
residuals	2.97E-02	**<0.0001**			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	-0.127	0.061	-2.084	0.0372	
priming	-0.048	0.086	-0.562	0.5743	
triggering	0.050	0.093	0.540	0.5894	
priming:triggering	0.125	0.127	0.987	0.3239	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	-0.077	0.101	27.4	-0.759	0.4546
C-T	-0.175	0.093	25.4	-1.895	0.0696
C-PT	-0.127	0.093	25.4	-1.372	0.1821
P-T	-0.099	0.101	27.4	-0.975	0.3381
P-PT	-0.050	0.101	27.4	-0.497	0.6234
T-PT	0.048	0.093	25.4	0.523	0.6056
PG4 Leaf 5					
Random effect	*variance*	*SD*			
replicate	0	0			
residuals	1.102	1.05			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	0.254	0.397	0.639	0.5228	
priming	-0.369	0.584	-0.633	0.5270	
triggering	-1.525	0.584	-2.612	0.0090	
priming:triggering	1.641	0.842	1.950	0.0511	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	-1.272	0.681	24.8	-1.867	0.0738
C-T	-0.116	0.672	22.7	-0.173	0.8645
C-PT	0.253	0.652	23.5	0.389	0.7011
P-T	1.156	0.672	22.7	1.721	0.0989
P-PT	1.525	0.652	23.5	2.338	0.0282
T-PT	0.369	0.65	23.5	0.568	0.5755
HCT Leaf 0					
Random effect	*variance*	*SD*			
replicate	0.0078	0.0882			
residuals	0.1294	0.3597			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	-0.221	0.131	-1.688	0.0914	
priming	0.043	0.180	0.237	0.8124	
triggering	0.161	0.195	0.826	0.4090	
priming:triggering	0.017	0.265	0.065	0.9478	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	-0.060	0.212	27.1	-0.284	0.7789
C-T	-0.178	0.193	25.4	-0.923	0.3648
C-PT	-0.221	0.193	25.4	-1.144	0.2634
P-T	-0.118	0.212	27.1	-0.559	0.5810
P-PT	-0.161	0.212	27.1	-0.760	0.4537
T-PT	-0.043	0.193	25.4	-0.221	0.8269

Continues

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HCT Leaf 5					
Random effect	*variance*	*SD*			
replicate	0.112	0.335			
residuals	0.314	0.561			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	1.244	0.245	5.081	**<0.0001**	
priming	0.316	0.319	0.989	0.3230	
triggering	-1.726	0.319	-5.413	**<0.0001**	
priming:triggering	0.246	0.462	0.532	0.5950	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	-0.561	0.375	23.2	-1.497	0.1480
C-T	1.480	0.365	21.8	4.056	0.0005
C-PT	1.164	0.355	22.2	3.281	0.0034
P-T	2.041	0.365	21.8	5.595	**<0.0001**
P-PT	1.726	0.355	22.2	4.863	0.0001
T-PT	-0.316	0.356	22.4	-0.888	0.3842
PR1 Leaf 0					
Random effect	*variance*	*SD*			
replicate	0.035	0.187			
residuals	0.065	0.255			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	-0.164	0.112	-1.465	0.1429	
priming	0.030	0.128	0.231	0.8170	
triggering	0.268	0.140	1.916	0.0554	
priming:triggering	-0.134	0.189	-0.706	0.4803	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	-0.104	0.151	26.3	0.688	0.4974
C-T	-0.134	0.137	25.4	-0.979	0.3370
C-PT	-0.164	0.137	25.4	-1.194	0.0882
P-T	-0.238	0.151	26.3	-1.576	0.1270
P-PT	-0.268	0.151	26.3	-1.771	0.0882
T-PT	-0.030	0.137	25.4	-0.215	0.8313
PR1 Leaf 5					
Random effect	*variance*	*SD*			
replicate	0	0			
residuals	0.714	0.845			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	0.206	0.319	0.645	0.5190	
priming	0.027	0.470	0.058	0.9540	
triggering	-0.925	0.470	-1.969	0.0490	
priming:triggering	0.692	0.677	1.022	0.3070	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	-0.719	0.548	24.8	-1.312	0.2017
C-T	0.233	0.541	22.7	0.431	0.6703
C-PT	0.206	0.525	23.5	0.392	0.6985
P-T	0.952	0.541	22.7	1.762	0.0916
P-PT	0.925	0.525	23.5	1.762	0.0911
T-PT	-0.027	0.524	23.5	-0.052	0.9589
PRX2 Leaf 0					
replicate	0.203	0.450			
residuals	0.084	0.290			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	-0.288	0.194	-1.48	0.1390	
priming	-0.011	0.152	-0.08	0.9400	
triggering	0.013	0.167	0.08	0.9360	
priming:triggering	0.286	0.221	1.29	0.1970	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	-0.274	0.173	24.7	-1.585	0.1258
C-T	-0.299	0.157	24.5	-1.910	0.0679
C-PT	-0.288	0.164	24.6	-1.756	0.0916
P-T	-0.025	0.173	24.7	-0.144	0.8868
P-PT	-0.013	0.181	24.9	-0.074	0.9415
T-PT	0.012	0.164	24.6	0.070	0.9448

Continues

Continued

LOX Leaf 5					
Random effect	*variance*	*SD*			
replicate	0.004	0.065			
residuals	0.563	0.750			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	3.608	0.285	12.679	**<0.0001**	
priming	-0.396	0.418	-0.948	0.3430	
triggering	-3.299	0.418	-7.897	**<0.0001**	
priming:triggering	0.085	0.602	0.141	0.8880	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.311	0.488	24.8	0.637	0.5298
C-T	3.214	0.480	22.6	6.689	**<0.0001**
C-PT	3.610	0.467	23.4	7.735	**<0.0001**
P-T	2.903	0.480	22.6	6.042	**<0.0001**
P-PT	3.299	0.467	23.4	7.069	**<0.0001**
T-PT	0.396	0.465	23.5	0.851	0.4036

Table A10: Statistical models assessing the effect of oviposition and larval feeding by *S. exigua* on phytohormonal accumulation in *S. dulcamara* leaves local to larval feeding respectively corresponding leaf positions (Leaf 5; Exp. 5). Summaries of linear mixed models (LMMs) testing the effect of priming (oviposition) and triggering (larval feeding) on the accumulation of salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA) and jasmonic acid-isoleucine (JA-Ile). LMMs included priming (oviposition), triggering (larval feeding) and their interaction as fixed factors and the replicate block as random factor (blocked experimental design). Significant *p*-values are highlighted in bold.

SA Leaf 5				
Random effect	*variance*	*SD*		
replicate	85.35	9.238		
residuals	432.2	20.789		
Fixed effect	*estimate*	*SE*	*Z*	*p*
intercept	36.577	5.518	6.629	**<0.0001**
priming	-1.522	7.255	-0.210	0.8340
triggering	11.057	7.131	1.551	0.1210
priming:triggering	-4.759	10.474	-0.454	0.6500
ABA Leaf 5				
Random effect	*variance*	*SD*		
replicate	2843	53.32		
residuals	10069	100.34		
Fixed effect	*estimate*	*SE*	*Z*	*p*
intercept	364.393	27.559	13.222	**<0.0001**
priming	40.078	35.032	1.144	0.2526
triggering	-112.767	34.417	-3.276	**0.0011**
priming:triggering	7.484	50.606	0.148	0.8824
JA Leaf 5				
Random effect	*variance*	*SD*		
replicate	28572	169		
residuals	416895	645.7		
Fixed effect	*estimate*	*SE*	*Z*	*p*
intercept	1063.7	161.9	6.571	**<0.0001**
priming	375.9	225.1	1.670	0.0950
triggering	-1063.1	221.5	-4.800	**<0.0001**
priming:triggering	-380.2	324.5	-1.172	0.2410
JA-Ile Leaf 5				
Random effect	*variance*	*SD*		
replicate	18.77	4.332		
residuals	489.9	22.134		
Fixed effect	*estimate*	*SE*	*Z*	*p*
intercept	34.194	5.470	6.251	**<0.0001**
priming	12.610	7.714	1.635	0.1020
triggering	-33.492	7.592	-4.412	**<0.0001**
priming:triggering	-12.557	11.115	-1.130	0.2590

Table A11: Statistical models assessing the effect of oviposition and larval feeding by *S. exigua* on phytohormonal and transcriptional accumulation in *S. dulcamara* leaves (Exp. 6). Summaries of linear mixed models (LMMs) testing the effect of priming (oviposition) and triggering (larval feeding) on the accumulation of salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA) and jasmonic acid-isoleucine (JA-Ile) respectively the transcriptional accumulation of cytokinin oxidase/dehydrogenase (*CDH*), hydroxycinnamoyl-CoA shikimate/quinate-hydroxycinnamoyl transferase (*HCT*), and lipoxygenase (*LOX*), proteinase inhibitor 1 (*PI*1), polyphenol oxidase (*PPO*) and 12-oxophytodienoate reductase 3 (*OPR3*) within three different leaf positions. Leaf material of the oviposited leaf (leaf 0) and the leaf exposed to larval feeding (leaf 5) was analysed (respectively corresponding leaf positions). LMMs included priming (oviposition), triggering (larval feeding) and their interaction as fixed factors and the replicate block as random factor (blocked experimental design). Significant *p*-values are highlighted in bold.

SA Leaf 0					
Random effect	*variance*	*SD*			
replicate	105.7	10.28			
residuals	175.6	13.25			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	23.64	6.34	3.729	**0.0002**	
priming	-3.737	7.083	-0.528	0.5977	
triggering	6.255	7.083	0.883	0.3772	
priming:triggering	5.886	10.017	0.588	0.5568	
ABA Leaf 0					
Random effect	*variance*	*SD*			
replicate	10779	103.82			
residuals	3015	54.91			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	177.601	44.391	4.001	**0.0001**	
priming	-12.554	29.348	-0.428	0.6690	
triggering	8.315	29.348	0.283	0.7770	
priming:triggering	-24.995	41.504	-0.602	0.5470	
JA Leaf 0					
Random effect	*variance*	*SD*			
replicate	0.222	0.471			
residuals	10.657	3.265			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	3.975	1.247	3.189	**0.0014**	
priming	-2.551	1.745	-1.462	0.1437	
triggering	-1.468	1.745	-0.841	0.4003	
priming:triggering	1.191	2.468	0.483	0.6294	
JA-Ile Leaf 0					
Random effect	*variance*	*SD*			
replicate	0.014	0.120			
residuals	0.123	0.350			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	1.162	0.140	8.305	**<0.0001**	
priming	-0.280	0.187	-1.494	0.1350	
triggering	-0.035	0.187	-0.189	0.8500	
priming:triggering	0.052	0.265	0.195	0.8450	
SA Leaf 5					
Random effect	*variance*	*SD*			
replicate	2242	47.35			
residuals	3419	58.48			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	83.48	28.44	2.935	**0.0033**	
priming	-22.49	31.26	-0.720	0.4718	
triggering	54.63	31.26	1.748	0.0805	
priming:triggering	-33.46	44.2	-0.757	0.4491	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.398	0.303	24.5	1.314	0.2011
C-T	-0.484	0.303	24.5	-1.598	0.1228
C-PT	-1.015	0.303	24.5	-3.353	**0.0026**
P-T	-0.086	0.303	24.5	-0.285	0.7783
P-PT	-0.617	0.303	24.5	-2.039	0.0524
T-PT	0.531	0.303	24.5	1.754	0.0919

Continues

Continued

ABA Leaf 5					
Random effect	*variance*	*SD*			
replicate	12452	111.59			
residuals	8490	92.14			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	285.6	54.7	5.222	**<0.0001**	
priming	-57.44	49.25	-1.166	0.2440	
triggering	199.85	49.25	4.058	**<0.0001**	
priming:triggering	-46.12	69.65	-0.662	0.5080	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	57.4	53.2	24.5	1.080	0.2908
C-T	-153.7	53.2	24.5	-2.890	**0.0079**
C-PT	-257.3	53.2	24.5	-4.837	**0.0001**
P-T	-96.3	53.2	24.5	-1.810	0.0826
P-PT	-199.9	53.2	24.5	-3.757	**0.0009**
T-PT	103.6	53.2	24.5	1.947	0.0631
JA Leaf 5					
Random effect	*variance*	*SD*			
replicate	19831	140.8			
residuals	82493	287.2			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	1.036	120.904	0.009	0.993	
priming	0.268	153.524	0.002	0.999	
triggering	1614.140	153.524	10.514	**<0.0001**	
priming:triggering	-335.241	217.115	-1.544	0.123	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	-0.268	166	24.5	-0.002	0.9987
C-T	-1278.899	166	24.5	-7.712	**<0.0001**
C-PT	-1613.872	166	24.5	-7.712	**<0.0001**
P-T	-1279.166	166	24.5	-7.714	**<0.0001**
P-PT	-1614.140	166	24.5	-7.714	**<0.0001**
T-PT	334.974	166	24.5	2.020	0.0544
JA-Ile Leaf 5					
Random effect	*variance*	*SD*			
replicate	44.62	6.68			
residuals	158.84	12.6			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	0.864	5.391	0.16	0.873	
priming	-0.073	6.737	-0.011	0.991	
triggering	66.563	6.737	9.881	**<0.0001**	
priming:triggering	-4.867	9.527	-0.511	0.609	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.073	7.28	24.5	0.01	0.9921
C-T	-61.697	7.28	24.5	-8.479	**<0.0001**
C-PT	-66.636	7.28	24.5	-9.158	**<0.0001**
P-T	-61.623	7.28	24.5	-8.469	**<0.0001**
P-PT	-66.561	7.28	24.5	-9.148	**<0.0001**
T-PT	4.940	7.28	24.5	0.679	0.5036
HCT leaf 0					
Random effect	*variance*	*SD*			
replicate	0	0			
residuals	6.121	2.474			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	2.321	0.825	2.814	**0.0049**	
priming	-2.321	1.202	-1.930	**0.0536**	
triggering	0.918	1.202	0.764	0.4451	
priming:triggering	-1.048	1.725	-0.608	0.5434	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	2.321	1.290	28.300	1.802	0.0823
C-T	0.130	1.330	29.200	0.098	0.9227
C-PT	-3.239	1.330	29.200	-2.432	**0.0214**
P-T	2.451	1.290	28.300	1.903	0.0673
P-PT	-0.918	1.290	28.300	-0.713	0.4819
T-PT	3.369	1.330	29.200	2.530	**0.0171**

Continues

Continued

HCT leaf 5					
Random effect	*variance*	*SD*			
replicate	0	0			
residuals	0.6093	0.7806			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	0.555	0.319	1.741	0.082	
priming	-0.555	0.434	-1.278	0.201	
triggering	-0.178	0.414	-0.432	0.666	
priming:triggering	0.791	0.577	1.372	0.170	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.555	0.470	25.700	1.181	0.2484
C-T	-0.613	0.439	27.500	-1.395	0.1742
C-PT	-0.377	0.426	26.600	-0.885	0.3839
P-T	-0.058	0.462	28.700	-0.126	0.9006
P-PT	0.178	0.448	27.500	0.397	0.6948
T-PT	-0.236	0.410	25.500	-0.576	0.5695
CDH leaf 0					
Random effect	*variance*	*SD*			
replicate	2.254	1.501			
residuals	1.404	1.185			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	0.608	0.638	0.954	0.34	
priming	-0.608	0.559	-1.089	0.276	
triggering	0.336	0.559	0.601	0.548	
priming:triggering	0.157	0.790	0.198	0.843	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.608	0.592	30.4	1.026	0.3128
C-T	-0.493	0.592	30.4	-0.831	0.4122
C-PT	-0.944	0.592	30.4	-1.593	0.1214
P-T	0.116	0.592	30.4	0.195	0.8466
P-PT	-0.336	0.592	30.4	-0.567	0.5750
T-PT	0.451	0.592	30.4	0.762	0.4520
CDH leaf 5					
Random effect	*variance*	*SD*			
replicate	0.823	0.907			
residuals	1.189	1.091			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	0.592	0.494	1.198	0.2308	
priming	-0.592	0.533	-1.109	0.2673	
triggering	-1.272	0.533	-2.384	0.0171	
priming:triggering	0.781	0.741	1.054	0.2918	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.592	0.568	29.7	1.042	0.3060
C-T	0.491	0.546	29.4	0.898	0.3763
C-PT	0.68	0.546	29.4	1.245	0.2231
P-T	1.082	0.568	29.7	1.906	0.0664
P-PT	1.272	0.568	29.7	2.239	**0.0328**
T-PT	-0.189	0.546	29.4	-0.346	0.7316
LOX leaf 0					
Random effect	*variance*	*SD*			
replicate	0.177	0.421			
residuals	0.327	0.572			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	0.082	0.237	0.349	0.7272	
priming	-0.083	0.269	-0.306	0.7593	
triggering	0.681	0.269	2.527	**0.0115**	
priming:triggering	-1.009	0.381	-2.649	**0.0081**	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.083	0.286	30.4	0.289	0.7747
C-T	0.329	0.286	30.4	1.150	0.2590
C-PT	-0.763	0.286	30.4	-2.671	**0.0120**
P-T	0.411	0.286	30.4	1.439	0.1604
P-PT	-0.681	0.286	30.4	-2.382	**0.0237**
T-PT	1.092	0.286	30.4	3.821	**0.0006**

Continues

Continued

LOX leaf 5					
Random effect	*variance*	*SD*			
replicate	0.066	0.257			
residuals	0.219	0.468			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	-0.054	0.188	-0.288	0.7730	
priming	0.054	0.228	0.237	0.8130	
triggering	4.338	0.228	19.01	**<0.0001**	
priming:triggering	-0.142	0.317	-0.447	0.6550	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	-0.054	0.243	29.9	-0.222	0.8258
C-T	-4.196	0.243	29.9	-17.916	**<0.0001**
C-PT	-4.284	0.243	29.9	-18.291	**<0.0001**
P-T	-4.250	0.243	29.9	-17.477	**<0.0001**
P-PT	-4.338	0.243	29.9	-17.838	**<0.0001**
T-PT	0.088	0.243	29.9	0.375	0.7105
OPR3 leaf 0					
Random effect	*variance*	*SD*			
replicate	0.097	0.312			
residuals	0.317	0.563			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	0.489	0.215	2.279	**0.0227**	
priming	-0.489	0.266	-1.841	0.0656	
triggering	0.752	0.266	2.832	**0.0046**	
priming:triggering	-0.708	0.375	-1.887	0.0592	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.489	0.282	30.4	1.736	0.0927
C-T	-0.044	0.282	30.4	-0.155	0.8779
C-PT	-1.241	0.282	30.4	-4.406	**0.0001**
P-T	0.445	0.282	30.4	1.581	0.1242
P-PT	-0.752	0.282	30.4	-2.670	**0.0121**
T-PT	1.197	0.282	30.4	4.251	**0.0002**
OPR3 leaf 5					
Random effect	*variance*	*SD*			
replicate	0.005	0.071			
residuals	0.241	0.491			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	-0.054	0.175	-0.310	0.757	
priming	0.054	0.239	0.228	0.820	
triggering	3.356	0.239	14.054	**<0.0001**	
priming:triggering	-0.215	0.333	-0.646	0.518	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	-0.054	0.255	30.2	-0.213	0.8324
C-T	-3.141	0.246	29.4	-12.767	**<0.0001**
C-PT	-3.301	0.246	29.4	-13.42	**<0.0001**
P-T	-3.195	0.255	30.2	-12.545	**<0.0001**
P-PT	-3.356	0.255	30.2	-13.176	**<0.0001**
T-PT	0.161	0.246	29.4	0.653	0.5189
PI1 leaf 0					
Random effect	*variance*	*SD*			
replicate	13.798	3.715			
residuals	3.197	1.788			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	1.626	1.374	1.183	0.2368	
priming	-2.710	0.877	-3.091	**0.0020**	
triggering	1.567	0.843	1.859	0.0630	
priming:triggering	-0.801	1.216	-0.659	0.5100	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	2.710	0.933	29.5	2.906	**0.0069**
C-T	-0.766	0.933	29.5	-0.821	0.4181
C-PT	-4.277	0.933	29.5	-4.587	**0.0001**
P-T	1.944	0.896	29.4	2.196	**0.0383**
P-PT	-1.567	0.896	29.4	-1.749	0.0908
T-PT	3.511	0.896	29.4	3.918	**0.0005**

Continues

Continued

PI1 leaf 5					
Random effect	*variance*	*SD*			
replicate	24.770	4.977			
residuals	1.047	1.023			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	-2.312	1.707	-1.354	0.176	
priming	0.603	0.535	1.126	0.260	
triggering	4.960	0.528	9.388	**<0.0001**	
priming:triggering	-0.512	0.721	-0.710	0.478	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	-0.603	0.572	27.4	-1.053	0.3014
C-T	-4.448	0.539	27.5	-8.250	**<0.0001**
C-PT	-4.357	0.539	27.5	-8.080	**<0.0001**
P-T	-5.051	0.565	27.5	-8940.000	**<0.0001**
P-PT	-4.960	0.565	27.5	-8.779	**<0.0001**
T-PT	-0.091	0.516	27.4	-0.177	0.8608
PPO leaf 0					
Random effect	*variance*	*SD*			
replicate	0.168	0.410			
residuals	0.648	0.805			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	0.778	0.301	2.584	**0.0098**	
priming	-0.778	0.380	-2.050	**0.0403**	
triggering	0.200	0.380	0.527	0.5983	
priming:triggering	-0.123	0.537	-0.228	0.8194	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.778	0.403	30.4	1.933	0.0626
C-T	-0.077	0.403	30.4	-0.192	0.8487
C-PT	-0.978	0.403	30.4	-2.430	**0.0212**
P-T	0.701	0.403	30.4	1.741	0.0918
P-PT	-0.200	0.403	30.4	-0.497	0.6229
T-PT	0.901	0.403	30.4	2.238	**0.0327**
PPO leaf 5					
Random effect	*variance*	*SD*			
replicate	0.668	0.817			
residuals	0.223	0.472			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	0.123	0.321	0.382	0.702	
priming	-0.123	0.231	-0.530	0.596	
triggering	2.128	0.231	9.197	**<0.0001**	
priming:triggering	0.190	0.321	0.592	0.554	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.123	0.246	29.5	0.499	0.6218
C-T	-2.318	0.237	29.4	-9.789	**<0.0001**
C-PT	-2.250	0.237	29.4	-9.798	**<0.0001**
P-T	-2.195	0.246	29.5	-8.918	**<0.0001**
P-PT	-2.128	0.246	29.5	-8.918	**<0.0001**
T-PT	-0.067	0.237	29.4	-0.284	0.7782

Table A12: Statistical comparisons to assess the effect of oviposition on larval survival and to assess plant fitness of *N. attenuata* in response to *S. exigua* (Exp. 7). Plants were either kept untreated (C), exposed to larval feeding only (T) or a combination of oviposition followed by larval feeding (PT). Certain parameters (larval survival, number of open flowers per plant and stalk length) were considered at different time points, while differences were only compared within the corresponding measurement time point. Significant *p*-values are highlighted in bold.

Comparison	*t*	*df*	*p*
Larval survival (Two Sample t-test)			
P-PT 2d	0.0000	6	1.0000
P-PT 4d	2.1828	6	0.0718
P-PT 6d	1.9932	6	0.0933
P-PT 8d	0.8321	6	0.4372
Duration of flowering (Two Sample t-test)			
C-T	4.5826	6	**0.0038**
C-PT	3.6742	6	**0.0104**
T-PT	-0.7746	6	0.4680
Cumulative number of flowers per plant (Two Sample t-test)			
C-T	4.6568	6	**0.0035**
C-PT	4.7929	6	**0.0030**
T-PT	-0.4533	6	0.6663
Number of capsules per plant (Two Sample t-test)			
C-T	4.9591	6	**0.0026**
C-PT	2.3935	6	0.0538
T-PT	-1.9596	6	0.0978
Seed weight per plant (Two Sample t-test)			
C-T	4.8081	6	**0.0030**
C-PT	2.7261	6	**0.0344**
T-PT	-1.6217	6	0.1560
Number of open flowers per plant (Welch Two Sample t-test)			
C-T 45d	0.5108	5.6177	0.6290
C-PT 45d	0.4611	5.9700	0.6611
T-PT 45d	-0.1309	5.4245	0.9005
C-T 46d	1.5962	5.6830	0.1643
C-PT 46d	1.9980	3.1949	0.1340
T-PT 46d	0.0000	3.3149	1.0000
C-T 47d	2.2478	3.8887	0.0898
C-PT 47d	2.0494	3.9730	0.1102
T-PT 47d	-0.2182	5.9864	0.8345
C-T 48d	2.1997	5.8479	0.0713
C-PT 48d	2.0430	4.5473	0.1021
T-PT 48d	-0.5774	5.0103	0.5887
C-T 49d	4.1457	4.6362	**0.0105**
C-PT 49d	2.4198	5.9448	0.0523
T-PT 49d	-1.3720	4.9122	0.2294
C-T 50d	3.8105	4.1028	**0.0181**
C-PT 50d	9.6667	5.8011	**0.0001**
T-PT 50d	1.2439	3.7725	0.2852
C-T 51d	7.6667	3.7385	**0.0020**
C-PT 51d	5.0932	5.9793	**0.0023**
T-PT 51d	-0.6325	3.6585	0.5644
Comparison	*t*	*df*	*p*
Stalk length (Two Sample t-test)			
C-T 36d	0.3470	6	0.7405
C-PT 36d	0.3583	6	0.7324
T-PT 36d	-0.0867	6	0.9337
C-T 38d	0.1763	6	0.8658
C-PT 38d	0.2966	6	0.7768
T-PT 38d	0.1273	6	0.9028
C-T 40d	0.2044	6	0.8448
C-PT 40d	0.3874	6	0.7118
T-PT 40d	0.2673	6	0.7982
C-T 42d	0.2159	6	0.8362
C-PT 42d	0.4249	6	0.6858
T-PT 42d	0.2762	6	0.7916
C-T 44d	0.0410	6	0.9686
C-PT 44d	0.0822	6	0.9371
T-PT 44d	0.0611	6	0.9532
C-T 46d	0.3821	6	0.7156
C-PT 46d	0.4220	6	0.6877
T-PT 46d	0.0995	6	0.9240

Table A13: Statistical comparisons (Two Sample t-test) to assess the effect of oviposition on larval survival and larval weight of *S. exigua* (Exp. 8). Larvae feed on plants either kept exposed to larval feeding only (T) or a combination of oviposition followed by larval feeding (PT). Differences were only compared within the corresponding measurement time point.In case the data did not meet the requirements of the specified parametric tests, the data was transformed (TF) as listed.

Comparison	*TF*	*t*	*df*	*p*
Larval survival				
T-PT 2d		0	30	1
T-PT 4d		-0.5647	30	0.5765
T-PT 6d		-0.8537	30	0.4
T-PT 8d		-0.5308	30	0.5995
T-PT 10d		-0.3854	30	0.7027
T-PT 12d		-0.7265	30	0.4732
Mean larval weight				
T-PT 8d	log	1.7062	30	0.0983
T-PT 12d	log	1.5647	30	0.1281

Table A14: Statistical models assessing the effect of oviposition and larval feeding by *S. exigua* on different growth and fitness parameter of *N. attenuata* (Exp. 8). Summaries of two-way ANOVA testing the effect of priming (oviposition), triggering (larval feeding) and their interaction on the specified growth and fitness parameter. Certain parameters (number of open flowers per plant and stalk length) were considered at different time points, while differences were only compared within the corresponding measurement time point. Significant p-values are highlighted in bold.

Effect	*df*	*sum sq*	*mean sq*	*F*	*p*
Number of individual flowers					
priming	1	47	47.3	0.645	0.4250
triggering	1	2903	2902.5	39.587	**<0.0001**
priming:triggering	1	1	0.8	0.010	0.9190
residuals	60	4399	73.3		
Duration of flowering					
priming	1	0.02	0.016	0.005	0.9457
triggering	1	23.77	23.766	7.114	**0.0098**
priming:triggering	1	1.27	1.266	0.379	0.5405
residuals	60	200.44	3.341		
Number of capsules per plant					
priming	1	42	42	1.362	0.2480
triggering	1	4225	4225	136.208	**<0.0001**
priming:triggering	1	0	0	0.002	0.9640
residuals	60	1861	31		
Seed weight per plant					
priming	1	6153	6153	0.680	0.4130
triggering	1	2852599	2852599	315.288	**<0.0001**
priming:triggering	1	17289	17289	1.911	0.1720
residuals	60	542856	9048		
Number of open flowers 40d					
priming	1	0.39	0.391	0.096	0.7580
triggering	1	0.02	0.016	0.004	0.9510
priming:triggering	1	0.77	0.766	0.189	0.6660
residuals	60	243.69	4.061		
Number of open flowers 41d					
priming	1	0.4	0.391	0.057	0.8120
triggering	1	0.8	0.766	0.112	0.7390
priming:triggering	1	5.6	5.641	0.827	0.3670
residuals	60	409.2	6.82		
Number of open flowers 42d					
priming	1	1	1	0.293	0.5910
triggering	1	4	4	1.171	0.2840
priming:triggering	1	4	4	1.171	0.2840
residuals	60	205	3.417		

Continues

Continued

Effect	*df*	*sum sq*	*mean sq*	*F*	*p*
Number of open flowers 43d					
priming	1	2.6	2.641	0.480	0.4910
triggering	1	0	0.016	0.003	0.9580
priming:triggering	1	2.6	2.641	0.480	0.4910
residuals	60	329.9	5.499		
Number of open flowers 45d					
priming	1	0	0	0.000	1.0000
triggering	1	189.1	189.06	9.562	**0.0030**
priming:triggering	1	0.6	0.56	0.028	0.8666
residuals	60	1186.4	19.77		
Number of open flowers 46d					
priming	1	5.1	5.063	0.849	0.3610
triggering	1	0.1	0.062	0.010	0.9190
priming:triggering	1	0	0	0.000	1.0000
residuals	60	357.9	5.965		
Number of open flowers 47d					
priming	1	3.1	3.06	0.294	0.5896
triggering	1	169	169	16.227	**0.0002**
priming:triggering	1	0.1	0.06	0.006	0.9385
residuals	60	624.9	10.41		
Number of open flowers 48d					
priming	1	0	0.02	0.002	0.9680
triggering	1	178.9	178.89	18.026	**0.0001**
priming:triggering	1	0.8	0.77	0.077	0.7820
residuals	60	595.4	9.92		
Number of open flowers 49d					
priming	1	3.5	3.5	0.242	0.6250
triggering	1	356.3	356.3	24.522	**<0.0001**
priming:triggering	1	0.1	0.1	0.010	0.9220
residuals	60	871.7	14.5		
Number of open flowers 50d					
priming	1	9	9	1.084	0.3020
triggering	1	225	225	27.088	**<0.0001**
priming:triggering	1	0.6	0.56	0.068	0.7960
residuals	60	498.4	8.31		
Number of open flowers 51d					
priming	1	0	0.02	0.002	0.9660
triggering	1	185.6	185.64	22.021	**<0.0001**
priming:triggering	1	17	17.02	2.018	0.1610
residuals	60	505.8	8.43		
Number of open flowers 52d					
priming	1	0.1	0.06	0.009	0.9260
triggering	1	138.1	138.06	19.148	**<0.0001**
priming:triggering	1	0.3	0.25	0.035	0.8530
residuals	60	432.6	7.21		
Number of open flowers 54d					
priming	1	0.56	0.562	0.167	0.6839
triggering	1	10.56	10.563	3.143	0.0813
priming:triggering	1	6.25	6.25	1.860	0.1777
residuals	60	201.62	3.36		
Number of open flowers 55d					
priming	1	5.06	5.062	3.839	0.0547
triggering	1	4	4	3.033	0.0867
priming:triggering	1	1.56	1.562	1.185	0.2807
residuals	60	79.13	1.319		
Stalk length 31d					
priming	1	0.69	0.6875	0.580	0.4510
triggering	1	0.05	0.0511	0.043	0.8370
priming:triggering	1	0.05	0.0511	0.043	0.8370
residuals	40	47.41	1.1852		
Stalk length 33d					
priming	1	0.2	0.25	0.041	0.8390
triggering	1	1.6	1.563	0.259	0.6130
priming:triggering	1	5.6	5.641	0.934	0.3380
residuals	60	362.3	6.038		

Continues

Continued

Effect	*df*	*sum sq*	*mean sq*	*F*	*p*
Stalk length 35d					
priming	1	4	4	0.337	0.5640
triggering	1	0.1	0.141	0.012	0.9140
priming:triggering	1	13.1	13.141	1.106	0.2970
residuals	60	713	11.883		
Stalk length 37d					
priming	1	0.5	0.473	0.032	0.8590
triggering	1	1.1	1.129	0.076	0.7840
priming:triggering	1	18.6	18.598	1.250	0.2680
residuals	60	892.7	14.878		
Stalk length 39d					
priming	1	9.8	9.77	0.784	0.3795
triggering	1	70.1	70.14	5.630	0.0209
priming:triggering	1	23.8	23.77	1.908	0.1723
residuals	60	747.4	12.46		
Stalk length 41d					
priming	1	6.2	6.25	0.814	0.3706
triggering	1	105.1	105.06	13.678	0.0005
priming:triggering	1	20.2	20.25	2.636	0.1097
residuals	60	460.9	7.68		

Table A15: Statistical models assessing the effect of oviposition and larval feeding by *M. sexta* on different growth and fitness parameter of *N. attenuata* (Exp. 9). Summaries of two-way ANOVA testing the effect of priming (oviposition), triggering (larval feeding) and their interaction on the specified growth and fitness parameter. Certain parameters (number of open flowers per plant and stalk length) were considered at different time points, while differences were only compared within the corresponding measurement time point. Significant p-values are highlighted in bold.

Effect	*df*	*sum sq*	*mean sq*	*F*	*p*
Number of flowers					
priming	1	0.5	0.48	0.061	0.8060
triggering	1	220.2	220.17	28.050	**<0.0001**
priming:triggering	1	12	12.02	1.531	0.2220
residuals	48	376.8	7.85		
Duration of flowering					
priming	1	0.08	0.077	0.055	0.8150
triggering	1	30.77	30.769	22.069	**<0.0001**
priming:triggering	1	1.92	1.923	1.379	0.2460
residuals	48	66.92	1.394		
Number of capsules					
priming	1	0.48	0.48	0.103	0.7500
triggering	1	96.94	96.94	20.674	**<0.0001**
priming:triggering	1	0.94	0.94	0.201	0.6560
residuals	48	225.08	4.69		
Seed weight per plant					
priming	1	3.68	3.68	0.865	0.3569
triggering	1	32.26	32.26	7.593	**0.0083**
priming:triggering	1	3.07	3.07	0.723	0.3992
residuals	48	203.93	4.25		
Number of open flowers 40d					
priming	1	0.48	0.4808	0.289	0.5880
triggering	1	0.48	0.4808	0.289	0.5880
priming:triggering	1	0.17	0.1731	0.107	0.7450
residuals	48	77.54	1.6154		
Number of open flowers 41d					
priming	1	0.31	0.308	0.059	0.8090
triggering	1	2.77	2.769	0.534	0.4680
priming:triggering	1	1.92	1.923	0.371	0.5450
residuals	48	248.77	5.183		

Continues

Continued

Effect	*df*	*sum sq*	*mean sq*	*F*	*p*
Number of open flowers 42d					
priming	1	0.48	0.481	0.079	0.7800
triggering	1	0.02	0.019	0.003	0.9550
priming:triggering	1	0.02	0.019	0.003	0.9550
residuals	48	291.23	6.067		
Number of open flowers 43d					
priming	1	0.08	0.077	0.015	0.9030
triggering	1	1.23	1.231	0.241	0.6250
priming:triggering	1	0	0	0.000	1.0000
residuals	48	244.77	5.099		
Number of open flowers 44d					
priming	1	15.08	15.077	2.616	0.1120
triggering	1	0.69	0.692	0.120	0.7300
priming:triggering	1	4.92	4.923	0.854	0.3600
residuals	48	276.62	5.763		
Number of open flowers 45d					
priming	1	0.31	0.308	0.075	0.7850
triggering	1	4.92	4.923	1.199	0.2790
priming:triggering	1	2.77	2.769	0.674	0.4160
residuals	48	197.08	4.106		
Number of open flowers 46d					
priming	1	10.17	10.173	2.423	0.1261
triggering	1	12.02	12.019	2.863	0.0971
priming:triggering	1	6.94	6.942	1.653	0.2047
residuals	48	201.54	4.199		
Number of open flowers 47d					
priming	1	7.69	7.69	1.397	0.2431
triggering	1	48.08	48.08	8.731	**0.0048**
priming:triggering	1	7.69	7.69	1.397	0.2431
residuals	48	264.31	5.51		
Number of open flowers 48d					
priming	1	7.69	7.69	1.264	0.2665
triggering	1	99.69	99.69	16.379	**0.0002**
priming:triggering	1	3.77	3.77	0.619	0.4352
residuals	48	292.15	6.09		
Number of open flowers 49d					
priming	1	3.77	3.77	1.210	0.2770
triggering	1	73.92	73.92	23.730	**<0.0001**
priming:triggering	1	0	0	0.000	1.0000
residuals	48	149.54	3.12		
Number of open flowers 50d					
priming	1	0.08	0.77	0.048	0.8282
triggering	1	27.77	27.769	17.190	**0.0001**
priming:triggering	1	0.31	0.308	0.190	0.6645
residuals	48	77.54	1.615		
Number of open flowers 51d					
priming	1	0.077	0.0769	0.329	0.5691
triggering	1	0.692	0.6923	2.959	0.0918
priming:triggering	1	0.077	0.0769	0.329	0.5691
residuals	48	11.231	0.234		
Stalk length 31d					
priming	1	0.17	0.173	0.027	0.8710
triggering	1	0.02	0.019	0.003	0.9570
priming:triggering	1	1.56	1.558	0.242	0.6250
residuals	48	309.58	6.45		
Stalk length 33d					
priming	1	0.1	0.077	0.004	0.9500
triggering	1	0.5	0.481	0.025	0.8750
priming:triggering	1	3.8	3.769	0.196	0.6600
residuals	48	925.3	19.278		
Stalk length 35d					
priming	1	1.9	1.923	0.067	0.7970
triggering	1	8.5	8.481	0.295	0.5900
priming:triggering	1	2.8	2.769	0.096	0.7580
residuals	48	1381.7	28.784		

Continues

Continued

Effect	*df*	*sum sq*	*mean sq*	*F*	*p*
Stalk length 37d					
priming	1	0.8	0.812	0.028	0.8670
triggering	1	27	27.043	0.937	0.3380
priming:triggering	1	5.2	5.236	0.181	0.6720
residuals	48	1385.8	28.87		
Stalk length 39d					
priming	1	1.6	1.56	0.068	0.7950
triggering	1	40.7	40.69	1.775	0.1890
priming:triggering	1	0.3	0.31	0.013	0.9080
residuals	48	1100.3	22.92		
Stalk length 41d					
priming	1	1.2	1.23	0.070	0.7924
triggering	1	81.3	81.25	4.625	**0.0366**
priming:triggering	1	2.8	2.77	0.158	0.6931
residuals	48	843.3	17.57		
Stalk length 43d					
priming	1	0.2	0.24	0.020	0.8881
triggering	1	143.9	143.89	12.213	**0.0010**
priming:triggering	1	0.8	0.81	0.069	0.7940
residuals	48	565.5	11.78		
Stalk length 45d					
priming	1	0	0.04	0.005	0.9420
triggering	1	154	154.04	18.909	**0.0001**
priming:triggering	1	1.1	1.08	0.133	0.7170
residuals	48	391	8.15		
Stalk length 47d					
priming	1	0.8	0.81	0.097	0.7570
triggering	1	175.4	175.39	20.850	**<0.0001**
priming:triggering	1	1.4	1.39	0.165	0.6860
residuals	48	403.8	8.41		
Stalk length 49d					
priming	1	0.5	0.48	0.072	0.7900
triggering	1	169.9	169.92	25.382	**<0.0001**
priming:triggering	1	1.9	1.92	0.287	0.5940
residuals	48	321.3	6.69		
Stalk length 51d					
priming	1	0	0.04	0.006	0.9390
triggering	1	171.7	171.74	23.125	**<0.0001**
priming:triggering	1	3	3	0.405	0.5280
residuals	48	356.5	7.43		

Table A16: Statistical models assessing the effect of oviposition by *M. sexta* and simulated herbivory on different fitness parameter of *N. attenuata* (Exp. 10). Summaries of two-way ANOVA testing the effect of priming (oviposition), triggering (simulated herbivory) and their interaction on the specified growth and fitness parameter. The stalk length were considered at different time points, while differences were only compared within the corresponding measurement time point. Significant *p*-values are highlighted in bold.

Effect	***df***	***sum sq***	***mean sq***	***F***	***p***
Number of cumulative flowers					
priming	1	2.6	2.6	0.097	0.75831
triggering	1	358.6	358.6	13.392	**0.00108**
priming:triggering	1	0.7	0.7	0.026	0.87256
residuals	27	723.1	26.8		
Number of capsules					
priming	1	2.7	2.7	0.064	0.803
triggering	1	86.9	86.93	2.043	0.164
priming:triggering	1	4.5	4.5	0.106	0.747
residuals	27	1148.7	42.54		
Seed weight					
priming	1	0.1	0.103	0.011	0.918
triggering	1	22.3	22.296	2.351	0.138
priming:triggering	1	7.66	7.659	0.808	0.377
residuals	27	237.1	9.484		
Stalk length 41d					
priming	1	12	11.997	0.603	0.444
triggering	1	0	0.007	0	0.985
priming:triggering	1	1.9	1.851	0.093	0.763
residuals	27	537	19.888		
Stalk length 43d					
priming	1	11.6	11.634	0.518	0.478
triggering	1	1.3	1.271	0.057	0.814
priming:triggering	1	2.3	2.289	0.102	0.752
residuals	27	606	22.444		
Stalk length 45d					
priming	1	8.4	8.376	0.405	0.53
triggering	1	8.5	8.479	0.41	0.527
priming:triggering	1	2	2.048	0.099	0.755
residuals	27	558.6	20.691		
Stalk length 47d					
priming	1	0.7	0.695	0.045	0.833
triggering	1	24.8	24.799	1.623	0.213
priming:triggering	1	2.3	2.256	0.148	0.704
residuals	27	412.5	15.277		

Table A17: Statistical models assessing the effect of oviposition and larval feeding by *M. sexta* on the fitness parameters of regrown *N. attenuata* plants after defoliation (Exp. 11 & 12). Data of both experiments were combined. Summaries of generalized linear mixed models (GLMMs) and linear mixed models (LMMs) which included priming (oviposition), triggering (larval feeding) and their interaction as fixed factors and the replicate block as random factor (blocked experimental design). Significant *p*-values are highlighted in bold.

Number of flowers				
Random effect	*variance*	*SD*		
replicate	0.009	0.095		
Fixed effect	*estimate*	*SE*	*Z*	*p*
intercept	2.613	0.070	37.537	**<0.0001**
priming	-0.242	0.099	-2.447	**0.0144**
triggering	-0.186	0.112	-1.664	0.0961
priming:triggering	0.128	0.159	0.806	0.42

Continues

Number of flowers continued

Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.114	0.124		0.914	0.3606
C-T	0.058	0.112		0.516	0.6060
C-PT	0.299	0.107		2.802	**0.0051**
P-T	-0.056	0.117		-0.477	0.6334
P-PT	0.186	0.112		1.664	0.0961
T-PT	0.242	0.099		2.447	**0.0144**
Number of cumulative flowers					
Random effect	*variance*	*SD*			
replicate	0.031	0.178			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	3.401	0.062	55.164	**<0.0001**	
priming	-0.259	0.066	-3.890	**0.0001**	
triggering	-0.231	0.077	-2.992	**0.0028**	
priming:triggering	0.134	0.110	1.215	0.2245	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.125	0.088		1.417	0.1566
C-T	0.098	0.078		1.258	0.2084
C-PT	0.356	0.074		4.828	**<0.0001**
P-T	-0.027	0.081		-0.335	0.7375
P-PT	0.231	0.077		2.992	**0.0028**
T-PT	0.259	0.067		3.890	**0.0001**
Duration of flowering					
Random effect	*variance*	*SD*			
replicate	0.013	0.115			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	2.971	0.062	48.289	**<0.0001**	
priming	-0.141	0.080	-1.761	0.0782	
triggering	-0.198	0.095	-2.075	**0.038**	
priming:triggering	0.194	0.130	1.492	0.1357	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	-0.053	0.103		-0.516	0.6056
C-T	0.003	0.088		0.039	0.9692
C-PT	0.145	0.085		1.695	0.0901
P-T	0.056	0.098		0.577	0.5638
P-PT	0.198	0.095		2.075	**0.0380**
T-PT	0.141	0.080		1.761	0.0782
Number of capsules					
Random effect	*variance*	*SD*			
replicate					
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	2.068	0.107	19.289	**<0.0001**	
priming	-0.109	0.156	-0.700	0.4840	
triggering	-0.122	0.157	-0.780	0.4350	
priming:triggering	-0.132	0.245	-0.539	0.5900	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	0.241	0.189		1.276	0.2019
C-T	0.254	0.189		1.348	0.1778
C-PT	0.363	0.185		1.964	**0.0496**
P-T	0.013	0.161		0.080	0.9360
P-PT	0.122	0.156		0.780	0.4352
T-PT	0.109	0.156		0.700	1.4837
Seed weight					
Random effect	*variance*	*SD*			
replicate	6.730	2.594			
residuals	525.760	22.929			
Fixed effect	*estimate*	*SE*	*Z*	*p*	
intercept	72.888	5.597	13.023	**<0.0001**	
priming	-5.681	7.865	-0.722	0.4700	
triggering	-4.212	8.884	-0.474	0.6350	
priming:triggering	-6.789	12.148	-0.559	0.5760	
Comparison	*estimate*	*SE*	*df*	z.ratio	*p*
C-P	12.47	9.72	54.20	1.283	0.2050
C-T	11.00	8.61	47.70	1.278	0.2075
C-PT	16.68	8.61	47.70	1.937	0.0586
P-T	-1.47	9.28	50.60	-0.158	0.8749
P-PT	4.21	9.23	50.60	0.454	0.6519
T-PT	5.68	8.15	45.30	0.697	0.4892

Table A18: Statistical comparisons to assess the growth of *N. attenuata* before and after defoliation (Exp. 11). Plants were either kept untreated (C), exposed to larval feeding only (T) or a combination of oviposition followed by larval feeding (PT) before removing all aboveground plant parts. Considered parameters: stalk length (before defoliation) or cumulative stalk length (sum of all stalks of a regrowth plant, after defoliation). Differences were only compared within the corresponding measurement time point between the different treatments, utilizing two sample t-test. Significant p-values are highlighted in bold.

Comparison	*t*	*df*	*p*
Stalk length 46d			
C-T	-0.6269	10	0.5448
C-PT	0.4837	10	0.6390
T-PT	1.0364	10	0.3244
Stalk length 48d			
C-T	-0.6951	10	0.5028
C-PT	-0.3536	10	0.7310
T-PT	0.3830	10	0.7097
Stalk length 50d			
C-T	0.6747	10	0.5151
C-PT	0.5766	10	0.5770
T-PT	-0.1120	10	0.9131
Stalk length 52d			
C-T	0.7470	10	0.4723
C-PT	1.2934	10	0.2250
T-PT	0.4769	10	0.6437
Stalk length 54d			
C-T	2.6864	10	**0.0228**
C-PT	3.1491	10	**0.0104**
T-PT	-0.0738	10	0.9426
Cumulative stalk length after defoliation 19d			
C-T	-1.0622	10	0.3131
C-PT	-2.3805	10	**0.0386**
T-PT	-0.4193	10	0.6839
Cumulative stalk length after defoliation 21d			
C-T	-1.1024	10	0.2961
C-PT	-2.7058	10	**0.0221**
T-PT	-0.8400	10	0.4205
Cumulative stalk length after defoliation 23d			
C-T	-1.3862	10	0.1958
C-PT	-2.5206	10	**0.0304**
T-PT	-0.2437	10	0.8124
Cumulative stalk length after defoliation 25d			
C-T	-1.3195	10	0.2164
C-PT	-2.6713	10	**0.0234**
T-PT	-0.7982	10	0.4433
Cumulative stalk length after defoliation 27d			
C-T	-1.3202	10	0.2162
C-PT	-2.5370	10	**0.0295**
T-PT	-0.8790	10	0.4000
Cumulative stalk length after defoliation 29d			
C-T	-1.2343	10	0.2453
C-PT	-2.2651	10	**0.0470**
T-PT	-0.8388	10	0.4212
Cumulative stalk length after defoliation 31d			
C-T	-1.3568	10	0.2047
C-PT	-2.4473	10	**0.0344**
T-PT	-0.7855	10	0.4504
Cumulative stalk length after defoliation 33d			
C-T	-1.1713	10	0.2686
C-PT	-2.4425	10	**0.0347**
T-PT	-1.1596	10	0.2732
Cumulative stalk length after defoliation 35d			
C-T	-1.1798	10	0.2654
C-PT	-2.4126	10	**0.0365**
T-PT	-0.9726	10	0.3537

Table A19: Statistical comparisons to assess the growth of *N. attenuata* before and after defoliation (Exp. 12). Summaries of two-way ANOVA testing the effect of priming (oviposition), triggering (larval feeding) and their interaction on the specified growth parameter before and after removal of all aboveground plant parts. Considered parameters: stalk length (before defoliation) or cumulative stalk length (sum of all stalks of a regrowth plant, after defoliation). Differences were only compared within the corresponding measurement time point. Significant p-values are highlighted in bold.

Effect	*df*	*sum sq*	*mean sq*	*F*	*p*
Stalk length 44d					
priming	1	5.45	5.446	4.388	**0.0431**
triggering	1	0.03	0.029	0.023	0.8799
priming:triggering	1	0.12	0.117	0.094	0.7603
residuals	37	45.92	1.241		
Stalk length 46d					
priming	1	22.32	22.321	4.517	**0.0403**
triggering	1	3.29	3.293	0.666	0.4195
priming:triggering	1	3.31	3.312	0.67	0.4182
residuals	37	182.83	4.941		
Stalk length 48d					
priming	1	35	34.96	3.695	0.0623
triggering	1	6.9	6.91	0.731	0.3982
priming:triggering	1	2.6	2.57	0.272	0.6052
residuals	37	350	9.46		
Stalk length 50d					
priming	1	17.9	17.941	1.949	0.171
triggering	1	13.3	13.263	1.441	0.238
priming:triggering	1	2.6	2.572	0.279	0.6
residuals	37	340.6	9.205		
Stalk length 52d					
priming	1	19.2	19.158	2.017	0.164
triggering	1	11	11.046	1.163	0.288
priming:triggering	1	1.2	1.208	0.127	0.723
residuals	37	351.5	9.499		
Stalk length 54d					
priming	1	14.5	14.496	1.869	0.18
triggering	1	7.78	7.776	1.003	0.323
priming:triggering	1	0.01	0.006	0.001	0.977
residuals	37	279.2	7.755		
Cumulative stalk length 17d after defoliation					
priming	1	25.1	25.15	0.715	0.4031
triggering	1	249	248.97	7.083	**0.0114**
priming:triggering	1	2.5	2.51	0.072	0.7906
residuals	37	1300.6	35.15		
Cumulative stalk length 21d after defoliation					
priming	1	19.2	19.19	0.371	0.5462
triggering	1	236.1	236.09	4.565	**0.0393**
priming:triggering	1	5.7	5.74	0.111	0.7408
residuals	37	1913.7	51.72		
Cumulative stalk length 23d after defoliation					
priming	1	2.4	2.43	0.037	0.848
triggering	1	201	201.04	3.071	0.088
priming:triggering	1	0.1	0.1	0.002	0.969
residuals	37	2421.8	65.45		
Cumulative stalk length 25d after defoliation					
priming	1	0.7	0.67	0.009	0.926
triggering	1	73.6	73.65	0.976	0.33
priming:triggering	1	0.2	0.18	0.002	0.961
residuals	37	2793.3	75.49		
Cumulative stalk length 27d after defoliation					
priming	1	6	6.32	0.07	0.793
triggering	1	20	19.78	0.219	0.643
priming:triggering	1	4	4.37	0.048	0.827
residuals	37	3344	90.38		

Continues

Continued

Effect	*df*	*sum sq*	*mean sq*	*F*	*p*
Cumulative stalk length 29d after defoliation					
priming	1	10	10.34	0.102	0.751
triggering	1	0	0.02	0	0.99
priming:triggering	1	9	8.89	0.088	0.768
residuals	37	3740	101.09		
Cumulative stalk length 33d after defoliation					
priming	1	23	23.49	0.212	0.648
triggering	1	8	7.65	0.069	0.794
priming:triggering	1	14	14.45	0.13	0.72
residuals	37	4102	110.87		

Table A20: Statistical models assessing the effect of oviposition and larval feeding by *M. sexta* on the fitness parameters of regrown *N. attenuata* plants after defoliation in early rosette or flowering state (Exp. 13). At the time of oviposition plants were either four-week-old young rosette plants or eight-week-old elongated flowering plants. Summaries of two-way ANOVA testing the effect of priming (oviposition), triggering (larval feeding) and their interaction on the specified growth and fitness parameter. In this analysis only the data of one of the two developmental stages were compared with each other. Significant p-values are highlighted in bold.

Effect	*df*	*sum sq*	*mean sq*	*F*	*p*
Young rosette plants: Number of flowers					
priming	1	52.9	52.9	4.295	**0.0454**
triggering	1	0.4	0.4	0.032	0.858
priming:triggering	1	0.9	0.9	0.073	0.7885
residuals	36	443.4	12.32		
Flowering plants: Number of flowers					
priming	1	57.6	57.6	4.334	**0.0445**
triggering	1	1.6	1.6	0.12	0.7306
priming:triggering	1	0	0	0	1
residuals	36	478.4	13.29		
Young rosette plants: Duration of flowering					
priming	1	2.5	2.5	0.575	0.4533
triggering	1	32.4	32.4	7.448	**0.0098**
priming:triggering	1	8.1	8.1	1.862	0.1809
residuals	36	156.6	4.35		
Flowering plants: Duration of flowering					
priming	1	11.02	11.025	1.621	0.211
triggering	1	0.63	0.625	0.092	0.764
priming:triggering	1	4.22	4.225	0.621	0.436
residuals	36	244.9	6.803		
Young rosette plants: Number of capsules					
priming	1	0.1	0.1	0.022	0.882
triggering	1	0.9	0.9	0.2	0.657
priming:triggering	1	2.5	2.5	0.557	0.46
residuals	36	161.6	4.489		
Flowering plants: Number of capsules					
priming	1	9.02	9.025	1.413	0.242
triggering	1	5.62	5.625	0.881	0.354
priming:triggering	1	7.22	7.225	1.131	0.295
residuals	36	229.9	6.386		
Young rosette plants: Seed weight					
priming	1	1400	1400	2.139	0.1522
triggering	1	9257	9257	14.148	**0.0006**
priming:triggering	1	516	516	0.789	0.3802
residuals	36	23553	654		
TukeyHSD		*diff*	*lwr*	*upr*	*p*
	C-P	-4.644	-35.4518	26.1638	0.9770
	C-T	37.611	6.8033	68.4188	**0.0116**
	C-PT	18.594	-12.2138	49.4018	0.3775
	P-T	42.255	11.4472	73.0628	**0.0039**
	P-PT	23.238	-7.5698	54.0458	0.1956
	T-PT	-19.017	-49.8248	11.7908	0.3579

Continues

Continued

Effect	*df*	*sum sq*	*mean sq*	*F*	*p*
Flowering plants: Seed weight					
priming	1	0	0.1	0	0.995
triggering	1	3	2.9	0.002	0.963
priming:triggering	1	346	345.9	0.261	0.612
residuals	36	47643	1323.4		

Table A21: Statistical models assessing the effect of oviposition and larval feeding by *M. sexta* on the growth of regrown *N. attenuata* plants after defoliation in early rosette or flowering state (Exp. 13). At the time of oviposition plants were either young rosette plants or elongated flowering plants. Summaries of two-way ANOVA testing the effect of priming (oviposition), triggering (larval feeding) and their interaction on the specified growth parameter. Considered parameters: stalk length (before defoliation) or cumulative stalk length (sum of all stalks of a regrowth plant, after defoliation). Differences were only compared within the corresponding measurement time point. Significant p-values are highlighted in bold.

Effect	*df*	*sum sq*	*mean sq*	*F*	*p*
Young rosette plants: Stalk length 67d					
priming	1	47.3	47.31	4.696	**0.0369**
triggering	1	2.8	2.76	0.274	0.6041
priming:triggering	1	0.1	0.06	0.006	0.9408
residuals	36	362.6	10.07		
Flowering plants: Stalk length 43d					
priming	1	0.23	0.225	0.088	0.769
triggering	1	4.22	4.225	1.649	0.207
priming:triggering	1	0.40	0.400	0.156	0.695
residuals	36	92.25	2.563		
Young rosette plants: Cumulative stalk length 17d after defoliation					
priming	1	372.1	372.1	7.544	**0.00934**
triggering	1	1416.1	1416.1	28.71	**<0.0001**
priming:triggering	1	38	38	0.771	0.38575
residuals	36	1775.7	49.3		
Flowering plants: Cumulative stalk length 17d after defoliation					
priming	1	37.1	37.07	2.87	0.1
triggering	1	32.2	32.25	2.497	0.125
priming:triggering	1	9.3	9.33	0.723	0.402
residuals	33	387.4	12.91		
Young rosette plants: Cumulative stalk length 19d after defoliation					
priming	1	486.5	486.5	5.666	**0.0227**
triggering	1	2814	2814	32.775	**<0.0001**
priming:triggering	1	17.6	17.6	0.204	0.6538
residuals	36	3090.9	85.9		
Flowering plants: Cumulative stalk length 19d after defoliation					
priming	1	32.3	32.27	1.311	0.26
triggering	1	41.9	41.95	1.704	0.201
priming:triggering	1	15.1	15.14	0.615	0.439
residuals	33	812.3	24.62		
Young rosette plants: Cumulative stalk length 21d after defoliation					
priming	1	562	562	4.359	**0.0439**
triggering	1	3940	3940	30.535	**<0.0001**
priming:triggering	1	4	40	0.033	0.8574
residuals	36	4645	129		
Flowering plants: Cumulative stalk length 21d after defoliation					
priming	1	110.6	110.6	2.557	0.119
triggering	1	117	116.96	2.704	0.11
priming:triggering	1	27.6	27.64	0.639	0.43
residuals	33	1427.2	43.25		
Young rosette plants: Cumulative stalk length 23d after defoliation					
priming	1	462	462	2.885	0.0981
triggering	1	4516	4516	28.17	**<0.0001**
priming:triggering	1	7	7	0.045	0.8331
residuals	36	5771	160		

Continues

Continued

Effect	***df***	***sum sq***	***mean sq***	***F***	***p***
Flowering plants: Cumulative stalk length 23d after defoliation					
priming	1	149.4	149.41	1.942	0.173
triggering	1	247.5	247.53	3.217	0.082
priming:triggering	1	113.8	113.8	1.479	0.233
residuals	33	2538.9	76.94		
Young rosette plants: Cumulative stalk length 25d after defoliation					
priming	1	325	325	1.661	0.206
triggering	1	4601	4601	23.517	**<0.0001**
priming:triggering	1	63	63	0.319	0.575
residuals	36	7043	196		
Flowering plants: Cumulative stalk length 25d after defoliation					
priming	1	174	173.9	1.729	0.198
triggering	1	182	181.6	1.806	0.188
priming:triggering	1	225	225.2	2.238	0.144
residuals	33	3319	100.6		
Young rosette plants: Cumulative stalk length 29d after defoliation					
priming	1	64	64	0.298	0.58844
triggering	1	3735	3735	17.462	**0.0001**
priming:triggering	1	170	170	0.796	0.378335
residuals	36	7699	214		
Flowering plants: Cumulative stalk length 29d after defoliation					
priming	1	324	324.1	2.763	0.106
triggering	1	72	71.6	0.61	0.44
priming:triggering	1	260	259.6	2.213	0.146
residuals	33	3870	117.3		
Young rosette plants: Cumulative stalk length 31d after defoliation					
priming	1	0	0.2	0.001	0.97457
triggering	1	2418	2418	11.076	**0.00203**
priming:triggering	1	185	184.9	0.847	0.36355
residuals	36	7859	218.3		
Flowering plants: Cumulative stalk length 31d after defoliation					
priming	1	125	124.6	0.897	0.35
triggering	1	65	65.2	0.47	0.498
priming:triggering	1	382	382.4	2.754	0.106
residuals	33	4582	138.8		

B.3 Index

B.3.1 List of Tables

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B.3.3 List of Abbreviations

ABA	abscisic acid
ACX	acyl-CoA oxidase
AOC	allene oxide cyclase
AOS	allene oxide synthase
ARG	accessory reproductive gland
BA	benzoic acid
BAZH	benzoic acid 2-hydroxylase
BOP1	blade on petiole 1
C	control
C3H	p-coumarate 3-hydroxylase
C4H	cinnamate 4-hydroxylase
CA	chorismate / chorismic acid
CAC	clathrin adaptor complexes medium subunit
CDH	cytokinin oxidase / dehydrogenase
cDNA	complementary DNA
CGA	caffeoyl quinate / chlorogenic acid
CHI	chalcone isomerase
CHS	chalcone synthase
cis-(+)-OPDA	(9S, 13S)-12-oxo-phytodienoic acid
CK	cytokinin
COI1	coronatine insensitive 1
CP	caffeoylputesine
CPDK	calmodulin-like proteins and calcium-dependent protein kinase
C_T	threshold cycle
DAMP	damage associated molecular pattern
EAMP	egg associated molecular pattern
eATP	extracellular adenosine 5‘-triphosphate
EDS5	enhanced disease susceptibility 5
ELF1	elongation factor 1
eNAD(P)	extracellular nicotinamide adenine dinucleotide (phosphate)
ERF	ethylene response factor
ET	ethylene
Exp.	experiment
ESI	electrospray ionization
FAC	fatty acid-amino acid conjugate
Fig	figure
GA	glycolalkaloids
GAGE	generally applicable gene set enrichment
GAPDH	glycerinaldehyd-3-phosphat-dehydrogenase
GOI	gene of interest
HAMP	herbivore-associated molecular pattern
HCGQT	hydroxycinnamoyl glucose:quinate hydroxycinnamoyl transferase

HCT	hydroxycinnamoyl-CoA shikimate hydroxycinnamoyl transferase
IC	isochorismate
IC-9-Glu	isochorismate-9-glutamate
ICS1	isochorismate synthase
JA	jasmonic acid
JA-Ile	jasmonic acid-isoleucine
JAR1	jasmonoyl amino acid synthetase
JAZ	jasmonate ZIM-domain
KAT	L-3-ketoacyl-CoA thiolase
LOX	lipoxygenease
MAPK	mitogen-activated protein kinase
MeJA	methyl jasmonate
NO	nitric oxide
NPR1	nonexpressor of PR genes 1
NRQ	normalized relative quantity
o-coumaric acid	ortho- coumaric acid
OD	optical density
OIPV	oviposition induced plant volatile
OPC-8	3-oxo-2- (2‘[Z]-pentenyl)-cyclopentan-1-octanoic acid
OPR3	OPDA reductase 3
OS	oral secretion
P	primed
PAL	phenylalanine ammonia-lyase
PBS3	avrPphB susceptible 3
PC	phosphatidylcholines
PG4	polygalacturonase 4
Phe	L-phenylalanine
PI	proteinase inhibitor
PI1	protease inhibitor 1
PPC	phenylpropanoid-polyamine conjugates
PPO	polyphenol oxidase
PR	pathogenesis-related
PR1	pathogenesis-related gene 1
PRR	pattern recognition receptor
PRX2	peroxidase 2
PT	primed and triggered
PYL	pyrabactin resistance 1-like
ROS	reactive oxygen species
RQ	relative quantity
SA	salicylic acid
SEM	standard error of the mean
T	triggered
t-CA	trans-cinnamic acid
Tab.	table
TOF-MS/MS	time of flight mass spectrometer
TPI	trypsin protease inhibitor

UGCT	UDP-glucose:cinnamate glucosyl transferase
UPLC	ultra-high-performance-liquid-chromatography
Vm	plasma transmembrane potential
VOC	volatile organic compound
W+OS	wounding and oral secretion
α-LeA	α-linolenic acid
13(S)-HPOT	(13S)-hydroperoxy octadecatrienoic acid
13-LOX	13-lipoxygenase
2HNG	2-hydroxy-acryloyl-N-glutamate
4CL	4-coumaroyl:CoA-ligase

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Peppinchen (+4) <3