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Plant-mediated interactions between spatially and temporally separated above- and belowground herbivores

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Contents

Foreword

This dissertation is a cumulative work of manuscripts, either published or submitted for publication in international peer-reviewed journals. This dissertation, therefore, is based on the following articles:

1. **Kafle D**, Krämer A, Naumann A, Wurst S. 2014. Genetic variation of the host plant species matters for interactions with above- and belowground herbivores. Insects 5(3): 651-667. (Submitted: 15 May 2014, Accepted: 8 August 2014, Published: 29 August 2014)
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2. **Kafle D**, Hänel A, Lortzing T, Steppuhn A, Wurst S. 2016. Sequential above- and belowground herbivory modifies plant responses depending on herbivore identity. Submitted to BMC Ecology.

3. **Kafle D,** Naumann A, Wurst S. 2016. Herbivory in the parental generation affects root traits and herbivore performance on progeny plants. Submitted to Oikos.

Summary

Both above- and belowground herbivores rely on plants for food and shelter; in response plants employ different defense strategies to minimize the adversity of herbivory on their fitness. Herbivore-induced changes on plant traits in systemic tissue may lead to indirect interaction between spatially, and in some cases, temporally separated above- and belowground herbivores. The main aim of this thesis is to investigate the aboveground herbivore-induced changes in plant traits to elucidate their potential role in interaction between spatially and temporally separated above- and belowground herbivores. The consequence of such plant-mediated interactions between above- and belowground herbivores, and factors which may shape such interactions were also examined.

In the first experiment (**chapter 2**), two genotypes (M4 an E9) of the perennial bittersweet nightshade plant (*Solanum dulcamara*, Solanaceae) were challenged with foliar feeding caterpillars (*Spodoptera exigua*, Noctuidae) and root-feeding wireworms (*Agriotes* spp., Elateridae) sequentially. Here, I investigated how the genetic variation of a plant species affects the plants' interaction with above- and belowground herbivores. Aboveground herbivory had genotype-specific effect on plant traits such as shoot and root biomass, root C/N ratio, and protein content. However, such altered traits had no effect on the performance of the belowground herbivores. On the other hand, belowground herbivory increased the root N concentration of M4 plants which promoted the growth of conspecific larvae as found in choice and non-choice feeding bioassays. *Agriotes* larvae also tended to prefer roots of M4 over E9, irrespective of the aboveground herbivore treatment in bioassays. Further analysis documented the differences in metabolic profiles of the two plant genotypes. The results demonstrate the genotype-specific effects of above- and belowground herbivory on quantitative and qualitative root traits.

In the second experiment (**chapter 3**), tomato (*Solanum lycopersicum*, Solanaceae) plants were challenged with either of chewing beet armyworms (*S. exigua*, Noctuidae) or sap-sucking green peach aphids (*Myzus persicae,* Aphididae) to investigate their indirect impact on performance of belowground root-knot nematodes (*Meloidogyne incognita*, Heteroderidae). Both chewing and sucking aboveground herbivory altered plant traits such as plant growth, resource allocation, and phytohormone contents in both shoot and root tissue; however, overall performance of nematodes was unaffected. Herbivore-induced changes in several parameters were different in sequential herbivory depending upon the identity of the aboveground herbivore. For example, the shoot biomass of the plant was reduced by transient aboveground herbivory by aphids or *S. exigua* when added alone, but aphid-treated plants when challenged with nematodes later showed compensatory growth while plants challenged with *S. exigua* and nematodes did not show such effects. The results demonstrate that the earlier aboveground herbivory can modify plant responses to subsequent belowground herbivores depending upon identity of the aboveground herbivores.

In the studies presented in **chapter 4**, wild tobacco (*Nicotiana attenuata,* Solanaceae) plants were challenged with aboveground chewing caterpillars (*Manduca sexta*, Sphingidae) and belowground root-knot nematodes (*M. incognita*). Conducting three different experiments varying in their temporal scale, I investigated short-term, long-term and transgenerational legacy effects of the transient aboveground herbivory on performance and fitness of plants and subsequent nematodes. Earlier transient aboveground herbivory had significant impact on plant traits such as plant biomass, nutrient content, and plant fitness in short-term and long-term experiments. Aboveground herbivory had a facilitating effect on the nematode performance in the long-term and transgenerational experiments. Progeny plants whose parents were challenged with sequential above- and belowground herbivory were resistant against aboveground herbivores, while nematode herbivory in the parental generation increased the root biomass of progeny plants. These results show that herbivory can have a long-term impact on plant fitness by improving the resistance and tolerance traits of the progeny plants suggesting a transgenerational priming response.

Zusammenfassung

Sowohl oberirdisch als auch im Boden lebende Herbivoren sind auf Pflanzen als Nahrungsquelle und Rückzugsorte angewiesen; im Gegenzug bedienen sich Pflanzen verschiedener Abwehrstrategien, um die negativen Auswirkungen von Herbivorie auf ihre Fitness zu minimieren. Herbivoreninduzierte systemische Veränderungen von Pflanzeneigenschaften können zu indirekten Wechselwirkungen zwischen räumlich voneinander getrennten Herbivoren führen, sowie in manchen Fällen auch zwischen zeitlich voneinander getrennten. Hauptziel dieser Arbeit ist die Untersuchung der durch oberirdische Herbivorie induzierten Veränderungen von Pflanzeneigenschaftenim Hinblick auf derenpotenzielle Rolle im Zusammenspiel räumlich und zeitlich voneinander getrennter, oberirdisch und im Boden lebender Herbivoren. Die Auswirkung einer solchen pflanzenvermittelten Wechselwirkung zwischen oberirdisch- und bodenlebenden Herbivoren wurdeebenso untersucht wie Faktoren, die eine solche Interaktion möglicherweise prägen.

Im ersten Experiment (**Kapitel 2**) wurden zwei Genotypen (M4 und E9) des mehrjährigen Bittersüßen Nachtschattens (*Solanum dulcamara*, Solanaceae) nacheinander Blattfraß durch Raupen (*Spodoptera exigua*, Noctuidae) und Wurzelfraß durch Drahtwürmer (*Agriotes* spp., Elateridae) ausgesetzt. Hierbei habe ich untersucht, wie sich die genetische Variabilität innerhalb einer Pflanzenart auf die Wechselwirkungenzwischen Pflanze und Spross- und Wurzelherbivoren auswirkt. Sprossherbivorie hatte genotypspezifische Auswirkungen auf Pflanzeneigenschaften wie Spross- und Wurzelgewicht, C/N-Verhältnis in Wurzeln und den Proteingehalt. Dennoch hatten diese Veränderungen keinen Einfluss auf die Entwicklung der Wurzelherbivoren. Andererseits führte die Wurzelherbivorie zu erhöhten N-Konzentrationen in den Wurzeln der M4-Pflanzen, was in Fraßversuchen mit und ohne Wahlmöglichkeit das Wachstum von konspezifischen Larven förderte. *Agriotes*-Larven tendierten in Biotests außerdem zu einer Präferenz für Wurzeln von M4-gegenüber E9-Pflanzen, und zwar unabhängig von der oberirdischen Herbivorenbehandlung. Weitere Analysen offenbarten die Unterschiede in Stoffwechselprofilen der zwei pflanzlichen Genotypen. Die Ergebnisse veranschaulichen die genotypspezifischen Effekte von Spross- und Wurzelherbivorie auf quantitative und qualitative Wurzeleigenschaften.

Im zweiten Experiment (**Kapitel 3**) wurden Tomatenpflanzen (*Solanum lycopersicum*, Solanaceae) entweder Fraß durch Larven der Zuckerrübeneule(*S. exigua*, Noctuidae) ausgesetzt oder pflanzensaftsaugenden Grünen Pfirsichblattläusen (*Myzus persicae*, Aphididae), um einen indirekten Einfluss auf die Entwicklung von Wurzelgallennematoden(*Meloidogyne incognita*, Heteroderidae) zu untersuchen. Sowohl stechend-saugende als auch beißend-kauende Herbivorie an oberirdischen Pflanzenteilen führte zu Veränderungen beim Wachstum der Pflanzen, zu Resourcenverschiebung und zu Änderungen bei Phytohormongehalten in Wurzel- wie Sprossgewebe; dennoch blieb die Entwicklung der Nematoden davon insgesamt unberührt. Herbivoreninduzierte Änderungen etlicher Parameter bei zeitlich getrennter Herbivorenabfolge waren unterschiedlich je nach oberirdisch eingesetzter Herbivorenart. Beispielsweise wiesen Pflanzen, die vorübergehend oberirdisch nur von Blattläusen oder nur von *S. exigua* befallen waren, ein verringertes Sprossgewicht auf. Ursprünglich blattlausbefallene Pflanzen jedoch zeigten bei späterem Befall der Wurzeln mit Nematoden kompensatorisches Wachstum, wobei Pflanzen, die zuvor anstelle von Blattläusen *S. exigua* ausgesetzt waren, keine solchen Effekte aufwiesen. Die Ergebnisse belegen, dass vorausgegangene oberirdische Herbivorie, abhängig von der Art des Herbivoren, die pflanzliche Reaktion auf nachfolgende Wurzelherbivorie modifizieren kann.

Bei den in **Kapitel 4** vorgestellten Studien wurde Wilder Tabak (*Nicotiana attenuata*, Solanaceae) oberirdisch mit beißend-kauenden Raupen (*Manduca sexta*, Sphingidae) und im Boden mit Wurzelgallennematoden (*M. incognita*) konfrontiert. Mit der Durchführung dreier Experimente unterschiedlicher zeitlicher Ausdehnung habe ich kurzzeitige, langfristige und generationenübergreifende Effekte der zeitweiligen oberirdischen Herbivorie auf die Entwicklung und Fitness der Pflanzen und nachfolgender Nematoden untersucht. Vorausgegangene zeitweise Herbivorie am Spross hatte signifikanten Einfluss auf Pflanzeneigenschaften wie Biomasse, Nährstoffgehalt und Fitness in den Kurzzeit- und Langzeitexperimenten. Oberirdische Herbivorie hatte einen unterstützenden Effekt auf die Nematodenentwicklung im langfristigen und im generationenübergreifenden Experiment. Die Nachkommen derjenigen Pflanzen, die einer zeitlich getrennten Abfolge aus Spross- und Wurzelherbivorie ausgesetzt waren, wiesen eine Resistenz gegen die Sprossherbivoren auf, während Nematodenbefall der Parentalgeneration in der Filialgeneration zu mehr Wurzelbiomasse führte. Diese Ergebnisse zeigen, dass Herbivorie langfristige Auswirkungen auf die Fitness von Pflanzen haben kann in Form von verbesserten Widerstands- und Toleranzeigenschaften in der Folgegeneration, was auf einen generationenübergreifenden Primingeffekt hinweist.

Chapter 1

General introduction

In the evolutionary arms race, spanning millions of years, plants and herbivores have been interacting with each other at various levels and intensities. Selection pressure exerted on both plants and herbivores from their long-standing co-evolutionary relationship has resulted in an enormous diversity of both plants and herbivores. Herbivory reduces the fitness of the plants; in response, plants employ different defense strategies to deal with herbivores. Resistance and tolerance are two major defense strategies of the plants which help to deter the herbivores or withstand the adversity of herbivory (Gatehouse 2002). Herbivore-induced plant responses also lead to indirect interaction between herbivores that share the host plant at the same or different plant tissue, simultaneously or consecutively (Price et al. 1980; Bardgett et al. 1998; van der Putten 2001; Wardle et al. 2004; Bezemer and van Dam 2005). Such interactions may extend to higher trophic levels, such as predators and parasitoids (Soler et al. 2007).

Plant response to herbivory

Plants are naturally equipped with several forms of resistance strategies to combat biotic stresses such as insect herbivory and microbial infection. Broadly, plants constitute two general defense modes: constitutive and induced (Gatehouse 2002; Howe and Jander 2008). Constitutive defense is always present in a “ready-to-use” state in the plant’s tissue, irrespective of herbivory, and it includes both physical defenses (thorns, trichome, and spines) and preformed chemical defenses (secondary metabolites and volatiles). An induced defense is activated upon the incidence of herbivory (Swain 1977; Pieterse and Dicke 2007). Despite the differences in the timing of availability in plants, similar defense compounds facilitate both constitutive and induced defenses (Gatehouse 2002).

Some major defense compounds include proteinase inhibitors (PIs) and secondary metabolites such as flavonoids, lignans, glucosinolates, phenylpropanoids, terpenoids, steroids, and alkaloids (Swain 1977). These compounds have either an antibiosis or an antixenosis mode of action on herbivores through which they deter the growth and survival of herbivores via several mechanisms, such as cell membrane disruption, inhibition of metabolism, and disruption in the central nervous system (Wittstock and Gershenzon 2002). Several plant species are also known to emit volatile organic compounds (terpenoids and fatty acid derivatives), which play a defensive role in two ways: directly, by having repellent or toxic effects on herbivores, and indirectly, by attracting natural enemies of herbivores, such as parasitoids or predators (Pare and Tumlinson 1999; Kessler and Baldwin 2001; Pichersky and Gershenzon 2002). In general, defense response involves a complex chain of events starting with the plant's perception and recognition of herbivory up to the activation of herbivore-specific signaling pathways and the induction of herbivore-specific defensive compounds (Halitschke and Baldwin 2004). The activation of defense-regulatory signaling pathways due to herbivory stress may also lead to the systemic induction of a defense response in plant tissues that are spatially separated from the site of herbivory (Gatehouse 2002). Hence, aboveground (AG) herbivory may induce changes in defense traits of the root tissue, with potential impacts on the belowground (BG) herbivores that share a common host plant.

Resistance strategies employed by plants against herbivores are often costly in terms of plant resources that would otherwise be invested in plant's growth and reproduction (Herms and Mattson 1992; Strauss et al. 2002), resulting in a trade-off between resistance and plant fitness. Plants, therefore, may exhibit tolerance strategies as an alternative, or exhibit them complementarily with resistance, to fine-tune their overall response against herbivory and lower incurred fitness costs (van der Meijden et al. 1988; van Dam 2009; Carmona and Fornoni 2013). Some common tolerance responses include altered photosynthesis and growth rates, compensatory growth, increased tillering, and reallocation of primary metabolites and minerals (van der Meijden et al. 1988; Strauss and Agrawal 1999). Translocation of resources away from the site of herbivory is a common tolerance response by which a plant shields valuable nutrients for later use in growth and development. The lower nutritional quality at the feeding site may lead to lower performance of the herbivores (Babst et al. 2005; Kaplan et al. 2008a). Such shifts in the nutritional quality of local or systemic tissue may further result in indirect interaction between AG and BG herbivores.

Another potential mechanism which may mediate the interaction between above- and belowground is the priming of plant response. A growing body of evidence suggests that biotic or abiotic stresses may prime plants to "get ready for the next battle" (Conrath et al. 2006; Frost et al. 2008; Karban 2011). Primed plants, that is, plants with previous stress experience, are more efficient in defending themselves against future stresses than naïve plants with no previous stress experience. Priming involves the induced response but differs from induction: induction occurs following the single stress event, whereas a dual-stress events and storage of information about the first stress event is prerequisite for priming response (Hilker et al. 2015). In a novel priming model proposed by Hilker et al. (2015), organisms without a nervous system, such as plants or microorganisms, are suggested to be primed by a first stress (priming stimulus) for their modified response to a second stress (triggering stimulus). Therefore, priming of plant response, if occurred due to aboveground herbivory, may result in indirect interaction between spatially and temporally separated AG and BG herbivores.

While there are a very limited numbers of studies to help draw the conclusion, priming is considered to be more effective and efficient than constitutive or induced defense in terms of the associated fitness cost (Hilker et al. 2015). Constitutive defenses are always present and need to be maintained in plant tissue regardless of herbivory; therefore, they are considered biosynthetically and ecologically costly for plants (Karban and Myers 1989; Dicke and Hilker 2003). On the other hand, inducible defenses are activated after receiving herbivory cues and are therefore considered a cost-saving plant strategy compared to constitutive defense (Karban and Myers 1989; Zangerl 2003). Priming may even have two major benefits over induction: first, the fitness costs of priming can be lower than those of induced defense (van Hulten et al. 2006), and second, once primed, the duration of plant vulnerability to herbivores is expected to be shorter (Heil and Kost 2006) than in the case of induced defense, where the plant is expected to need more time to activate its defense (Heil and Baldwin 2002). Plants have been shown to be primed by several biotic factors, such as damage by herbivores, insect egg deposition, and volatile organic compounds (VOCs) emitted by the damaged tissue of neighboring plants (Engelberth et al. 2004; Kessler and Baldwin 2004; Heil and Kost 2006; Hilker and Meiners 2006; Kessler et al 2006; Heil and Bueno 2007; Stork et al. 2009; Kim et al. 2012). A limited number of studies so far provide some insight into the existence of the priming and transgenerational priming of plant response, but several ecological, physiological, and molecular aspects still need to be assessed to fully understand the underlying mechanism and adaptive value of such phenomena. Previous studies have suggested phenotypic, hormonal, epigenetic, or cellular changes in local and/or systemic plant tissue due to biotic or abiotic stresses as the possible mechanism of priming

(Conrath et al. 2015; Hilker et al. 2015). However, they have ruled out the alteration of the plant tissue’s genetic structure during priming.

While priming has drawn scientific attention as an ideal strategy for plants to deal with recurring herbivory stress, few studies have also provided evidence of priming across plant generations (Pieterse 2012; Rasmann et al. 2012b; Walters and Paterson 2012). In such transgenerational priming or induction, mother plants, based on the stress they have experienced, bestow their progeny with the ability to modify their response to similar stress in their generation (Agrawal et al. 1999; Rasmann et al. 2012a). For example, in latter study by Rasmann et al. (2012a), it was shown that Arabidopsis (*Arabidopsis thaliana*) and tomato (*Solanum lycopersicum*) plants whose parents were challenged with caterpillars (*Pieris rapae* and *Helicoverpa zea* respectively), the application of methyl jasmonate, or mechanical damage were more resistant to conspecific caterpillars as compared to the progeny of control plants. Two possible mechanisms have been suggested for transgenerational priming effects: maternal effects such as seed provisioning and enrichment, and epigenetic effects such as DNA methylation (Holeski et al. 2012).

Plant-mediated interaction between above- and belowground herbivores

The herbivory-induced changes in plant traits, such as production or translocation of primary and secondary compounds, plant architecture by feeding damage or compensatory growth, and phenological changes such as the onset of flowering, may lead to the indirect interaction between spatially separated herbivores such as AG and BG herbivores (Masters et al. 1993; Bardgett et al. 1998; van der Putten 2001; Wardle et al. 2004). For example, Kaplan et al. (2008a) showed that the translocation of photoassimilates from shoot to root in response to foliar feeding by *Manduca sexta* caterpillars facilitated the performance of the BG plant parasitic nematode *Meloidogyne incognita*. Nematodes, on the other hand, interfered with the biosynthesis of the defensive compound nicotine, enhancing the performance of AG caterpillars. In another experiment, Johnson et al. (2009) found that the foliar-feeding aphid *Rhopalosiphum padi* increased the concentration of root minerals in barley (*Hordeum vulgare*), thereby enhancing the performance of root-feeding wireworms (*Agriotes* spp.). For their part, wireworms increased the level of essential amino acid in the leaves, which resulted in increased aphid population. In addition to the effects on spatially separated herbivores, altered plant characteristics may also impact subsequent herbivores, resulting in the indirect interaction between temporally separated herbivores (Ohgushi

2005). For example, Petersen and Sandström (2001) showed that the earlier herbivory of pecan (*Carya illinoensis*) seedlings by aphid *Melanocallis caryaefoliae* had a negative effect on the performance of later-coming conspecific aphids by altering the amino acid content in the phloem tissue of the plant. To my knowledge, however, there are no studies that have investigated the interaction between temporally separated AG and BG herbivores.

Plant roots are responsible for the uptake of nutrients and water necessary for the growth and development of the plant and are therefore an integral part of the plant. Like shoots, roots are equally challenged by a wide range of herbivores and pathogenic microbes. They receive much less consideration in ecological studies, however, probably because of the technical difficulties associated with the observation and analysis of BG interactions (van der Putten et al. 2001). Nevertheless, in recent years there have been a growing number of studies investigating a previously unseen BG world of root-herbivore interaction; several studies have already highlighted the defensive ability of roots at both the constitutive and the induced level (Bezemer et al. 2003; Bezemer and van Dam 2005; Rasmann et al. 2005; Kaplan et al. 2008b; Rasmann and Agrawal 2008; van Dam 2009; Wurst et al. 2010; Erb et al. 2012). A majority of plant species contain similar defensive compounds in roots as their AG counterparts (Rasmann and Agrawal 2008). In some plant species, roots function as a site for the biosynthesis of such compounds; for example, alkaloid nicotine is biosynthesized in the root tissue of tobacco plants (Baldwin 1989). As a BG plant part is less likely to be attacked by same foliar herbivores, roots might be a strategically safer site for the storage of primary and secondary metabolites and even their biosynthesis (Kaplan et al. 2008a; Babst et al. 2005). Therefore, the prominence of roots for the outcome of AG and BG plant-herbivore interaction cannot be overlooked. One of the main aims of this thesis is to investigate the AG herbivore-induced changes in root traits and their potential role in establishing indirect interaction between spatially and temporally separated AG and BG herbivores.

Factors affecting above- and belowground herbivore interactions

Several biotic and abiotic factors shape the direction and intensity of the outcome of plant-mediated interaction between herbivores. Such interaction may be facilitative, neutral, or detrimental to the herbivores, depending upon several factors. These include herbivore species, feeding guilds and their sequence of arrival on host plant; plant types, genotypes, and their defense strategy; availability of resources in the soil; and competition for acquisition of available resources (Bezemer et al. 2003; Wurst and van der Putten 2007; Kaplan et al. 2008a; Wurst et al. 2008; Erb et al. 2009, 2011; Johnson et al. 2012; Kutyniok and Müller 2012, 2013).

Plant's characteristics

It is not uncommon for different plant species to have evolved with different forms of defense strategies, but different genotypes of the same plant species have also been found to differ when interacting with herbivores. Such genetic variation within a single species increases the plants' ability to deal with diverse herbivores, thereby increasing the fitness of a plant species for survival (Karban 1989; Johnson and Agrawal 2005). On the other hand, genetic variations in plant species also exert selection pressure on herbivores to shape their competitive strength to co-exist with plants (Smith et al. 2008). While some studies have examined the significance of plant genotype for the specificity of AG plant-herbivore interactions (English-Loeb et al. 1998; Bingham and Agrawal 2010; Uesugi et al. 2013), very few studies have examined if it contributes to the outcome of AG and BG herbivore interactions (Hol et al. 2004; Uesugi et al. 2013; Wurst et al. 2008).

Herbivore's identity

Like plants, the dietary preferences and feeding modes of herbivores also play a crucial role in plant-insect interactions. The induction of a plant's response to herbivory results from a complex chain of physiological events following the first physical contact with herbivores. Plants perceive the presence of herbivores by movement, wound trauma inflicted by feeding, and chemical cues or elicitors present in herbivores' oral secretions that come in contact with wounded plant cells (Howe and Jander 2008; Bonaventure 2012). Therefore, the nature and intensity of a feeding injury and the chemical composition of the herbivore's saliva, which differs among herbivore species, are important indicators of a particular plant response. Based on dietary preference, herbivores are categorized in two major classes, *generalists* and *specialists*; based on the nature of their feeding, they are classified in two major feeding guilds, *chewing* and *sucking*.

Along with plant defense, herbivores have also evolved with the ability to counterdefend themselves and discriminate among suitable host plants, resulting in their dietary specialization as generalists or specialists (Ali and Agrawal 2012). Generalist herbivores are polyphagous with wider diet breadth, and they are generally considered to be sensitive to defensive compounds (Ali and Agrawal 2012). Hence, plants respond with broad-spectrum defense traits such as secondary metabolites, which are toxic to generalist herbivores even in small quantities. On the other hand, specialist herbivores (monophagous and oligophagous) have evolved with the ability to cope with plant defense by detoxifying or attenuating the milder concentration of defensive compounds, even using them as a chemical cue to locate the host plant (Whittaker and Feeny1971; Bernays and Chapman 2000; Wittstock et al., 2004; Ramsey et al., 2010; Karban and Agrawal 2002). To deal with the specialist herbivores, plants need to induce higher concentrations of dose-dependent defensive compounds, such as anti-nutritive PIs (Siemens and Mitchell-Olds 1996). Thus, feeding strategy is another determinant of plant-mediated interaction among herbivores, because plants optimize their response in accordance with their evolutionary relationship with herbivore species.

Another major classification of insect herbivores is based on their feeding modes. Coleopteran and lepidopteran insects, which make up two-thirds of herbivorous insect species, are equipped with chewing-, snipping-, or tearing-type mouthparts and thus cause noticeable wounding injuries (Walling 2000; Fürstenberg-Hägg et al. 2013). On the other hand, hemipteran insects, such as aphids and whiteflies, have piercing- and sucking-type mouthparts, which use their stylet to feed on phloem sap, causing minimal tissue injury (Walling 2000; Goggin 2007; Kempema et al. 2007). Plants are known to discriminate among these herbivores and thereby activate specific signaling pathways that regulate the defense response accordingly. Three major plant phytohormones, namely jasmonic acid (JA), salicylic acid (SA), and ethylene, are known to regulate the induction of plant defense. Furthermore, several previous studies have found that the chewing herbivores predominantly induce JA-dependent defense pathways, while sucking herbivores and plant pathogenic microbes induce SA- along with JA- and ethylene-dependent defenses (Walling 2000; Bari and Jones 2009). Many studies have also suggested cross talk between JA- and SA-dependent defense pathways, while a few have also evidenced their synergistic effect in defense regulation (Mewis et al. 2005; Bari and Jones 2009; Thaler et al. 2012). Therefore, the herbivores' feeding guild can be expected to have a significant impact on plant-mediated interaction between AG and BG herbivores. For example, a study by Wurst and van der Putten (2007) showed the negative effect of root-feeding nematodes (*Pratylenchus penetrans*) on the performance of phloem-feeding aphids (*Myzus persicae*), while no such effect was found on chewing *Chrysodeixis chalcites* larvae.

Sequence of arrival of herbivores

Another important factor that determines the outcome of interaction between AG and BG herbivores is the sequence of arrival of the herbivores on the plant. A meta-analysis of the studies on AG and BG herbivore interaction investigated the effect of sequence of arrival and concluded that the arrival of AG herbivores first on the host plants usually has negative effects on the performance of BG herbivores (Johnson et al. 2012). For example, in one of such study, Erb et al. (2011) found the negative effect of foliar herbivore *Spodoptera frugiperda* on root-feeding *Diabrotica virgifera virgifera* larvae only when *S. frugiperda* were added first on the teosinte (*Zea mays mexicana*) and cultivated maize (*Zea mays mays*) plants. Early arrival on the plant may be an advantageous strategy of the herbivore to avoid competition and induced defense, as the plant is in a naïve state before the commencement of herbivory. On the other hand, the activation of defense by early herbivores is more likely to be detrimental for the subsequent herbivore that feeds on the same plant. In addition to induction of defense, earlier herbivory may also prime the plant for an accelerated and efficient response to subsequent herbivores (Conrath et al. 2006; Karban 2011; Hilker et al. 2015).

Thesis outlines and research objectives

In recent years, the importance of studies on plant-mediated interaction between AG and BG herbivores has gathered greater recognition in the field of ecology, but the majority of the research has focused on the plant-mediated indirect effect of BG herbivores on AG herbivores. Few have studied such effects from another perspective. By testing several model systems under greenhouse conditions, I intended to provide significant advances in the field of plant-mediated indirect interactions between spatially and temporally separated AG and BG herbivores, specifically in AG to BG direction.

The main objectives of this thesis are the following: 1) to elucidate the plant-mediated indirect effect of earlier transient AG herbivory on spatially and temporally separated BG herbivores; 2) to investigate if such interactions between AG and BG herbivores depend upon factors such as plant genotypes and the herbivores' identity; and 3) to investigate a potential priming response of the plant as a result of sequential AG and BG herbivory.

To achieve these objectives, I challenged three different Solanaceous plant species with different sets of AG and BG herbivores in a series of greenhouse experiments. The events of AG and BG herbivory were separated with a lag phase (a period without any herbivory) of five or seven days, both to assess the effect of transient AG herbivory on temporally separated BG herbivory and to provide an opportunity for the plant to be primed. Upon harvest, the performance of BG herbivores was assessed by measuring changes in their mass (in the case of root-feeding larvae) or number of galls (in the case of root-knot nematodes). Depending upon the model organisms used in the experiment, different resistance and tolerance traits, such as secondary metabolites (protease inhibitors, nicotine), phytohormones (JA and SA), shoot and root biomass, and primary metabolites (proteins, C and N) were measured in the plants as these traits are known to mediate the interaction between plants and BG herbivores. I have discussed the results of my experiments in the following chapters:

Chapter 2

In the study presented in chapter 2, two genotypes of the perennial plant bittersweet nightshade (*Solanum dulcamara*) were used to determine whether genetic variation in the host plant species affects the outcome of AG and BG herbivore interactions. In a full factorial greenhouse experiment, plants were challenged with generalist foliar feeding caterpillars (*Spodoptera exigua*, Noctuidae) and root-feeding wireworms (*Agriotes* spp., Elateridae) sequentially with a lag phase of seven days.

Chapter 3

In the study presented in chapter 3, tomato (*Solanum lycopersicum*) plants were challenged with either of two AG herbivores from different feeding guilds, namely sap-sucking green peach aphids (*Myzus persicae*, Aphididae) and chewing beet armyworms (*S. exigua*), to see their indirect impact on performance of plant parasitic root-knot nematodes (*Meloidogyne incognita*, Heteroderidae). I also investigated whether the identity/feeding guilds of AG herbivores affect such interactions and potential priming responses. The events of AG and BG herbivory were separated by a lag phase of seven days.

Chapter 4

In the studies presented in chapter 4, wild tobacco (*Nicotiana attenuata*) plants were challenged with chewing caterpillars (*Manduca sexta*, Sphingidae) followed by root-knot nematodes (*M. incognita).* I performed three different experiments: short-term, long-term, and transgenerational. The first two experiments aimed to investigate the short- and long-term effects of transient AG herbivory on performance and fitness of plants and BG nematodes. In the transgenerational experiment, the progeny plants were grown from the seeds of the plants of the long-term experiment to test if herbivory in the parental plants increased the resistance of the progeny plants to herbivory via transgenerational priming response.

Chapter 2

Genetic variation of the host plant species matters for interactions with above- and belowground herbivores

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Chapter 2

Genetic variation of the host plant species matters for interactions with above- and belowground herbivores

Abstract

Plants are challenged by both above- and belowground herbivores which may indirectly interact with each other via herbivore-induced changes in plant traits; however, little is known about how genetic variation of the host plant shapes such interactions. We used two genotypes (M4 and E9) of *Solanum dulcamara* (Solanaceae) with or without previous experience of aboveground herbivory by *Spodoptera exigua* (Noctuidae) to quantify its effects on subsequent root herbivory by *Agriotes* spp. (Elateridae). In the genotype M4, due to the aboveground herbivory, shoot and root biomass was significantly decreased, roots had a lower C/N ratio and contained significantly higher levels of proteins, while the genotype E9 was not affected. However, aboveground herbivory had no effects on weight gain or mortality of the belowground herbivores. Root herbivory by *Agriotes* increased the nitrogen concentration in the roots of M4 plants leading to a higher weight gain of conspecific larvae. Also, in feeding bioassays, *Agriotes* larvae tended to prefer roots of M4 over E9, irrespective of the aboveground herbivore treatment. Fourier-Transform Infrared Spectroscopy (FT-IR) documented differences in metabolic profiles of the two plant genotypes and of the roots of M4 plants after aboveground herbivory. Together, these results demonstrate that previous aboveground herbivory can have genotype-specific effects on quantitative and qualitative root traits. This may have consequences for belowground interactions, although generalist root herbivores might not be affected when the root biomass offered is still sufficient for growth and survival.

Chapter 3

Sequential above- and belowground herbivory modifies plant responses depending on herbivore identity.

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Sequential above- and belowground herbivory modifies plant responses depending on herbivore identity.

Abstract

Herbivore-induced changes in plant traits can cause indirect interactions between spatially and/or temporally separated herbivores that share the same host plant. Feeding modes of the herbivores is one of the major factors that influence the outcome of such interactions. Here, we tested whether the effects of transient aboveground herbivory for 7 days by herbivores of different feeding guilds on tomato plants (*Solanum lycopersicum*) alters their interaction with spatially as well as temporally separated belowground herbivores. The transient aboveground herbivory by both chewing caterpillars (*Spodoptera exigua*) and sucking aphids (*Myzus persicae*) had significant impacts on plant traits such as plant growth, resource allocation and phytohormone contents. While the changes in plant traits did not affect the overall performance of the root-knot nematodes (*Meloidogyne incognita*) in terms of total number of galls, we found that the consequences of aboveground herbivory for the plants can be altered by the subsequent nematode herbivory. For example, plants that had hosted aphids showed compensatory growth when they were later challenged by nematodes, which was not apparent in plants that had hosted only aphids. In contrast, plants that had been fed by *S. exigua* larvae did not show such compensatory growth even when challenged by nematodes. The results suggest that the earlier aboveground herbivory can modify plant responses to subsequent herbivores, and such modifications may depend upon identity and/or feeding modes of the aboveground herbivores.

Introduction

Plants respond with morphological, physiological and biochemical changes in their resistance and tolerance traits to deal with herbivores and herbivory stress (Gatehouse 2002; Kessler and Baldwin 2002; Schwachtje et al. 2006; Howe and Jander 2008). Besides responses in local tissues which are being attacked, herbivory induces numerous changes in more distant systemic tissues, which can cause indirect interactions between spatially, and in some cases, temporally separated herbivores. Thereby, plants can even mediate indirect interactions between phytophagous organisms living above- and belowground (Masters et al. 1993; Blossey and Hunt-Joshi 2003; Bezemer et al. 2003; Bezemer and van Dam 2005; Ohgushi 2005; Wurst and Ohgushi 2015).

Although defensive quality of root has been analyzed less than that of aboveground plant parts, several plant species are known to systemically induce defensive compounds in roots following aboveground herbivory which may protect them from belowground herbivores (Rasmann and Agrawal 2008; Bezemer et al. 2003; Bezemer and van Dam 2005; van Dam 2009; Erb et al. 2012). Along with chemical defense, plants may also employ tolerance strategies to deal with herbivory, such as altered photosynthetic rates, compensatory growth, increased tillering, and reallocation of primary metabolites and minerals (van der Meijden et al. 1988; Strauss and Agrawal 1999). Plants fine-tune their resistance and tolerance ability in order to optimize plant fitness; therefore, they may or may not employ both strategies simultaneously (van Dam 2009). Any of the systemic changes in root tissue due to aboveground herbivory, either in resistance or tolerance traits, may significantly impact the performance of subsequent belowground herbivores (Kaplan et al. 2008a; Johnson et al. 2009; Huang et al. 2013). Recent studies also suggest that sequential herbivory events may result in the priming of plant responses which is a preconditioning by earlier herbivory that enables plants to deal with future herbivores more efficiently (Conrath et al. 2006; Frost et al. 2008; Karban 2011). Overall, the aboveground herbivore-induced changes in root tissue can be detrimental, neutral or facilitative to the belowground herbivores depending upon several factors such as herbivore species, their feeding guild, plant species, genotypes and defense strategies (Bezemer et al. 2003; Wurst and van der Putten 2007; Kaplan et al. 2008a; Wurst et al. 2008; Erb et al. 2008, 2011; Johnson et al. 2012; Kutyniok and Müller 2012, 2013).

One of the significant determinants of the outcome of above- and belowground herbivore interactions is the feeding mode of the herbivores. Chewing and sucking are two major feeding modes of herbivores. Coleopteran and lepidopteran insects are equipped with chewing or tearing-type mouthparts causing severe wounding injury whereas hemipteran insects such as aphids and whiteflies are equipped with piercing and sucking mouthparts to ingest the phloem-sap causing minimal injury on plant tissue (Walling 2000; Goggin 2007; Kempema et al., 2007). Wound trauma inflicted by the feeding damage and type of elicitors present in oral secretion of herbivores are two major cues that regulate the induction of specific resistance or tolerance responses of the plant (Howe and Jander 2008; Bonaventure 2012). Therefore, the feeding mode and the identity of the herbivore are key factors in plant-insect interactions as they determine specific activation patterns of plant signaling pathways that regulate a plant’s response. Plant responses upon herbivory are mainly regulated by three phytohormones, jasmonic acid (JA), salicylic acid (SA) and ethylene, which are also known to play essential roles for the growth and development of the plant. A large body of evidences suggests that chewing herbivores primarily activate JA-dependent defense pathways whilst sucking herbivores induce predominantly SA- along with JA- and ethylene-dependent pathways similar to the responses induced by plant pathogenic microbes (Walling 2000, Bari and Jones 2009). But, it is important to note that their activation is highly species-specific and not limited to particular feeding guilds. Several studies have shown the activation of SA-dependent responses upon chewing herbivores and activation of JA-dependent responses upon sucking herbivores; and the phytohormones may interact antagonistically or synergistically with each other (Mewis et al. 2005; Bari and Jones 2009; Thaler et al. 2012).

Here, we aimed to compare the effects of aboveground herbivory by insects from two feeding guilds (chewing caterpillars and sucking aphids) on the plant’s interaction with spatially and temporally separated belowground root-knot nematodes. Root-knot nematodes (*Meloidogyne*) are endoparasites which, with the help of special gland secretions, stimulate the root cells to grow into ‘giant cells’ (root-knots or galls) that serve as a feeding site (Williamson and Hussey 1996). By inducing galls and feeding on the root tissue, root-knot nematodes weaken the ability of the root to take up water and nutrients which impairs plant performance and fitness (Milligan et al. 1998). Although nematodes do not feed by sucking up phloem sap like aphids, their feeding strategies and salivary composition have noticeable similarities (Bird and Kaloshian 2003;

Carolan et al. 2011) and both are sensitive to plant resistance traits mediated by the same gene, *Mi-1* (Rossi et al. 1998) which is found in tomato (*Solanum lycopersicum*). Furthermore, subsequent studies found that the SA signaling pathway is essential for *Mi-1*-mediated defense responses, suggesting its inducibility (Branch et al. 2004; Li et al. 2006). Therefore, aphids, nematodes and tomato plants are an interesting model system to study plant-mediated interactions between above- and belowground herbivores.

Herbivory on tomato foliage induces a wide array of defensive proteins such as anti-nutritive polyphenol oxidase (PPO) and protease inhibitors (PIs), and volatile compounds that attract the natural enemies of herbivores as a mean of indirect defense (Duffey and Stout 1996; Bhonwong et al. 2009; Dicke et al. 1998). Commercial tomato cultivars are known to contain the *Mi* locus with two highly homologous genes, *Mi-1.1* and *Mi-1.2* (Milligan et al. 1998) which confer resistance against aphids (Rossi et al. 1998; Vos et al. 1998), whiteflies (Nombela et al. 2003) and root-knot nematodes including *Meloidogyne incognita* (Kaloshian et al. 1995; Milligan et al. 1998). Therefore, in our study, we hypothesized that earlier transient aboveground herbivory by aphids would have a more pronounced impact on nematodes because of activation of the same defense pathway than transient chewing herbivory by caterpillars.

To differentiate between the effects of plant responses to herbivores of different feeding modes on the plant's interaction with root-knot nematodes (*Meloidogyne incognita*, Heteroderidae), we used the sap-feeding green peach aphids (*Myzus persicae*, Aphididae) and the chewing beet armyworm (*Spodoptera exigua*, Noctuidae). Using tomato (*S. lycopersicum,* Solanaceae var. MicroTom) as a model plant, we aimed to investigate: i) if transient aboveground herbivory has any effect on spatially and temporally separated belowground herbivores; ii) if such sequential herbivory affects plant traits differently than single herbivory and whether a priming of plant responses may be involved; and iii) if such effects differ between the two herbivore species exhibiting different feeding modes. To answer these questions, we carried out a greenhouse experiment in which tomato plants were exposed to transient herbivory by either aphids, caterpillars or no aboveground herbivores, followed by nematode infestation or not. We separated the events of above- and belowground herbivory by a lag phase (a period without any herbivory) to assess the effect of transient aboveground herbivory on temporally separated belowground herbivores.

Materials and methods

Plant Material

Before germination, the seeds of tomato (*S. lycopersicum*) were surface-sterilized with 70% ethanol followed by mixture of 5.25% (w/v) sodium hypochlorite and 0.1% Polysorbate 20 (Tween 20). Then, the seeds were rinsed with deionized water and sown on paper towels in plastic boxes and kept in the greenhouse at 26°C for a week to germinate. The seedlings of about 2 cm size were transplanted to seedling trays for a month before finally being transferred to 1 l ($13\times11\times9$ cm^3) plastic pots (Pöppelmann GmbH & Co. KG, Lohne, Germany) containing 850 ml of steamed soil. The soil was collected from a research site of Freie Universität Berlin (Albrecht-Thaer-Weg) and sieved to remove the remains of roots and pebbles. The sieved soil was steamed for three hours at 90 °C using a Sterilo steamer (Harter Elektrotechnik, Schenkenzell, Germany) to exclude root herbivores. Pots were placed on individual plastic plates and the top layer of the soil was covered with sand grit to prevent the growth of green algae and infestation by fungus gnats (Sciaridae). The plants were assigned to different treatments after 3 weeks of growth in pots. During the experiment, plants were watered three times a week with 150 ml of water and randomized weekly to homogenize for variances due to abiotic factors such as light conditions.

Study Insects

The green peach aphid (*M. persicae*) individuals used in this experiment were obtained from the aphid rearing of the Julius Kühn-Institute, Berlin. The larvae of beet armyworm *S. exigua* were obtained from the laboratory cultures maintained at the Freie Universität Berlin. They were reared on artificial diet (wheat germ based basic diet with a vitamin mix) in a climate chamber at 24 °C and 70% humidity under 16/8 hour day/night light cycle. Second-stage juveniles (J2s) of root-knot nematodes *M. incognita* were obtained in aqueous suspension from a biological supply company, HZPC Holland B. V. (Hettema Zaaizaad en Pootgoed Coöperatie, Metslawier, The Netherlands).

Herbivory Treatments

For the herbivory treatments, a total of 90 healthy and homogeneous plants were selected. Plants were subjected to six different treatments with 15 replicates each: control with no herbivory (C), aboveground herbivory with *M. persicae* aphids (Aph) or *S. exigua* larvae (Spo), belowground herbivory with *M. incognita* nematodes (Nem), and sequential above- and belowground herbivory treatments (Aph+Nem and Spo+Nem) where nematodes were added to the root of the aboveground herbivore-treated plants following a lag phase of seven days. For the aboveground herbivory treatments, the three youngest, fully expanded leaves were chosen on every plant. In the treatments with the chewing herbivore, one third instar *S. exigua* larva was added in a mesh bag and allowed to feed on the first leaf for three days starting with the oldest among the three chosen leaves. The larva was then transferred successively to the second and the third leaf to feed for another two days on each. This way, larvae fed on three consecutive leaves for a total of seven days. In the treatments with the sucking herbivore, four individuals of *M. persicae* were added on each of the three leaves which were covered with a mesh bag. Aphids were allowed to feed on leaves for seven days and then removed carefully using a fine brush without damaging the leaves. After the removal of aboveground herbivores, the plants were kept for a lag phase of seven days without herbivory. Then, about 1875 second stage juveniles (J2's) of root-knot nematodes *M. incognita* were added per pot as belowground herbivore to the roots of half of the aboveground herbivore-treated and half of the control plants. The nematodes were applied in an aqueous suspension in three holes (depth 5 cm) perforated into the soil at a distance of 3 cm from the stem. These plants were treated for 14 days with the nematodes allowing them to infest the roots and induce root galls before harvest. Upon harvest, leaf and root subsamples were collected for the phytohormone analysis. The numbers of galls induced by the nematodes were counted in three different size classes (<1mm, 1-2 mm and >2mm) manually after keeping them submerged in water to facilitate the counting. The root (including galls) and shoot materials were then dried in an oven at 55 °C for three days before measuring the dry mass.

Sampling and Measurement of Phytohormone

For the phytohormone measurement, the roots of the harvested plants were washed immediately after harvest and 150–180 mg of representative fine root samples were separated and weighed. A similar amount was also collected of leaf samples from the youngest fully expanded leaf by cutting it transversely into small pieces. The leaf and root samples were kept in 2 mL screw-cap tubes, flash frozen in liquid nitrogen and stored at −80 °C until extraction. Extraction and quantification of ABA, SA, JA and JA-isoleucine (JA-Ile) was done following the procedure explained in Nguyen et al. (2016). In brief, root and leaf samples were homogenized within the tubes using FastPrep homogenizer (FastPrep®-24, MP Biomedicals, Santa Ana, CA, USA) along with 1 ml extraction solution, containing ethyl-acetate and internal deuterated standard mix: 20 ng of D4-SA, D6-ABA (OlChemIm Ltd., Olomouc, Czech Republic) and D6-JA-Ile and 60.4 ng of D6-JA (HPC Standards GmbH, Cunnersdorf, Germany). Supernatant was collected after centrifuging the homogenized samples for 5 min at high speed (18,000 x g). Samples were extracted a second time with 1 mL pure ethyl-acetate, then supernatants were combined and dried in a Vacufuge (Eppendorf, Hamburg, Germany). The dried samples were re-eluted in 400 µL of 70% (v/v) methanol (MeOH) and 0.1% acetic acid by shaking 10 minutes at room temperature. The re-eluted extracts were subjected to a UPLC-ESI-MS/MS Synapt G2-S HDMS (Waters, Milford, Massachusetts, USA) for identification and quantification of phytohormones as described in Nguyen et al. (2016). The peak area integration was performed using MassLynx Software v. 4.1 (Waters, Milford, Massachusetts, USA). The amount of hormone per g of sample fresh weight was calculated by comparing the peak area of the plant derived hormone in a given sample with the corresponding peak area of the deuterated internal standard in the same sample. From the pool of 15 replicates, eight replicates from each treatment were chosen randomly for hormonal measurement.

Carbon and Nitrogen Concentration Measurement

Dried leaf and root materials were ground in Eppendorf tubes by using a mixer mill (Mixer Mill MM 400, Retsch GmbH, Haan, Germany) and dried again for at least 24 hours. Then, their carbon and nitrogen concentration were determined by using an elemental analyzer (Euro EA, HEKAtech GmbH, Wegberg, Germany).

Statistical Analysis

All the statistical analyses were performed in ‘R’, version 3.2.2 (R Core Team 2015). One-way and two-way factorial ANOVAs were performed to test the significance of the treatments; aboveground herbivory (AGH), belowground herbivory (BGH) and their interactions (AGH*BGH). Statistical significance was set at $P < 0.05$. All the data were checked for normality and homogeneity of variances using Shapiro-Wilk test and Bartlett test, respectively, to make sure that they meet the assumptions of ANOVA. The data of number of galls and root C concentration were transformed using log and square transformation, respectively, while the data of shoot biomass and root C/N ratio was transformed using inverse transformation before being checked for assumptions of ANOVA. The phytohormone data were analyzed with Generalized Linear Models (GLM) assuming gamma distribution of errors as the data were not normally distributed. Means and standard errors (SE) are reported in the result section. To determine the effects of the particular aboveground herbivores, the means were additionally compared with Tukey HSD test as post-hoc analysis.

Results

Number of galls induced by nematodes

The total number of galls and number galls per mg of root tissue induced by nematodes did not differ between the treatments. There was a significant reduction of the number of small galls per mg of root tissue (< 1 mm) in the plants previously treated with aphids compared to plants treated with nematodes only ($p = 0.01$) while total number of small galls (not corrected for root mass) tended to be reduced ($p = 0.07$) (Suppl. fig.1).

Plant biomass

Shoot biomass: Both above- and belowground herbivory had significant main and interaction effects on shoot biomass (AGH: $F_{[2,\ 84]} = 16.58$, $p = 0.001$; BGH: $F_{[1,\ 84]} = 5.05$; $p = 0.027$; AGH*BGH: $F_{[2,\ 84]} = 9.08$; $p < 0.001$). When applied alone, both aphid and *S. exigua* herbivory reduced the shoot biomass. The negative effect of *S. exigua* remained stable under single or sequential herbivory exposure; the negative effect of aphid herbivory was abolished when followed by nematode infestation although nematode infestation alone did not significantly affect shoot biomass (fig. 1a).

Root biomass: Aboveground herbivory had no significant main effect on root biomass, while belowground herbivory significantly reduced root biomass, which was significantly affected by the interaction with aboveground herbivory (AGH: $F_{[2,\ 84]} = 1.13$, $p = 0.33$; BGH: $F_{[1,\ 84]} = 7.07$; $p = 0.001$; AGH*BGH: $F_{[2,\ 84]} = 6.20$; $p = 0.003$). Earlier *S. exigua* herbivory followed by the nematode treatment reduced the root biomass by about 25% as compared to *S. exigua* alone and control plants; aphids and nematodes alone and in combination did not significantly differ from the control (fig. 1b).

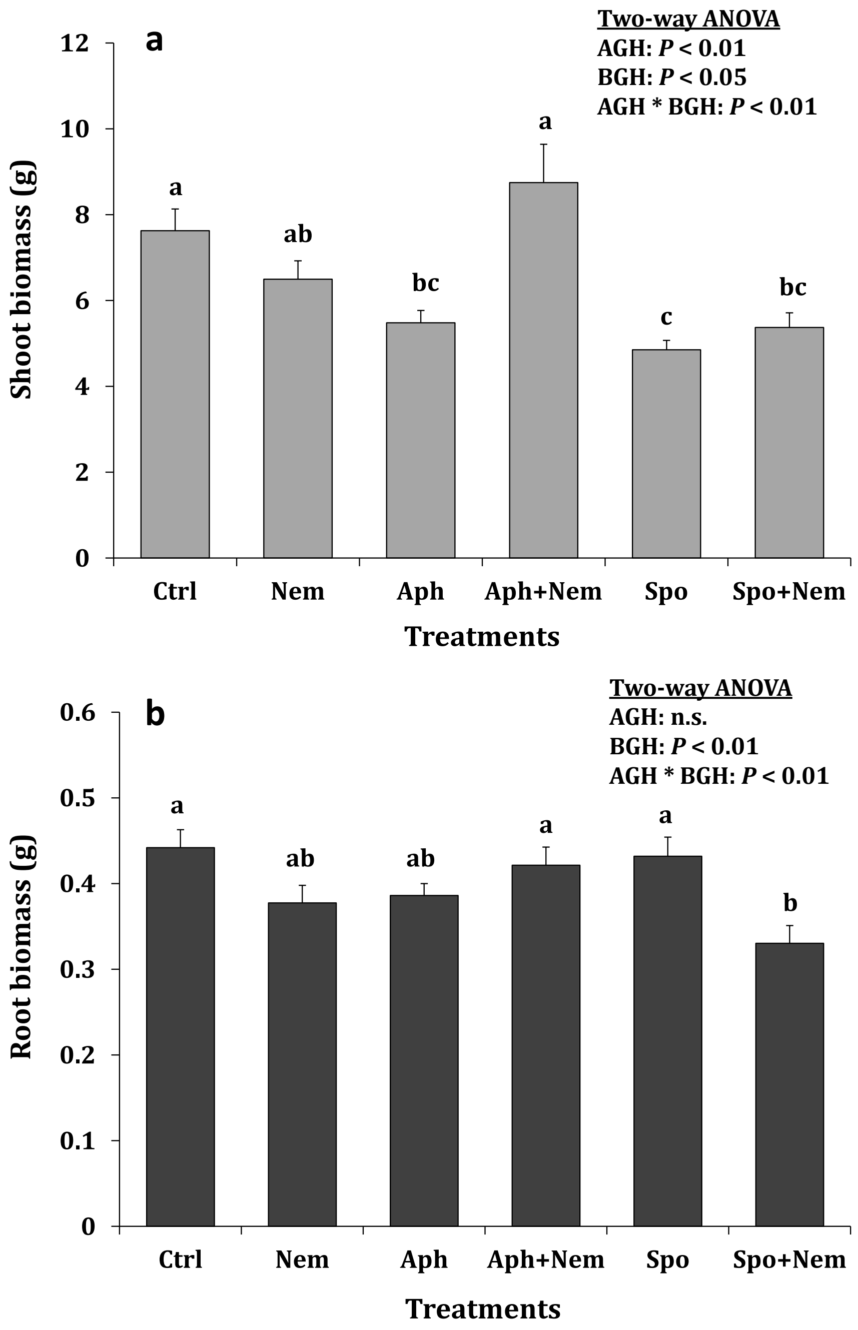

Figure 1. Shoot (a) and root (b) biomass (mean ± SE; n=15) of the tomato plants following herbivory treatments. Treatments: Ctrl: control (no herbivory), Nem: nematode only, Aph: aphids only, Aph+Nem: aphids followed by nematodes, Spo: *S. exigua* larvae only, Spo+Nem: *S. exigua* larvae followed by nematodes. Different letters above the bar indicate the significant difference in their mean (Tukey HSD test: P<0.05). Aboveground herbivory (AGH) was applied for a week and belowground herbivory (BGH) was applied for two weeks while there was a lag phase of a week between AGH and BGH in sequential herbivory treatments.

Carbon and Nitrogen Concentration

We measured the changes in C and N concentration in leaf and root tissue following herbivory to estimate changes in allocation of these major constituents of plant metabolites and because plant as well as herbivore performance parameters are known to depend on C/N contents.

Leaf C and N concentration: None of the herbivory treatments had any significant effect on the foliar C concentration. Aboveground herbivory had a significant main effect on leaf N concentration and a significant interaction effect with belowground herbivory as *S. exigua* feeding increased foliar N which was stronger and only significant when its herbivory was followed by nematode infestation. Belowground herbivory alone had no effect on foliar N concentration (table 1).

Root C and N concentration: Both above- and belowground herbivores had main and interaction effect in root C and N concentration (table 1). The *S. exigua* herbivory reduced the C concentrations in the root tissues, while the nematode treatment after *S. exigua* herbivory abolished this effect. Nematodes alone increased root N concentration compared to control plants. This effect was still present in plants previously damaged by *S. exigua*, but the nematodes had no effect on root N concentration if plant were fed by aphids earlier. Aphids or *S. exigua* alone had no effect on root N concentration.

C/N ratio: As the C concentration was similar in all treatments; the change in leaf C/N ratio was dependent on changes in leaf N concentration and therefore had similar patterns as leaf N concentration (table 1). Plants treated with *S. exigua* followed by nematode decreased the C/N ratio of the leaves but these herbivores alone had no effects. Similarly, there were significant main and interactive effects of both above- and belowground herbivores on the C/N ratio of the roots. Single herbivory by *S. exigua* and nematode, and sequential herbivory by *S. exigua* followed by nematodes decreased the C/N ratio in the roots as compared to control plants.

Table 1. The effect of above- and belowground herbivory treatments on C and N concentration (percentage) and their ratios in leaves and roots of the tomato plants

Tissue		Concentration (Mean ± SE; n=15)					
		Ctrl	Nem	Aph	Aph+Nem	Spo	Spo+Nem
Leaf	C	38.99±0.36 ^{a}	38.60±0.38 ^{a}	38.66±0.43 ^{a}	38.37±0.5 ^{a}	38.61±0.38 ^{a}	38.56±0.35 ^{a}
	N	2.89±0.08 ^{b}	2.92±0.10 ^{b}	2.98±0.08 ^{ab}	2.75±0.04 ^{b}	3.03±0.09 ^{ab}	3.27±0.08 ^{a}
	C/N	13.63±0.41 ^{a}	13.41±0.41 ^{a}	13.11±0.38 ^{ab}	14.00±0.25 ^{a}	12.94±0.41 ^{ab}	11.88±0.29 ^{b}
Root	C	41.86±0.56 ^{ab}	43.37±0.42 ^{a}	41.06±0.69 ^{b}	43.05±0.43 ^{ab}	37.94±0.51 ^{c}	42.64±0.56 ^{ab}
	N	2.46±0.08 ^{b}	3.02±0.04 ^{a}	2.61±0.07 ^{b}	2.68±0.06 ^{b}	2.53±0.06 ^{b}	3.08±0.05 ^{a}
	C/N	17.18±0.48 ^{a}	14.38±0.21 ^{cd}	15.81±0.36 ^{abc}	16.15±0.37 ^{ab}	15.1±0.39 ^{bcd}	13.88±0.21 ^{d}

		ANOVA results					
		AGH		BGH		AGH:BGH	
		F	*P*	*F*	*P*	*F*	*P*
Leaf	C	0.267	0.766	0.542	0.464	0.094	0.911
	N	7.127	**0.001**	0.047	0.8281	4.279	**0.017**
	C/N	6.418	**0.003**	0.194	0.6606	3.56	**0.033**
Root	C	9.706	**<0.001**	38.31	**<0.001**	4.751	**0.011**
	N	3.28	**0.042**	61.39	**<0.001**	10.45	**<0.001**
	C/N	12.58	**<0.001**	20.04	**<0.001**	9.95	**<0.001**

(AGH. *df*: 2, 84; BGH. *df*: 1, 84; AGH:BGH. *df*: 2, 84). Bold fonts indicate the significant effects (P<0.05) of the treatments. AGH and BGH stand for above- and belowground herbivory respectively. Means ± SE followed by different letters are significantly different from each other (Tukey HSD test: P<0.05).

Phytohormone induction

There were significant main effects of the above- and belowground herbivores on both salicylic acid (SA) and jasmonic acid (JA) content of the leaf tissues at a time point that was three weeks after the aboveground herbivory and after two weeks of exposure to nematodes. Nematodes had a significant negative main effect on leaf SA content (fig. 2a), while nematodes either alone or following *S. exigua* herbivory increased the leaf JA content, which did not occur on plants previously infested with aphids (fig. 2b). Above- and belowground herbivores had significant main effects and interaction effects on root SA content, while the root JA content was affected by the aboveground herbivores only. Whereas nematodes and *S. exigua* alone and in combination significantly reduced SA contents in the roots, previous aphid herbivory abolished this effect of nematodes on root SA (fig. 2c). On the other hand, *S. exigua* larvae alone or followed by nematodes decreased root JA content compared to control plants (fig. 2d).

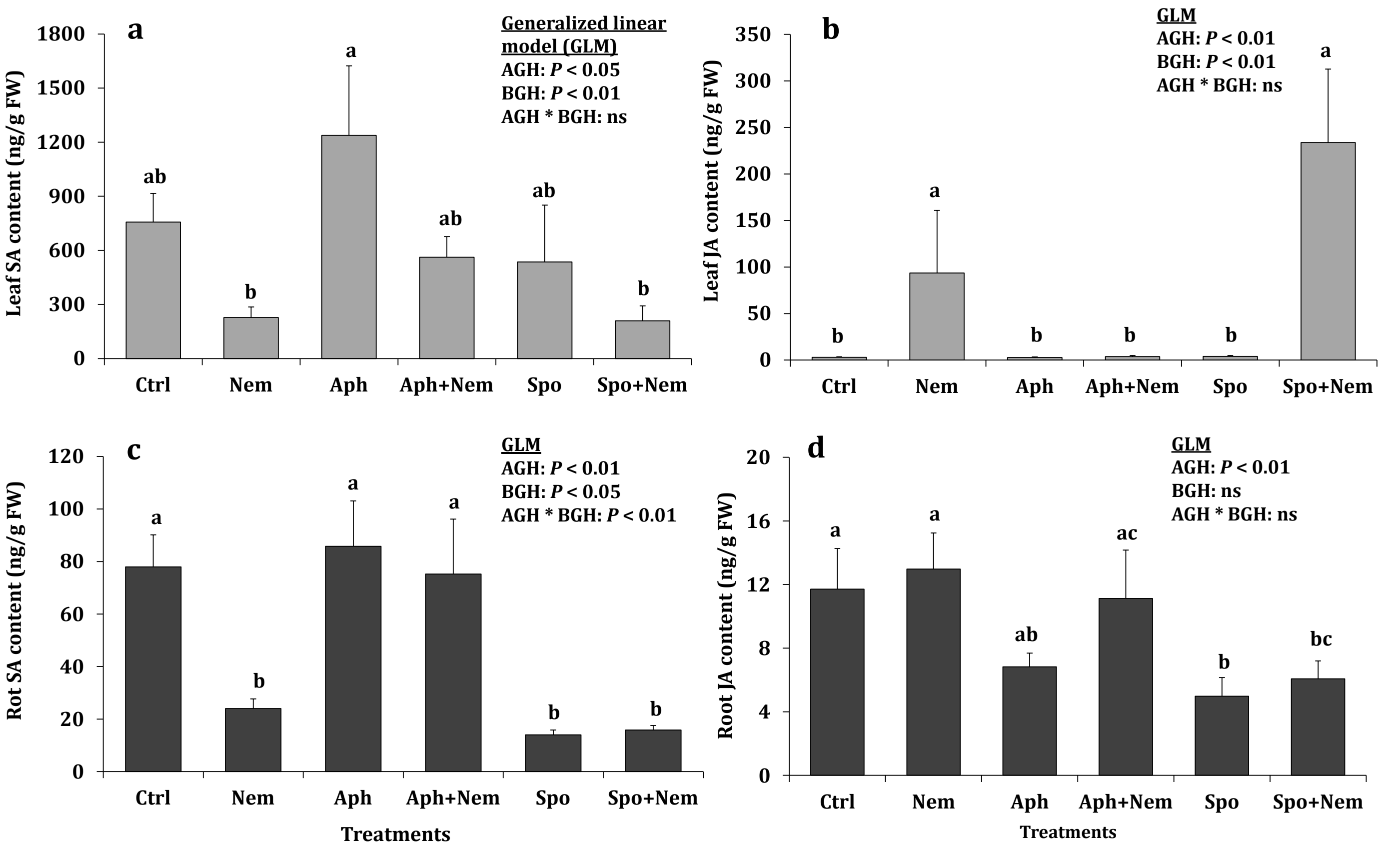

Figure 2. Leaf (a and b) and root (c and d) SA and JA content of the tomato plants (mean ± SE; n=8) following herbivory treatments. Treatments: Ctrl: control (no herbivory), Nem: nematode only, Aph: aphids only, Aph+Nem: aphids followed by nematodes, Spo: *S. exigua* larvae only, Spo+Nem: *S. exigua* larvae followed by nematodes. Different letters above the bar indicate the significant difference in their mean. Aboveground herbivory (AGH) was applied for a week and belowground herbivory (BGH) was applied for two weeks while there was a lag phase of a week between AGH and BGH in sequential herbivory treatments.

Discussion

Our study demonstrated that the effects of transient aboveground herbivory by both chewing and sucking herbivores on root and shoot parameters, nutrient allocation and the activation of signaling components (phytohormones) interacted with the effects of a later root infestation by nematodes. However, the consequences of aboveground herbivory on plant traits had no major effect on overall nematode performance (in terms of number of galls), despite plants previously exposed to aphids showed a reduced number of small galls per unit root mass. Further, sequential aphid and nematode herbivory led to compensatory growth suggesting a tolerance response. The way in which the plant response was altered by the sequential herbivory, was different for the two aboveground herbivores highlighting the significance of the herbivores' identities for plant-mediated interactions between them. As the two herbivores used in this experiment exhibit different feeding modes, the plants' distinct response to them could in part have resulted from the different feeding modes (Walling 2000, Bari and Jones 2009).

Effect of transient aboveground herbivory on belowground herbivores

A recent meta-analysis suggests that the aboveground herbivore, if it arrives first on the plant, is expected to have negative effects on the performance of belowground herbivores (Johnson et al. 2012); however we found no negative effects of aboveground herbivores on the performance of nematodes in terms of number of galls. For example, in an experiment with cultivated and wild maize plants (*Zea mays mays* and *Z. mays mexicana*), Erb et al. (2011) showed that feeding by the aboveground chewing herbivore *Spodoptera frugiperda* had a significant negative effect on the root chewing herbivore *Diabrotica virgifera* in terms of root colonization and weight gain, but only if *S. frugiperda* was added first to the plant. Thus, we expected an overall negative effect of aboveground herbivores, which were added first on the plant, on the belowground herbivore. Additionally, we expected even stronger responses of the plants treated first with aphids, as tomato plants are known to respond with a similar arsenal of defenses against aphids and nematodes, namely the *Mi-1* gene dependent defense responses which require SA signaling (Branch et al. 2004; Li et al. 2006). However, although *Mi-1* gene was found in commercial tomatoes, some tomato varieties lack it (Branch et al. 2004) and it remains unclear whether the MicroTom cultivar contains it. While we were not able to detect elevated SA levels in roots or shoots of plants that had been exposed to aphids three weeks earlier, we unexpectedly found reduced levels of root SA in nematode-infested plants and in plants that had been attacked by *S. exigua* and nematodes. Assuming that these lower SA levels in plants exposed to nematodes with and without earlier herbivory by *S. exigua* resulted in a reduced root resistance due to a lack of SA-mediated defense, the high basal root SA levels found in plants that had been previously attacked by aphids may have contributed to the reduced number of galls smaller than 1 mm on the plants with sequential aphid and nematode herbivory. The finding of a negative effect of nematodes on root SA that was negated on plants with earlier aphid herbivory highlights the effect of earlier aboveground herbivory on plant response to subsequent belowground herbivores.

Plant response upon above- and belowground herbivory

Both above- and belowground herbivores had significant effects on plant growth that differed in direction and magnitude. While *S. exigua* herbivory reduced the shoot biomass independent of a later nematode infestation, the negative effect of aphid herbivory on shoot biomass was abolished, when subsequently nematodes fed on the same plants. This suggests that aphid-treated plants showed a compensatory growth of shoots upon nematode herbivory, while *S. exigua* larvae-treated plants did not compensate for the loss in biomass upon nematode herbivory. Nematode addition may have facilitated a tolerance response of the tomato plants such as a compensatory growth to replenish the biomass loss due to aphid herbivory. Tolerance responses are considered as cost effective means to deal with biotic and abiotic stresses as compared to induction of costly resistance traits (van der Meijden et al. 1988). Some common tolerance responses include the increase in photosynthetic and growth rates, increased tillering, and reallocation of resources (Strauss et al. 1999).

Regarding the allocation pattern of C and N in leaf and root tissues, most noticeable effects were found in the N concentration of the plants subjected to sequential above- and belowground herbivory: plants previously exposed to *S. exigua* and followed by nematode infestation contained higher N concentration in both leaf and root tissue. In root tissue, nematodes also increased the N concentration but not when the plants had been previously exposed to aphid feeding. Interestingly, the direction of change in N concentration was opposite of the changes in biomass of the *S. exigua* followed by nematode infested plants. These results suggest that the nutritional quality may be improved in the shoot and root tissues of the plants whose biomass was decreased in the *S. exigua* followed by the nematode treatment. Systemic nutrient translocation to and away from the site of herbivory is another well-known tolerance response of the plants upon herbivory. Plants allocate carbon and nitrogen in specific cells and tissues to be used for compensatory growth or defense of valuable plant parts which are critical for survival and reproduction (Creelman and Mullet 1997). In addition, such diversion of nutrients results in poor nutritional quality of the feeding site with possible negative effects on growth of herbivores (Babst et al. 2005; Schwachtje et al. 2006; Kaplan et al. 2008a; Gomez et al. 2010, 2012). Further, increased N in both shoot and root tissues in plants treated with *S. exigua* and later with nematodes may indicate the acquisition of additional N from the soil pool to meet the increased demand of N for either compensatory growth or for biosynthesis of N-based defense compounds such as PIs. However, such potential increase in N compounds did not contribute to resistance against nematodes.

We measured the defense-regulatory phytohormones JA and SA which may allow some estimation on the level of induced defense in the leaf and root tissue upon above- and belowground herbivory. The defensive functions of SA and JA in tomato against herbivores has been studied in detail in previous studies. For example, SA was found to be an essential component of the *Mi-1* mediated resistance against both aphid and nematode in tomato plants (Branch et al. 2004; Li et al. 2006). Thaler et al. (2002) has demonstrated that the JA is also an essential and dominant regulatory component for the induction of not only direct plant defense compounds such as PPO but also indirect plant defense compounds such as volatiles. In addition, defense signaling pathways mediated by these phytohormones are known to coordinate with several other pathways in a complex regulatory network that governs growth and defense physiology of plants and understanding the role of each of such pathways is still a challenge in ecological studies. We found herbivore-specific alterations of phytohormone levels in both leaf and root tissues. Nematode herbivory increased the leaf JA content but not on plants that had been previously exposed to aphids, whereas prior *S. exigua* herbivory did not alter this JA-induction by nematodes. The roots of plants previously attacked by caterpillars had lower JA levels independent of a later nematode infestation. On the other hand, both *S. exigua* and nematode herbivory either alone or in combination decreased the root SA content, while previous aphid herbivory reversed the negative effect of nematodes on root SA, which might be related to the lower number of small nematode galls per root mass in previously aphid infested plants. Although speculative, this finding may indicate an increased nematode resistance of plants upon aphid exposure due to stronger SA-mediated defenses, which would be in line with the concept of defense priming (Conrath et al. 2015; Hilker et al. 2015). However, whether a priming of plant defense is involved in the interactions between above- and belowground herbivory that we determined would require further investigation. In general, plant defense is considered to be costly for example in terms of resources that are required for the production of defense compounds (Karban and Myers 1989). And if the costs of defense outweigh the cost of herbivory, plants may employ other strategies such as tolerance which is an alternative plant strategy to cope with herbivory stress (Strauss et al. 1999). In our study, tomato plants were able to compensate for the loss of shoot biomass due to aphid herbivory when they were later exposed to nematodes indicating a tolerance response that is only triggered by the sequential herbivory.

Role of herbivores' identity and feeding mode in plant-insect interaction

As we hypothesized, the herbivore identity was a key factor to bring specific changes in plant traits. All the changes in measured parameters such as biomass, C and N distribution and phytohormone content in both leaf and root tissue upon nematode herbivory were dependent on the identity of the shoot herbivores. For example, plants previously treated with *S. exigua* herbivory contained higher N concentration in both leaf and root tissue upon nematode infestation, while previous aphid feeding had no such effect. There are some evidence that the induced response of tomato differs upon herbivory by insects of different feeding guilds; for example, aphid (*Macrosiphum euphorbiae*) feeding was found to induce peroxidase and lipoxygenase, but not PPO and PIs, while noctuid insect *Helicoverpa zea* feeding induced PPO, PIs, and lipoxygenase, but not peroxidase (Stout et al. 1998). Similarly, Rodriguez-Saona et al. (2010) also showed that herbivory by *S. exigua* increased the PI activity by three times as compared to control plants, whereas aphid (*M. euphorbiae*) herbivory did not induce such effects in tomato plants. For the efficient use of limited resources, plants respond to herbivores by activating a specific array of resistance and tolerance to deter herbivores which share similar characteristics such as feeding mode. Therefore, such specific defense strategies targeted at herbivores with different feeding modes might explain the differences we find in the plant response to sequential attack by aphids, caterpillars and nematodes.

In summary, our study showed that transient aboveground herbivory modified the plant response to subsequent root herbivory. Herbivores' identity and probably the feeding mode of the aboveground attacker affected the interaction between above- and belowground herbivores. Although earlier transient herbivory had no detrimental effect on the belowground herbivore, the plant responded with compensatory shoot growth to sequential aphid and nematode herbivory. As plants in nature constantly respond to a multitude of organisms, the whole ecological community interacts indirectly through the plant responses they elicit. Our study provides a small glimpse on the complexity of these interactions and shows that it is important to study interactions between multiple organisms above- and belowground to complement our understanding of plant-herbivore ecology.

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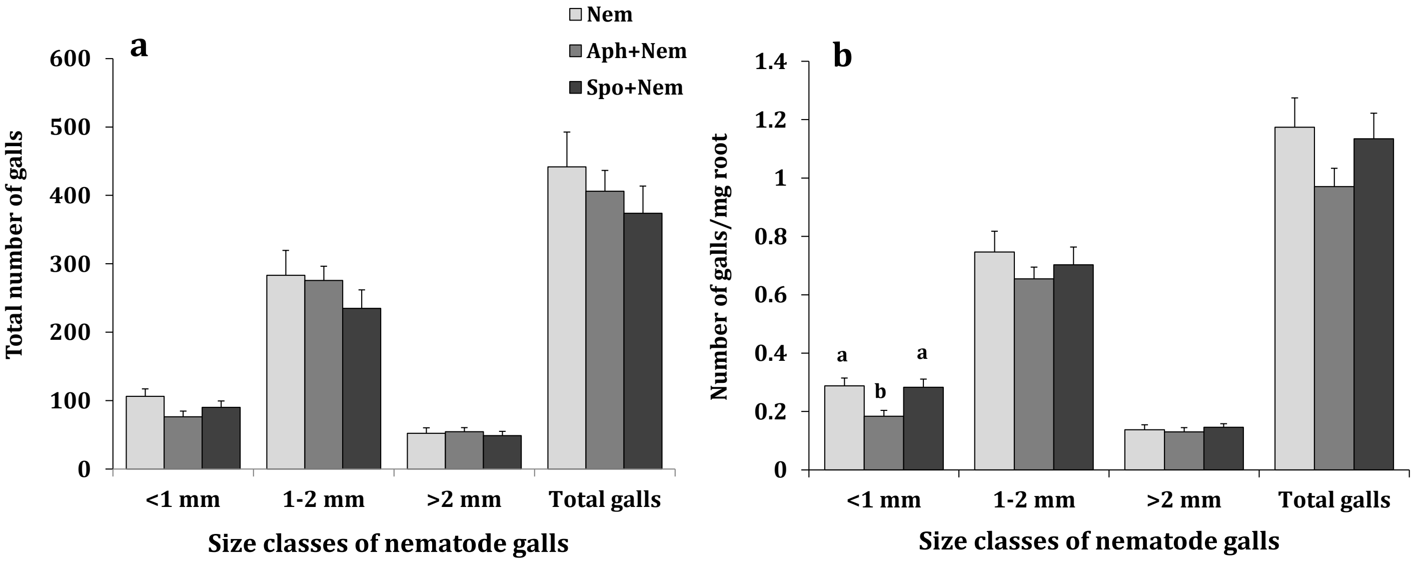
Appendix B. Supplementary Material

Appendix B: Supplementary material to chapter 3



Suppl. Fig. 1. Total number of galls of different size classes (mean ± SE; n=15) (a) and number of galls of different size classes per mg of roots (b) of tomato plants treated only with nematodes (Nem), treated with aphids followed by nematodes (Aph+Nem) and treated with *S. exigua* larvae followed by nematodes (Spo+Nem). Aboveground herbivory was applied for a week and nematode herbivory was applied for two weeks while there was a lag phase of a week between above- and belowground herbivory in sequential herbivory treatments. Different letters above the bar indicate the significant difference in their mean.

Chapter 4

Herbivory in the parental generation affects root traits and herbivore performance on progeny plants.

The following version has been submitted for consideration of publication in a peer-reviewed journal:

Kafle D, Naumann A, and Wurst S. 2016. Herbivory in the parental generation affects root traits and herbivore performance on progeny plants.

Herbivory in the parental generation affects root traits and herbivore performance on progeny plants.

Abstract

Herbivory-induced changes in plant resistance and tolerance traits can mediate the interaction between spatially and temporally separated above- and belowground herbivores. However, it is largely unknown how long the impact of transient herbivory events last and if they can be detected across plant generations. Here, we studied the impact of transient aboveground herbivory by the specialist caterpillar *Manduca sexta* on plant traits of wild tobacco *Nicotiana attenuata* and the consequences for belowground root-knot nematodes *Meloidogyne incognita* in short-term, long-term and transgenerational experiments. In the short- and long-term experiments, above- and belowground herbivory had significant, albeit independent impacts on key plant traits such as plant biomass, nutrient content and secondary metabolites. In addition, herbivory influenced fitness parameters such as seed yield and quality. In the long-term and the transgenerational experiments, transient aboveground herbivory had facilitating effects on nematodes irrespective of any treatments of the parental plants. We also found evidence for transgenerational effects of both above- and belowground herbivory on the performance of progeny plants. Sequential above- and belowground herbivory in the parental generation increased the resistance of progeny plants against aboveground herbivores, while nematode herbivory in the parental generation increased the root biomass of progeny plants. Our results suggest that herbivory may have long-term impact on plant fitness by improving the performance of progeny plants challenged by herbivory.

Introduction

Herbivory is an inevitable ecological event that drives adaptations in both interacting partners, i.e. the herbivores and the plants. Plants as sessile organisms employ two major strategies to cope with herbivory stress, namely resistance and tolerance. Resistance implies defense responses of the plants, such as local or systemic induction of defensive compounds which may negatively affect the metabolism and growth of the herbivores (Howe and Jander 2008). As an alternative of costly resistance strategies, plants may also exhibit tolerance responses to withstand the negative impacts of herbivory, such as shifts in resource allocation, altered nutritional quality, increased photosynthetic rates, compensatory growth and increased tillering (Strauss and Agrawal 1999; Tiffin 2000). Such herbivore-induced changes in plant traits are known to mediate the indirect interactions between spatially and in some case temporally separated herbivores such as above- and belowground herbivores (Masters et al. 1993; Bezemer and van Dam 2005; Ohgushi 2005; Wurst and Ohgushi 2015).

The unseen belowground parts of plants are challenged by a multitude of herbivores but often overlooked as compared to their aboveground counterparts. In recent years, research has been carried out to analyze the ecological and molecular aspects of belowground plant-insect interactions (e.g. Erb et al. 2012); and a step further, to analyze plant-mediated indirect interactions between above- and belowground herbivores (e.g. Soler et al. 2007; Wurst and van der Putten 2007). It has been shown that aboveground herbivores may affect the performance of belowground herbivores via herbivore-induced systemic changes in defensive compounds (e.g. Soler et al. 2007) or in plant nutritional resources (e.g. Kaplan et al. 2008a). Although studies on plant-mediated interaction between above- and belowground herbivores have been increasing in recent years, the majority of the research has focused on effect of belowground herbivory on aboveground herbivores. The few studies which investigated the effect of aboveground herbivory to belowground herbivores mainly focused on their spatial separation. However, herbivore-induced changes in plant traits may persist even after the herbivores are gone, and therefore, may also affect temporarily separated, subsequent herbivores (Wurst and Ohgushi 2015). For example, Barber et al. (2012) showed that cucumber (*Cucumis sativus*) plants that were treated with high levels of aboveground herbivorous cucumber beetles (*Acalymma vittatum*) in early season were less susceptible to subsequent above- and belowground herbivory by the conspecific herbivores in late season. So far, it is largely unknown how long the impact of transient herbivory lasts and if it can be detected across plant generations.

Consequences of transient herbivory may differ according to the developmental stage of the plant because its priorities for utilization of resources change with ontogeny. Younger plants invest their resources in growth and development, while mature plants invest in reproduction; thus, defensive strategies also vary with age (Boege and Marquis 2005; Barton and Koricheva 2010). Therefore, the effect of transient aboveground herbivory on plant traits can be expected to depend on the time passed since the herbivory event and the growth stage of the plant. Our study focuses on the effect of transient aboveground herbivory experienced by plants at an early developmental stage on plant traits at the following stage of vegetative growth, later at reproduction and at the early stage of the progeny generation. Further, we investigated the outcome of aboveground herbivore-induced changes in plant traits for subsequent belowground herbivores.

Generally, the outcome of plant-mediated herbivore interactions may be facilitative, detrimental or neutral to the herbivores depending upon several factors such as plant type and their defense strategy, identity, feeding guild and sequence of arrival of the herbivores (Bezemer et al. 2003; Wurst and van der Putten 2007; Kaplan et al. 2008a; Wurst et al. 2008; Erb et al. 2011; Johnson et al. 2012; Kutyniok and Müller 2013). Several studies have shown that the aboveground herbivore is more likely to have antagonistic effects on belowground herbivores, when it arrives first on the plant (Erb et al. 2011; Johnson et al. 2012). Early arrival on the host plant may be associated with competitive advantage to the ‘early birds’ because they forage naïve plants with minimal defense; and may eventually induce local and systemic plant responses with potential negative effect on latecomers. Thereby, such sequential herbivory events may even result in priming of the plant, i.e. earlier herbivory prepares the plant for a better or quicker response upon subsequent herbivory (Conrath et al. 2006; Frost et al. 2008; Hilker et al. 2015).

Priming of plant is simply described as a biological process of “getting ready for the next battle” (Conrath et al. 2006; Frost et al. 2008). Although the underlying mechanisms of priming are not fully understood, phenotypic, hormonal, epigenetic or cellular changes in local and/or systemic plant tissue due to biotic or abiotic stresses lead to priming. Per definition, these underlying mechanisms do not involve the alteration of the genetic structure of the plant (Conrath et al. 2015; Hilker et al. 2015). In addition to priming within a single generation, parental plants, based on the cues of stress events experienced in their generation, may differentially endow their offspring with changes in phenotype to withstand potential stresses through the process of transgenerational- induction or priming (Agrawal et al. 1999; Holeski et al. 2012; Rasmann et al. 2012a). For example, in one of the earliest studies on transgenerational effects, Agrawal et al. (1999) found that wild radish (*Raphanus raphanistrum*) plants whose parents were exposed to specialist caterpillars (*Pieris rapae*) were more resistant against conspecific caterpillars compared to progeny of undamaged parents (Agrawal et al. 1999). Primarily two mechanisms are suggested for such effects: maternal effects such as seed provisioning and/or epigenetic effects such as DNA methylation (Holeski et al. 2012). Since this is a relatively new topic in the field of ecology, the adaptive value (i.e. fitness consequences) and the mechanisms of the transgenerational effects are not fully understood.

Using wild tobacco (*Nicotiana attenuata* Torr. Ex Watson, Solanaceae) as a model plant and the specialist chewing caterpillar *Manduca sexta* (Sphingidae) and generalist root-knot nematodes *Meloidogyne incognita* (Heteroderidae) as above- and belowground herbivores, respectively, we examined how long the effects of transient aboveground herbivory last and if they can be detected even across plant generations. The following questions were asked by conducting short-, long-term and transgenerational experiments: 1) Does transient aboveground herbivory by *M. sexta* induce changes in plant traits which affect the performance of subsequent nematodes? 2) Do the effects of transient aboveground herbivory persist for long enough to have impact on lifetime fitness of plants? 3) Does above- and belowground herbivory in the parental plants increase the resistance of the progeny plants to herbivory?

Materials and methods

Plant

Nicotiana attenuata is an annual wild tobacco native to the southwestern United States (Baldwin and Ohnmeiss 1993). Several studies have confirmed that *N. attenuata* induces defense compounds such as nicotine and protease inhibitors and produces volatile compounds upon leaf herbivory (Baldwin et al. 1998; Kessler and Baldwin 2001; van Dam et al. 2001). The defensive alkaloid nicotine is known to be biosynthesized in the roots of tobacco plants as a response to shoot herbivory (Baldwin 1989); therefore, it is an interesting model plant to study interaction between above- and belowground herbivores. Wild type *N. attenuata* seeds, obtained from the

17th inbred line originating at the Great Basin Desert, Utah, USA (Baldwin 1998), were used for the short- and long-term experiments, while the seeds of the plants from the long-term experiment were used in the transgenerational experiment. Seeds were surface-sterilized and germinated on Gamborg's B5 medium as explained in Krügel et al. (2002). Eight days old seedlings were transferred to seedling trays. After growing them in seedling trays for 12 days, healthy and homogeneous plants were transferred to 2.5 l (16 cm diameter, 16 cm height) plastic pots (Pöppelmann GmbH and Co. KG, Lohne, Germany) containing 2.3 l of steamed soil:sand mixture. To obtain this mixture, standard potting soil 'type T' (Einheitserdewerke Werkverband e. V., Sinntal-Altengronau, Germany) was mixed with fine sand in the ratio of 3:2 (soil:sand by volume). The mixture was steamed for three hours at 90 °C using a Sterilo steamer (Harter Elektrotechnik, Schenkenzell, Germany) to exclude root herbivores. Pots were placed on individual plastic plates and the top layer of the soil was covered with sand grit to prevent the growth of green algae and infestation of fungus gnats (Sciaridae). Plants were randomized weekly to homogenize for variances due to abiotic factors such as light conditions.

Herbivores

Tobacco hornworm, *M. sexta* is a specialist herbivore feeding on plants of the Solanaceae family including *N. attenuata*. The larvae of *M. sexta* were obtained from the laboratory cultures maintained at the department of Applied Zoology, Freie Universität Berlin. They were reared on artificial diet (wheat germ based basic diet with a vitamin mix) in a climate chamber at 24 °C and 70% humidity under 16/8 hour day/night light cycle. Root-knot nematode, *M. incognita*, is a globally distributed plant parasite that feeds on roots of numerous host plants including commercial tobacco *N. tabacum* (Koenning et al. 1999; Barker 2003). It is also found in the natural habitat of *N. attenuata* plants (R. Machado, personal communication). Second-stage juveniles (J2s) of *M. incognita* (nematode hereafter) were obtained from a biological supply company (Hettema Zaaizaad en Pootgoed Coöperatie (HZPC Holland B.V.), Metslawier, The Netherlands).

Short-term Experiment (Experiment 1)

In the first full factorial experiment (Supplementary material Appendix C Table S1), a total of 60 six-week old healthy plants were selected. Half of the plants were treated with aboveground herbivore *M. sexta* (caterpillar hereafter), where a third instar larva was added in a mesh bag and allowed to feed on the first fully expanded leaf for two days. Then, a new larva was added on the second leaf for another two days and a third larva was allowed to feed on the third leaf for one day. This way, a larva fed on three consecutive leaves for a total of five days. The other half of the plants did not receive the caterpillars. After the removal of caterpillars, the plants were kept for a lag phase of five days without any herbivores. Then, 3000 second stage juveniles (J2's) of *M. incognita* were added as belowground herbivores to the roots of half of the caterpillar-treated and half of the untreated plants. Finally, there were four treatments; control (Ctrl), caterpillar only (Cat), nematode only (Nem) and caterpillar followed by nematode (Cat+Nem) with 15 replicate each. The plants were treated for 18 days with nematodes and then harvested. Leaf and root subsamples were collected to analyze the nicotine content. To assess the nematode performance following caterpillar feeding, we estimated the population growth of the nematodes by counting the number of nematode galls (in all experiments). The numbers of galls induced by the nematodes were counted in three different size classes (<1mm, 1-2 mm and >2mm) after keeping them submerged in water on a tray. Then, the root and shoot materials were dried in an oven at 55 °C for three days before measuring the dry mass.

Long-term Experiment (Experiment 2)

To test the long-term effect of the caterpillar herbivory on fitness of plants and nematodes, a full factorial experiment similar to experiment 1 was carried out in parallel, with 10 replicates per treatment, but the plants were harvested only after seed production. Thus, plants were kept for 90 days instead of 18 days after nematode inoculation, until the ripened seed capsules were produced (Supplementary material Appendix C Table S1). By this time, the nematodes had also reproduced and laid eggs in egg masses on/around the galls. The plants were harvested to count seed capsules, number of galls and egg masses on the root. The number of egg masses was counted to assess the reproductive success of the nematodes following caterpillar feeding. Fifteen fully ripened seed capsules were collected from each plant and seeds were counted with the help of the image processing program ImageJ (National Institutes of Health, Maryland, USA) to estimate the average number of seeds per capsule. Seeds were threshed from all capsules to measure total seed yield. A small volume of seeds was weighed and total number of seeds in this volume was counted using ImageJ to calculate the individual seed mass. Root and shoot biomasses were measured as described in experiment 1.

Transgenerational Experiment (Experiment 3)

The seeds collected from the plants in experiment 2 were germinated separately according to the treatments their respective parent received. Forty healthy plants (six-week old) were selected from each of the parental treatments making a total of 160 plants. Each progeny group received the identical set of treatments (Ctrl, Cat, Nem and Cat+Nem) that their parents had received following the same procedure (Supplementary material Appendix C Table S1), resulting in a total of 16 different treatments (four in the parental generation and four in the progeny generation) with 10 replicates each. The individual caterpillar mass was measured before and after the treatment to calculate the mass gain. The plants were harvested after 18 days of nematode inoculation. The numbers of galls induced by the nematodes were counted in three different size classes as described in experiment 1. Then, the roots and shoots were dried in an oven at 55 °C for three days before measuring the dry mass.

Nicotine Measurement

In experiment 1, the nicotine content induced by different treatments in the leaf and root tissue of the *N. attenuata* plants was measured by HPLC. The sample extraction procedure for nicotine measurement was modified after Gaquerel et al. (2012). In brief, the roots from the harvested plants were washed immediately and fine roots from five different parts of the root system were collected and mixed together. Then, 100-125 mg of these fresh root samples were weighed, placed in 1.5 ml FastPrep tubes, and flash frozen in liquid nitrogen. Similar amounts of leaf samples were taken from the youngest fully expanded rosette leaves. Samples were homogenized using FastPrep homogenizer (FastPrep®-24, MP Biomedicals, California, USA). To homogenize the sample, 1 ml of nicotine extraction buffer (40% methanol and 0.5% acetic acid) was added in the FastPrep tube along with 700 mg of lysing matrix-green (MP Biomedicals, California, USA) and one 1/4" Ceramic sphere bead (MP Biomedicals, California, USA), and then shaken at 6.0 ms^{-1} for 60 s. Supernatant was collected after centrifuging the sample for 10 min at 13000 rpm at room temperature. The supernatant was centrifuged again to obtain clean supernatant and stored at -20 °C until HPLC analysis. The nicotine contents in the root and shoot tissue were analyzed by high-performance liquid chromatography (HPLC) [Shimadzu degasser (DGU-20A3), 2x pumps (LC-20AD), diode array detector (SPD-20M20A), controller (CBM-20A), auto-sampler (SIL 10A), Shimadzu Corp., Kyoto, Japan] following the method described in Keinänen et al. (2001).

Carbon and nitrogen concentration in leaf and root tissue of the plants in experiment 1 and 2 was measured in the above- and belowground plant tissue. Dried leaf and root materials were ground in Eppendorf tubes by using a mixer mill (Mixer Mill MM 400, Retsch GmbH, Haan, Germany) and dried again for at least 24 hours. Then, their carbon and nitrogen concentration were determined by using an elemental analyzer (Euro EA, HEKAtech GmbH, Wegberg, Germany).

ATR-FTIR Spectroscopy and Sample Preparation

To gain a fast overview of the chemical composition and variations due to the herbivore treatments in the parental and progeny generation of plants from experiment 3, Fourier-Transform Infrared (FTIR) spectroscopy was used as a suitable and easily applicable method for non-target analyses (Schulz and Baranska 2007; Allwood et al. 2008; Schulz et al. 2014). Differences in the concentration of main components such as carbohydrates (cellulose, hemicelluloses, sugars), lignin, proteins, lipids, etc. can be observed by characteristic absorption patterns of the spectrum and evaluated by means of chemometric methods like hierarchical cluster analysis or principle component analysis (PCA) (Heraud et al. 2007; Naumann et al. 2010; Salzer and Siesler 2014). Hence, leaf and root material from the transgenerational experiment were analyzed with FTIR. Plant material was air dried after harvest and ground to fine powder by a mixer mill (Mixer Mill MM 400, Retsch GmbH, Haan, Germany). FTIR spectra were recorded with a Platinum ATR single reflection diamond FT-IR spectrometer (ALPHA Bruker Optics, Ettlingen, Germany) with a spectral resolution of 4 cm^{-1} and 32 scans from 4000–375 cm^{-1} using three subsamples. Mean spectra were calculated from three replicate measurements and vector normalization, offset correction, cluster and PCA analyses performed using the instrument software OPUS 7.2 (Bruker Optics, Ettlingen, Germany). For cluster analysis, first derivatives with 9 smoothing points were vector-normalized and the spectral ranges 3600–2800 and 1800–600 cm^{-1} used to construct the dendrogram by means of Euclidian distance (standard method OPUS 7.2) and the Ward's algorithm.

Statistical Analysis

All the statistical analyses were performed using statistical platform 'R', version 3.2.2 (R Core Team 2015). One-way and two-way factorial ANOVAs were performed to test the significance of the herbivory treatments 'Cat' and 'Nem' and their interactions (Cat*Nem). Statistical significance was set at $P < 0.05$. The data on number of nematode galls in experiment 2 was log-transformed in order to meet the assumptions of ANOVA. The individual seed mass data were analyzed with Generalized Linear Models (GLM) assuming gamma distribution of errors as the data were not normally distributed. Means and standard errors are reported in the result sections. Due to the big sample size in the transgenerational experiment, plants were germinated in blocks of two days and harvested in blocks of four days ensuring equal distribution of replicates from each treatment for each germination and harvest day. Therefore, the effects of the caterpillar, nematode and parental treatments on shoot and root biomass were analyzed using Linear Mixed-Effect (LME) Models including germination day and harvest day as random factors, while only germination day was included as random factor to analyze the parental effect on caterpillar performance on the progeny plants.

Results

Short-term experiment (Experiment 1)

Nematode Performance

Transient caterpillar feeding had no significant effect on the number of galls induced by nematodes in the short-term experiment (Figure 1a).

Nicotine Induction

Neither caterpillar nor nematode herbivory had any effect on the nicotine content in the leaves, while the nematode herbivory induced higher levels of nicotine in the root tissue (F [1, 55] = 15.76, $p < 0.001$) independent of the caterpillar feeding (Supplementary material Appendix C Figure S3).

Shoot and root biomass

Both shoot and root biomass were significantly reduced by the caterpillar feeding (shoots: F [1, 56] = 13.48, $p < 0.001$; roots: F [1, 56] = 11.61; $p = 0.001$). The nematode had no effect, and there was no interaction effect [Supplementary material Appendix C Figure S1 (a) and A2 (a)]

Carbon and Nitrogen Concentration

Caterpillar feeding increased C concentration in the leaf tissue in the short-term experiment. On the other hand, the nematode significantly altered the C and N concentrations in the root tissue without any changes in their concentrations in the leaves. Nematode decreased N while increasing C and C/N ratio in roots (Means and statistical test values are available in Supplementary material Appendix C Table S2).

Long-term experiment (Experiment 2)

Nematode Performance

In the long-term experiment, caterpillar herbivory had a marginally positive effect on nematode performance (Figure 1b). The number of galls of the size class bigger than 2 mm was significantly higher in plants previously treated with caterpillars compared to plants treated with nematodes only (F [1, 18] = 5.343, *p* = 0.033). Similarly, the total number of galls and total number of galls per mg of root tissue tended to be higher in plants previously treated with caterpillars (Total galls: F [1, 18] = 3.664, *p* = 0.072; Total galls/mg root: F [1, 18] = 3.256, *p* = 0.088), and a similar trend was observed for the total number of egg masses produced by nematodes but not for number of egg masses per mg of root tissue (Total egg masses: F [1, 18] = 3.288, *p* = 0.087; Total egg masses/mg root: F [1, 18] = 1.48, *p* = 0.239).

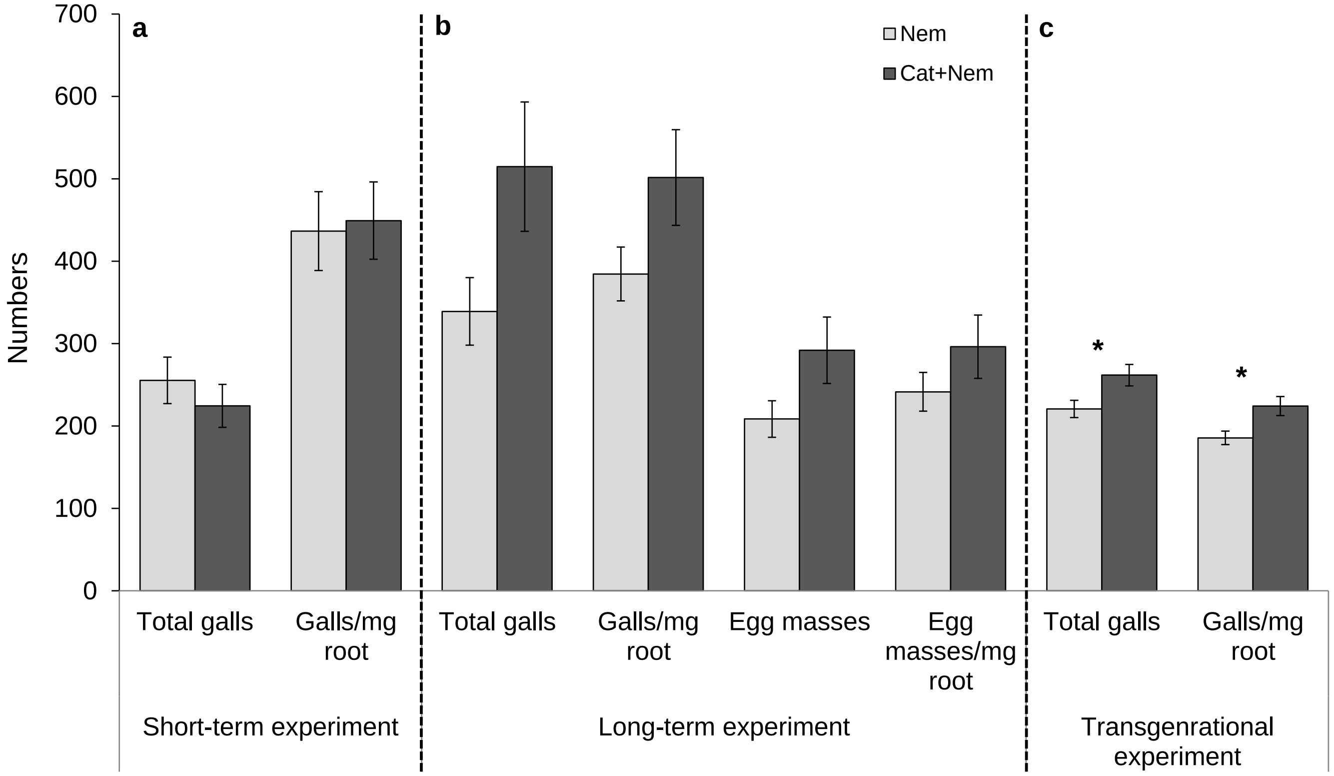


Figure 1. Total number of nematode (*Meloidogyne incognita*) galls and total number of galls per unit root biomass (mg) induced on plants treated with nematode only (Nem) and caterpillar

(*Manduca sexta*) followed by nematode (Cat+Nem) in experiment 1 (a), experiment 2 (b), experiment 3 (c), and number of egg masses (experiment 2) (mean ± SE). Asterisk (*) indicates significant difference between the mean number of galls at $P < 0.05$.

Shoot and root biomass

In the long-term experiment, there was no adverse effect of the caterpillar feeding in shoot and root biomass as found in the short-term experiment, while the nematodes decreased shoot biomass significantly (F [1, 36] = 78.44, $p < 0.001$); but had no effect on root biomass [Supplementary material Appendix C Figure S1 (b) and A2 (b)].

Carbon and Nitrogen Concentration

In the long-term experiment, caterpillar feeding had no significant effect on C and N concentration in leaf and root tissue of the plants. On the other hand, the nematodes significantly altered the C and N concentrations in the root tissue without any effect on their concentrations in the leaves. Nematode herbivory significantly increased both C and N concentration in the root tissue without any effect on C/N ratio (Means and statistical test values are available in Supplementary material Appendix C Table S2).

Plant Fitness

In the long-term experiment, we measured the number of seed capsules, seeds per capsules, total seed yield and seed characteristics to estimate plant fitness. Both caterpillar and nematode herbivory had significant main effects on the number of seed capsules (caterpillar: F [1, 36] = 10.34, $p = 0.002$; nematode: F [1, 36] = 53.67; $p < 0.001$). The plant produced less seed capsules upon herbivory from both above- and belowground herbivores without any interactive effect (Figure 2a). Despite having a negative impact on the number of seed capsules produced by the plants, the caterpillars increased the seeds per capsules (F [1, 36] = 6.96, $p = 0.012$); as a result total seed yield remained unchanged (Figures 2b and 2c). On the other hand, the nematodes significantly reduced both the number of seed capsules and the total seed yield (F [1, 36] = 48.17, $p < 0.001$).

The nematodes significantly increased the individual seed mass (F [1, 37] = 4.85, $p = 0.034$) [Supplementary material Appendix C Figure S4 (a)], but reduced the total seed yield (Figure 2c). There was a main effect of nematodes and an interaction between the caterpillars and the nematodes on the C/N ratio of the seeds (nematode: F [1, 36] = 4.761, $p = 0.036$; caterpillar*nematode: F [1, 36] = 7.918; $p = 0.008$). Both caterpillars and nematodes interacted to increase the seed C/N ratio which was even slightly higher than that of control plants [Supplementary material Appendix C Figure S4 (b)].

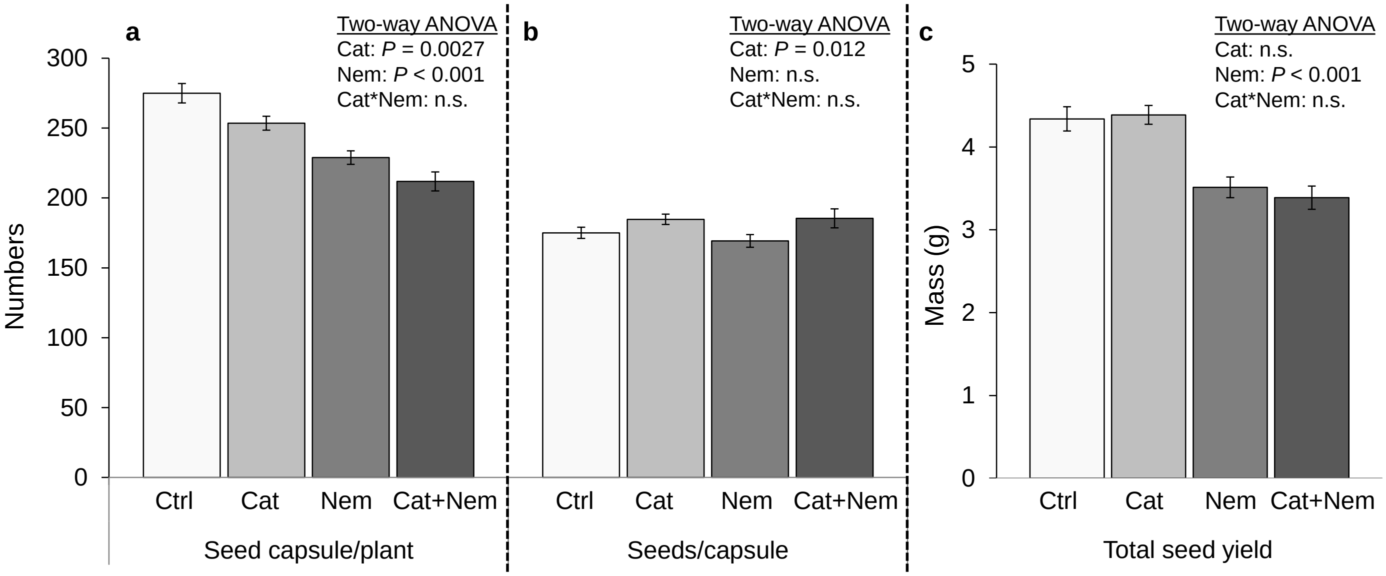


Figure 2. Total numbers of seed capsules (a), number of seeds per capsule (b) and total seed yield (g dry mass) (c) of the plants in experiment 2 (mean ± SE), n.s. stands for not significant. Treatments: Ctrl: control, Cat: Caterpillar only, Nem: Nematode only and Cat+Nem: Caterpillar followed by nematode herbivory with a lag phase of five days.

Transgenerational experiment (Experiment 3)

Caterpillar Performance

The sequential herbivory events (caterpillars followed by nematodes) in the parental generation resulted into poor growth of the caterpillar larvae on progeny plants. The daily mass gain (F [1, 75] = 7.20, $p = 0.009$) (Figure 3) and the daily percentage mass gain (F [1, 75] = 7.64, $p = 0.007$) (figure not shown) of the caterpillar was significantly lower on the plants whose parents received the above-and belowground herbivory (Cat+Nem treatment) while a single herbivory event in the parental generation had no such effects.

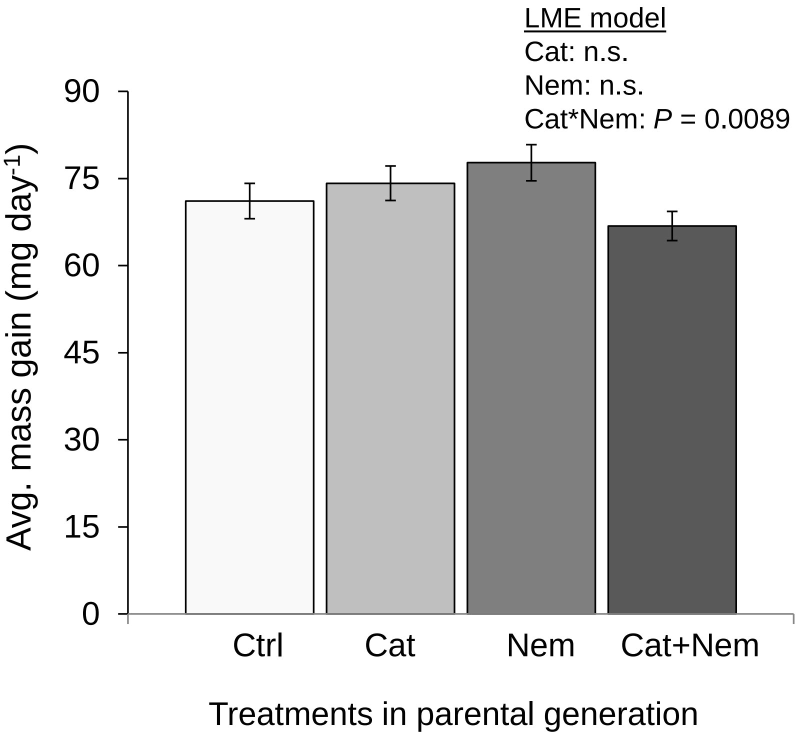


Figure 3. Average mass gain per day (mg) of the *M. sexta* larvae in experiment 3 (mean ± SE), each replication is the average of three larvae added on a plant as caterpillar treatment, n.s. stands for not significant. Treatments: Ctrl: control, Cat: Caterpillar only, Nem: Nematode only and Cat+Nem: Caterpillar followed by nematode herbivory with a lag phase of five days.

Nematode Performance

The parental treatments had no effect on the performance of the nematodes on progeny plants, however the aboveground herbivory by the caterpillars in the progeny generation had facilitative effects on nematode performance. Total number of galls and total number of galls per mg of the root tissue were significantly higher in plants previously treated with caterpillars (Total galls: F [1, 69] = 6.568, *p* = 0.013; Total galls/mg root: F [1, 69] = 8.303, *p* = 0.005) (Figure 1c). A similar positive effect of the caterpillar feeding was found in the number of galls of size class <1mm (F [1, 69] = 6. 595, *p* = 0.012) which comprised the majority (>90%) of the total number of galls, while no effects were found in the galls of size between 1 to 2mm and above 2mm (Figure 1c).

Shoot and root biomass

The parental treatments had no effect on the shoot biomass of the progeny plants, while the caterpillar feeding in the progeny generation had a marginally significant negative effect on total shoot biomass (F[1, 141]= 3.76, *p*=0.054). The nematode herbivory in the progeny generation, on the other hand, had no impact on shoot biomass. Nematode herbivory in both the parental and progeny generation had significant main effects on the root biomass (parental nematode treatments: F[1, 141]= 7.773, *p*=0.006; progeny nematode treatment: F[1, 141]= 7.532, *p*= 0.007). The nematode herbivory in the progeny generation reduced the root biomass (Figure 4b). In contrast, the plant which received the nematode treatment in the parental generation had higher root biomass in the progeny generation irrespective of the treatments in the progeny generation (Figure 4a). There was no interaction among any treatments.

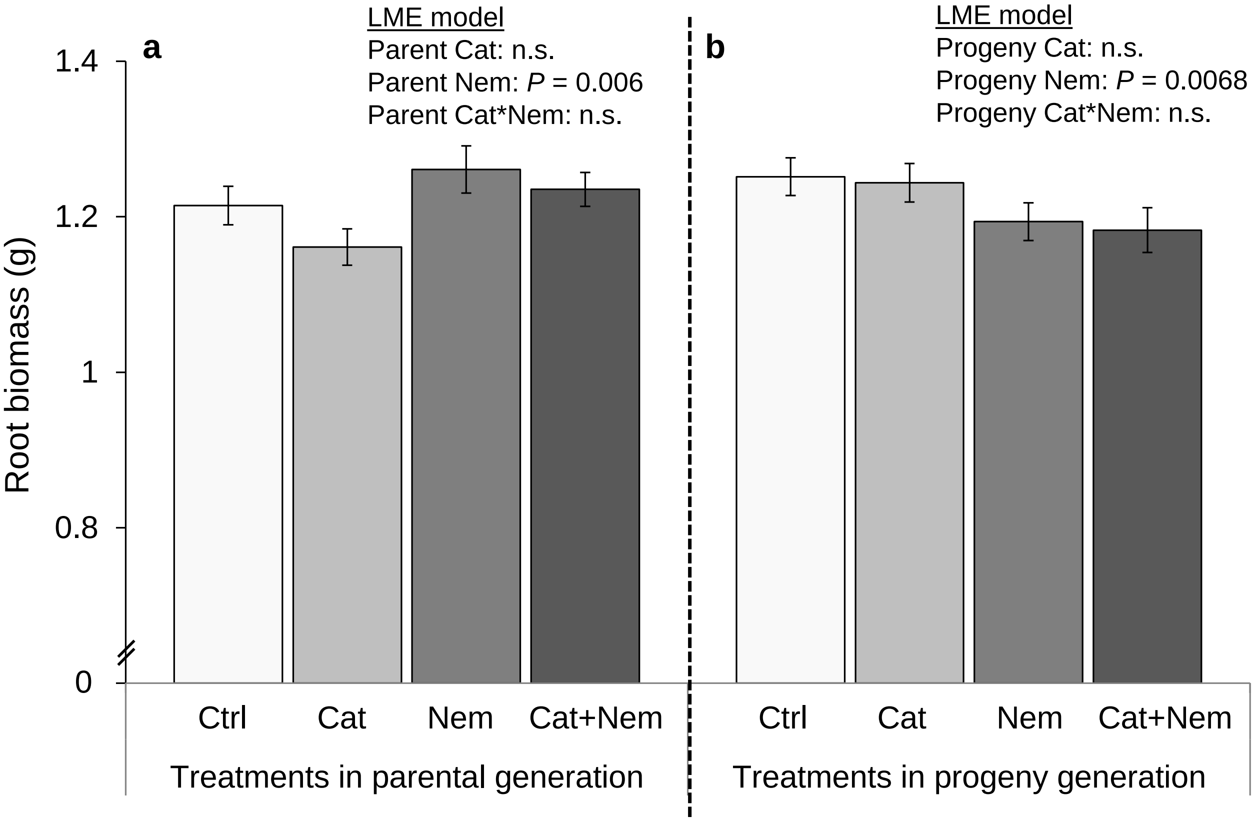


Figure 4. Root biomass (g dry mass) of the plants from the experiment 3, based on treatments in parental generation (a) and based on treatments in progeny generation (b) (mean ± SE), n.s. stands for not significant. Treatments: Ctrl: control, Cat: Caterpillar only, Nem: Nematode only and Cat+Nem: Caterpillar followed by nematode herbivory with a lag phase of five days.

FTIR Spectroscopy

Cluster and PCA analyses revealed no classification of leaf and root spectra according to treatments (not shown). Thus, parental and progeny treatments had no impact on spectra and the related chemical composition. However, clear differences in the chemical composition of leaves and roots were reflected in their distinct ATR-FTIR spectra (Supplementary material Appendix C Figure S5). Compared to roots, leaves showed higher absorbance in the spectral ranges tentatively assigned to proteins and lower absorbance in the ranges tentatively assigned to carbohydrates.

Discussion

In this study, we tested if earlier transient aboveground herbivory affects plant traits and subsequent belowground herbivores in the short-term, long-term and across a plant generation. In the short-term, transient aboveground herbivory reduced the shoot and root biomass of the plants, while the effect on the vegetative biomass disappeared in the long-term, but a significant impact on quantitative and qualitative seed traits was detected. Transient aboveground herbivory generally had a positive influence on nematode performance. Interestingly, above- and belowground herbivory in the parental generation increased plant resistance against aboveground herbivores in progeny plants, while nematode herbivory in the parental generation increased the root biomass of progeny plants. Such transgenerational effects of parental herbivory treatments may suggest priming responses as the changed plant traits are associated with increased plant performance.

Short- and long-term effects of transient aboveground herbivory on nematode performance

A meta-analysis of studies on plant-mediated above- and belowground herbivore interactions (Johnson et al. 2012) showed that aboveground herbivores when arriving first on the host plant generally have negative effects on survival of belowground herbivores, but increase their population growth rate and fecundity. We found contrasting results in the short- and the long-term experiment. In the short-term experiment, the effect was neutral, while in the long-term experiment we found generally positive effects of aboveground herbivores which significantly increased the number of galls above 2 mm in size and tended to increase the total number of galls and egg masses. This shows a long-term impact of the transient aboveground herbivory which lasted just for five days. In a study on plant-mediated linkages between above- and belowground herbivores in *N. tabacum*, a close relative of *N. attenuata*, Kaplan et al. (2008a) also showed that the shoot herbivores *M. sexta* and *Trichoplusia ni* facilitated the performance of nematodes (*M. incognita*) as a result of reallocation of photoassimilates from shoots to roots in response to the feeding damage. In another study, Johnson et al. (2009) documented that leaf sucking aphids (*Rhopalosiphum padi*) enhanced the growth of root feeding wireworms (*Agriotes* spp.) in barley (*Hordeum vulgare*) by increasing the concentration of root minerals, particularly sulfur.

Antagonistic interactions between above-and belowground herbivores are often associated with the induction of defensive compounds whereas facilitation is usually driven by nutrient reallocation. As a tolerance response, plants are known to protect their valuable nutrients by translocating them away from the site of herbivory which may later be used for regrowth; and in addition, such diversion of nutrients results in poor nutritional quality of the feeding site (Babst et al. 2005; Kaplan et al. 2008a; Gomez et al. 2012). Eventually such reallocation of nutrients, for example from shoot to root tissue, may positively affect belowground herbivores. In the short-term experiment, both above- and belowground herbivory independently increased the C concentration in the leaves and roots, respectively, while N concentration in roots was reduced by nematodes leading to a higher C/N ratio in the root tissue. In the long-term experiment, we found nematode derived nutrient accumulation in the roots leading to improved root quality as indicated by higher concentration of C and N in the roots. However, the better performance of nematodes in aboveground herbivore-treated plants may not be well linked increases in root nutrient contents, since increases of C and N in roots were only responses to nematode herbivory. In general, the allocations of C and N in the leaf and root tissues were only responses to the corresponding above- or belowground herbivory and independent of each other in our experiments. Contrastingly, Kaplan et al. (2008a) reported that *M. sexta* herbivory increased C allocation in the root of *N. tabacum* without altering leaf C, while nematodes had no significant impact on C contents. Both aboveground herbivores and nematodes have been found to increase microbial activities in the rhizosphere through increasing root exudation/nutrient leakage which may enhance nutrient cycling and mineralization, thereby increasing the nutrient availability in the rhizosphere (Bardgett et al. 1998; Tu et al. 2003). This might be a potential mechanism for the increase in C and N concentrations in roots due to nematode herbivory in the long-term experiment as suggested in an earlier study by Schöning and Wurst (2016).

Short- and long-term effects of herbivory on plant traits and fitness

Our study shows that both above- and belowground herbivory had significant, but different effects on the growth and fitness of *N. attenuata*. The transient aboveground herbivory by *M. sexta* reduced the shoot and root biomass in the short-term experiment. Similar reductions in shoot and root biomass of *S. dulcamara* plants by the generalist aboveground herbivore *S. exigua* were found in a previous experiment (Kafle et al. 2014). The negative effect of aboveground herbivory on plant biomass disappeared in the long-term experiment, while belowground herbivory by nematodes reduced the shoot biomass without altering the root biomass. The reduced shoot and root biomass due to above- and/or belowground herbivory may limit photosynthesis, nutrient uptake and allocation, with potential consequences for subsequent biotic interaction of the plants. For example, in a recent study, Machado et al. (2013) found that simulated *M. sexta* herbivory (mechanically wounded plants treated with *M. sexta* oral secretions) reduced the content of non-structural carbohydrates in roots which was correlated with a reduction in regrowth ability of *N. attenuata* plants.

We measured different quantitative and qualitative traits of the seeds produced by the plants under different herbivory treatments in the long-term experiment, as seed production best represents plant fitness for annual plants like *N. attenuata* (Baldwin 1998). Both above- and belowground herbivores had significant negative effects on the production of total number of seed capsules suggesting a long-term effect of the transient *M. sexta* herbivory, even 95 days after their removal from the plants. Although numbers of capsules were decreased by the transient aboveground herbivory, total numbers of seeds per capsule were higher in such plants which led to a compensation of the yield loss. In contrast, nematodes reduced the total seed yield, while increasing the individual seed mass. Baldwin et al. (1998) also reported a reduction in the seed yield of *N. attenuata* because of the trade-off between the costly production of nicotine and seed production, when the authors applied MeJA in root tissue to specifically induce nicotine. Consistently, nematodes induced nicotine in roots and reduced seed yield in the long-term experiment. Directing more nutrient reserves to the seeds could be a tolerance response of the plant upon herbivory, since maternal effects on seed size have been found to have profound effects on seed germination and seedling vigor (Roach and Wulff 1987). We also found significant changes in nutritional status of the seeds in the present study. There was an interactive effect of above- and belowground herbivory on the seed C/N ratio which was higher in those plants which received both herbivores. Individual seed mass and seed nutritional reserves are one of the few fundamental seed characteristics which are directly linked with the success of the next generation (Limami et al. 2002). Once established, deterring the nematodes that manipulate the root tissue to live and feed might be an ineffective effort for the plant in terms of resources. In that case, it might be an alternative strategy to change seed characteristics which may improve seed vigor and reproductive success.

Transgenerational effects of herbivory on plant and herbivore performance

We found transgenerational effects of above- and belowground herbivory in the parental generation on progeny plants. The *M. sexta* caterpillar performance on the progeny plants was significantly deterred due to sequential herbivory experience of their parental plants. It is important to note that such transgenerational priming response resulted only from sequential above- and belowground herbivory but not from single herbivory in the parental generation. This suggests that a certain threshold of herbivory might be required to elicit a transgenerational response in *N. attenuata* plants which was reached only by dual above- and belowground herbivory. The increased C/N ratio of the seeds due to sequential above- and belowground herbivory in the parental generation may have contributed to such enhanced resistance of the progeny plants towards caterpillars. Provisioning of the seeds with nutrients or altering the profile of defensive compounds in the seeds by the mother plant is considered as one of the major driving mechanism of such transgenerational induction or priming (Agrawal et al. 1999; Holeski et al. 2012). Similar reduction in the weight gain of *Pieris rapae* caterpillars was found in the progeny of wild radish plants (*Raphanus raphanistrum*) whose parents were treated with conspecific caterpillars (Agrawal et al. 1999). The authors suggested that the altered profile of defensive glucosinolates in seeds of caterpillar-treated plants might have resulted into such transgenerational induction of defense (referred to as ‘greater rapidity of induction of plant defences’ instead of priming possibly because the term ‘priming’ was not yet coined).

We also found a transgenerational effect of root herbivory by nematodes on the biomass of progeny plants: nematode herbivory in the parental generation significantly increased the root biomass of progeny plants. Root biomass is considered as a key plant trait associated with plant tolerance because of its significance in resource acquisition (Strauss et al. 2003). Therefore, it might be assumed that nematode herbivory experienced by parental plants primed the progeny plants to boost their tolerance ability because more roots are likely to be more tolerant to the same numbers of nematodes. This result also suggests that the transgenerational effect of herbivory is not only limited to chemical defense. Holeski et al. (2007) found that earlier simulated foliar herbivory on *Mimulus guttatus* plants resulted into increased trichome density on the leaves within the same and across generations. Another study showed that the induced status and N availability in parental *N. attenuata* plants prepare their progeny to adapt to the environmental condition that progeny plants may encounter with varying effect on their germination rate and fitness (van Dam and Baldwin 2001).

Independent of the treatments in parental plants, AG herbivory in the progeny generation tended to decrease the shoot biomass without any effect on root biomass, while BG herbivory reduced the root biomass without any effect on shoot biomass. Similarly, transient aboveground herbivory significantly facilitated the growth of the nematode as shown by significantly higher numbers of galls. Although the experimental duration and procedure were similar in the short-term and transgenerational experiment, the aboveground herbivory-induced responses of the plants varied in these two treatments. One possible reason could be the dissimilarity in seed source: seeds from naïve plants were used to grow plants for the short-term experiment, while seeds from the plants treated with above- and/or belowground herbivores in the long-term experiment were used in the transgenerational experiment. However, there was no significant impact of herbivory in the parental generation on the nematode performance in the progeny generation.

Leaf and root material of the plants in the transgenerational experiment was analyzed by ATR-FTIR spectroscopy to test for changes of major plant components due to herbivory in the current and the parental generation. However, cluster and PCA analyses of ATR-FTIR spectra revealed no such changes. Sensitivity of FTIR spectroscopy varies for specific substances and biomaterials, but generally the detection limit is in the mg/g range (Schulz and Baranska 2007); thus, herbivore-induced changes in plant quality may have been below detection limit in our experiment. In a former study, aboveground herbivory by *Spodoptera exigua* was found to influence the spectral features of root proteins in *Solanum dulcamara* plants (Kafle et al. 2014). Since we measured the FTIR spectra after harvesting the plants which was long after the transient aboveground herbivory, changes might have been induced temporarily following aboveground herbivory and not detectable anymore at harvest time.

Sequential above- and belowground herbivory events brought significant changes in both quantitative and qualitative plant traits without negative impacts on belowground herbivore performance. In the long run, earlier *M. sexta* herbivory had even favorable consequences for plant parasitic nematodes which tended to induce more galls in aboveground herbivore-treated plants suggesting facilitation of below- by aboveground herbivores. The considerable fitness costs of herbivory and altered seed traits together with evidence of transgenerational plasticity observed in our experiment suggest that herbivory in one generation may have significant implications for ecological interactions in the next generation. Further works on plant-mediated interaction between above- and belowground herbivore should focus equally on changes in tolerance, resistance and fitness traits of the plants to broaden our understanding of the long-term consequences of above- and belowground interactions.

Acknowledgments

The authors are grateful to Caspar Schöning for his suggestions and help during the entire research period. We thank Anke Steppuhn for her further suggestions on the model organisms. We also thank Annegret Plank, Monika Fünning and Cynthia Kienzle for their help in practical works and Michèle Bandoly for her help in HPLC. Technical assistance of Mario Harke in FTIR measurements is gratefully acknowledged. This research work was funded by The German Research Foundation (DFG), Collaborative Research Centre (CRC) 937 “Priming and Memory of Organismic Responses to Stress”.

Appendix C. supplementary Material

Appendix C: Supplementary material to chapter 4

Table S1. Time-line of the experiments, day 0 is the first day of addition of caterpillar on the leaf.

Day	Events	
	Experiment 1 and 3	Experiment 2
-42	*N. attenuata* seeds sown in Petri dishes	same as in exp. 1
-34	Seedlings transferred to seedling trays	”
-22	Seedlings transplanted on pots	”
0	First *M. sexta* larva added to first fully expanded leaf	”
2	First larva removed, second larva added to second leaf	”
4	Second larva removed, third larva added to third leaf	”
5	Third larva removed	”
5-10	Lag phase (period with no herbivores)	”
10	*M. incognita* added	”
28	Sub-sample collected and whole plant harvested	----
100	----	Whole plant harvested

Table S2. The effect of caterpillar and nematode herbivory treatments on C and N concentration (percentage) and their ratios in leaves and roots of the *N. attenuata* plants in experiment 1 and 2.

Experiments	Tissue	Compound	Concentration (Mean ± standard error)			
			Control	Caterpillar	Nematode	Cat+Nem
1	Leaf	C	34.55 ± 0.28	35.78 ± 0.31	35.17 ± 0.34	35.37 ± 0.35
		N	7.21 ± 0.09	7.27 ± 0.08	7.24 ± 0.08	7.21 ± 0.13
		C/N	4.81 ± 0.08	4.93 ± 0.06	4.87 ± 0.06	4.93 ± 0.09
	Root	C	42.58 ± 0.35	42.64 ± 0.24	43.96 ± 0.26	43.95 ± 0.24
		N	2.93 ± 0.06	2.97 ± 0.04	2.72 ± 0.05	2.92 ± 0.09
		C/N	14.65 ± 0.36	14.40 ± 0.22	16.27 ± 0.30	15.23 ± 0.48
2	Leaf	C	38.07 ± 0.36	37.54 ± 0.39	37.59 ± 0.37	37.74 ± 0.41
		N	3.10 ± 0.22	3.22 ± 0.09	2.89 ± 0.09	2.91 ± 0.15
		C/N	13.05 ± 1.22	11.76 ± 0.44	13.11 ± 0.40	13.38 ± 0.93
	Root	C	46.67 ± 0.27	46.17 ± 0.32	47.53 ± 0.32	47.46 ± 0.20
		N	2.46 ± 0.06	2.42 ± 0.04	2.55 ± 0.06	2.63 ± 0.10
		C/N	19.08 ± 0.54	19.16 ± 0.37	18.75 ± 0.45	18.30 ± 0.71

			ANOVA results					
			Caterpillar		Nematode		Cat+Nem	
			F	*P*	*F*	*P*	*F*	*P*
1	Leaf	C	4.95	**0.030**	0.12	0.730	2.57	0.114
		N	0.03	0.866	0.03	0.859	0.21	0.646
		C/N	1.52	0.223	0.18	0.671	0.18	0.675
	Root	C	0.01	0.938	23.45	**<0.001**	0.02	0.899
		N	3.84	0.055	4.65	**0.035**	1.52	0.223
		C/N	3.31	0.074	12.08	**<0.001**	1.25	0.268
2	Leaf	C	0.24	0.628	0.13	0.719	0.78	0.384
		N	0.25	0.618	2.97	0.093	0.11	0.739
		C/N	0.31	0.583	2.24	0.143	0.17	0.681
	Root	C	1.01	0.321	14.67	**<0.001**	0.58	0.452
		N	0.08	0.786	4.59	**0.039**	0.77	0.386
		C/N	0.12	0.728	1.25	0.271	0.24	0.627

(Short-term experiment, *df*: 1, 56 and Long-term experiment, *df*: 1, 36). Significant effects ($P<0.05$) are in bold for emphasis.

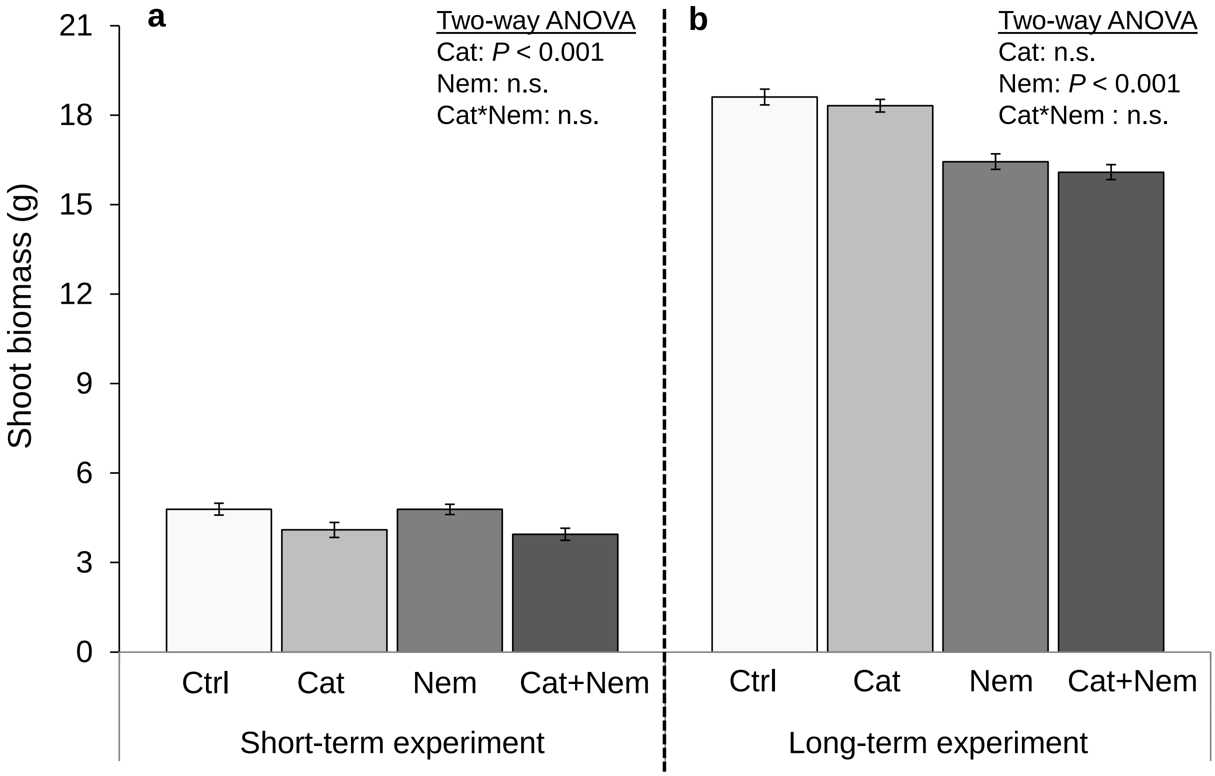


Figure S1. Shoot biomass (g dry mass) of the plants from experiment 1 (a) and experiment 2 (b) (mean ± SE), n.s. stands for not significant. Treatments: Ctrl: control, Cat: Caterpillar only, Nem: Nematode only and Cat+Nem: Caterpillar followed by nematode herbivory with a lag phase of five days.

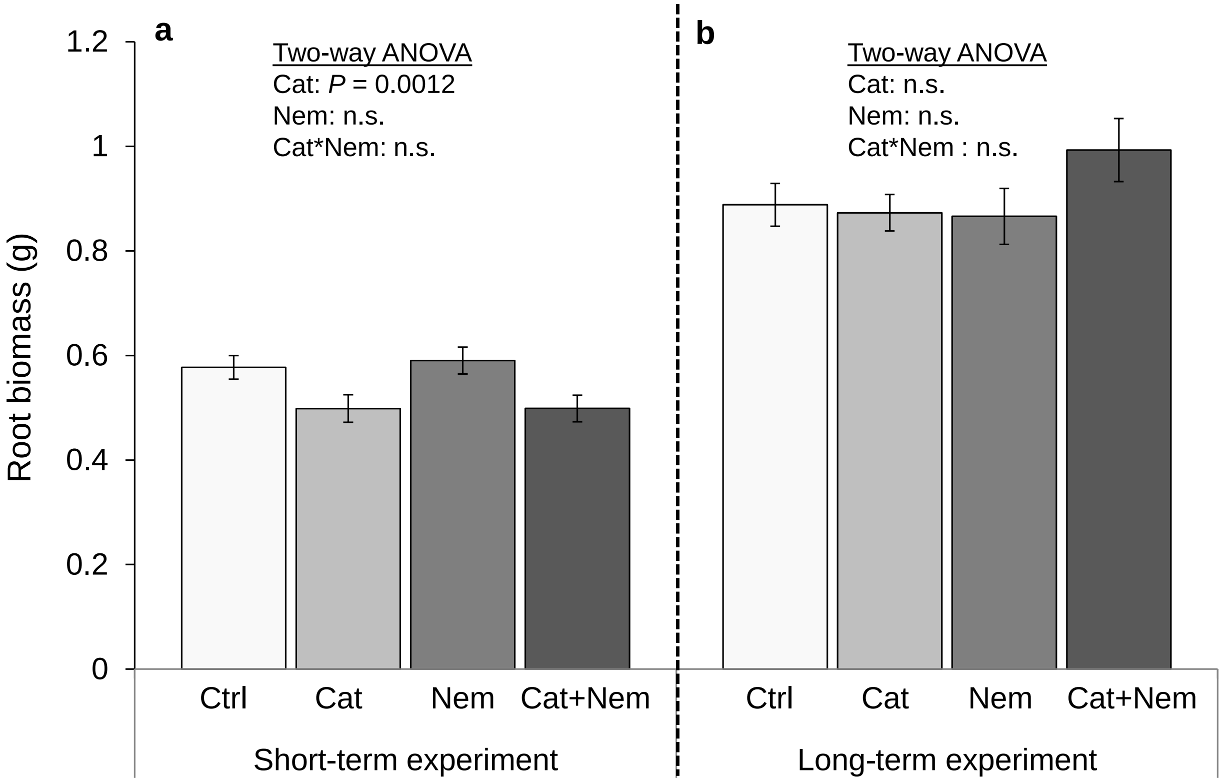


Figure S2. Root biomass (g dry mass) of the plants from experiment 1 (a) and experiment 2 (b) (mean ± SE), n.s. stands for not significant. Treatments: Ctrl: control, Cat: Caterpillar only, Nem: Nematode only and Cat+Nem: Caterpillar followed by nematode herbivory with a lag phase of five days.

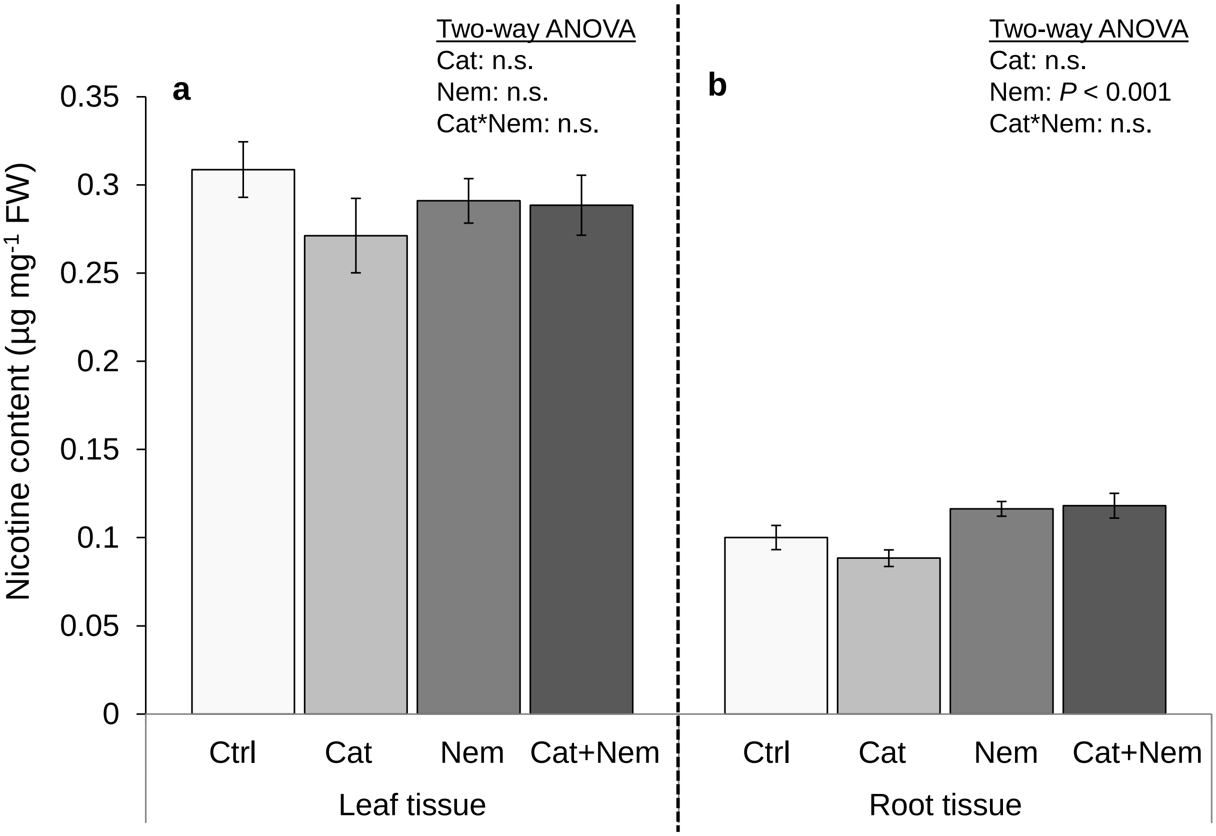


Figure S3. Total nicotine content (µg/mg fresh weight) in the leaf (a) and root tissue (b) of the plant in experiment 1 (mean ± SE); n.s. stands for not significant. Treatments: Ctrl: control, Cat: Caterpillar only, Nem: Nematode only and Cat+Nem: Caterpillar followed by nematode herbivory with a lag phase of five days. Nicotine content was measured at harvest which is 18 days after addition of the nematodes (thus 23 days after caterpillar feeding had ended).

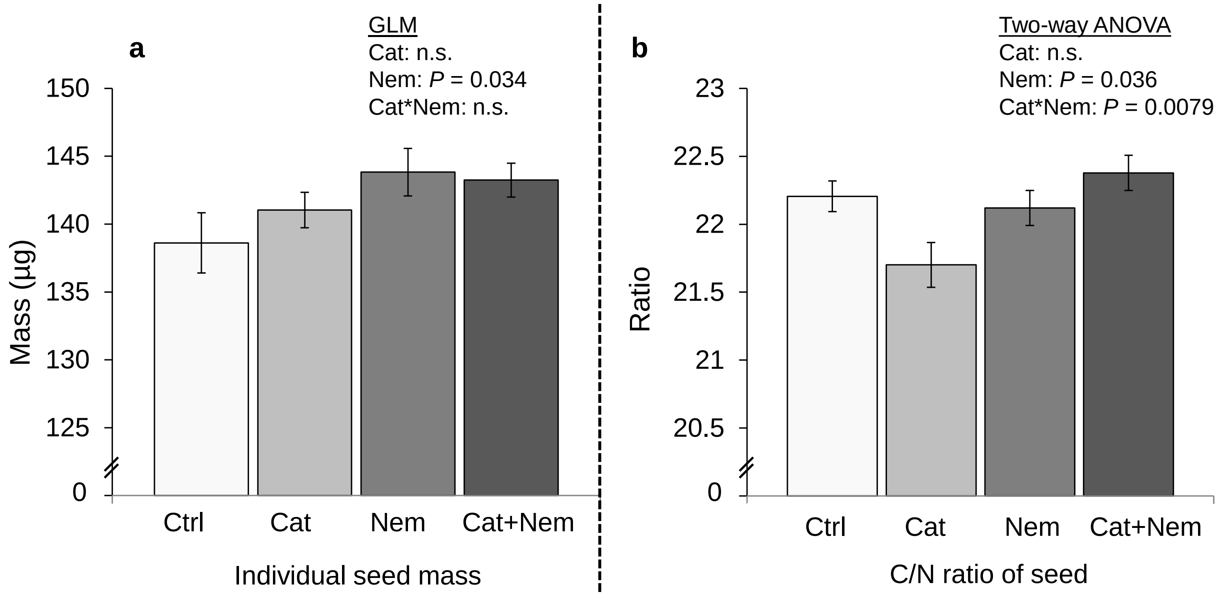


Figure S4. Individual seed mass (µg) (a) and C/N ratio of the seed (b) in experiment 2 (mean ± SE); n=10, n.s. stands for not significant. Treatments: Ctrl: control, Cat: Caterpillar only, Nem: Nematode only and Cat+Nem: Caterpillar followed by nematode herbivory with a lag phase of five days.

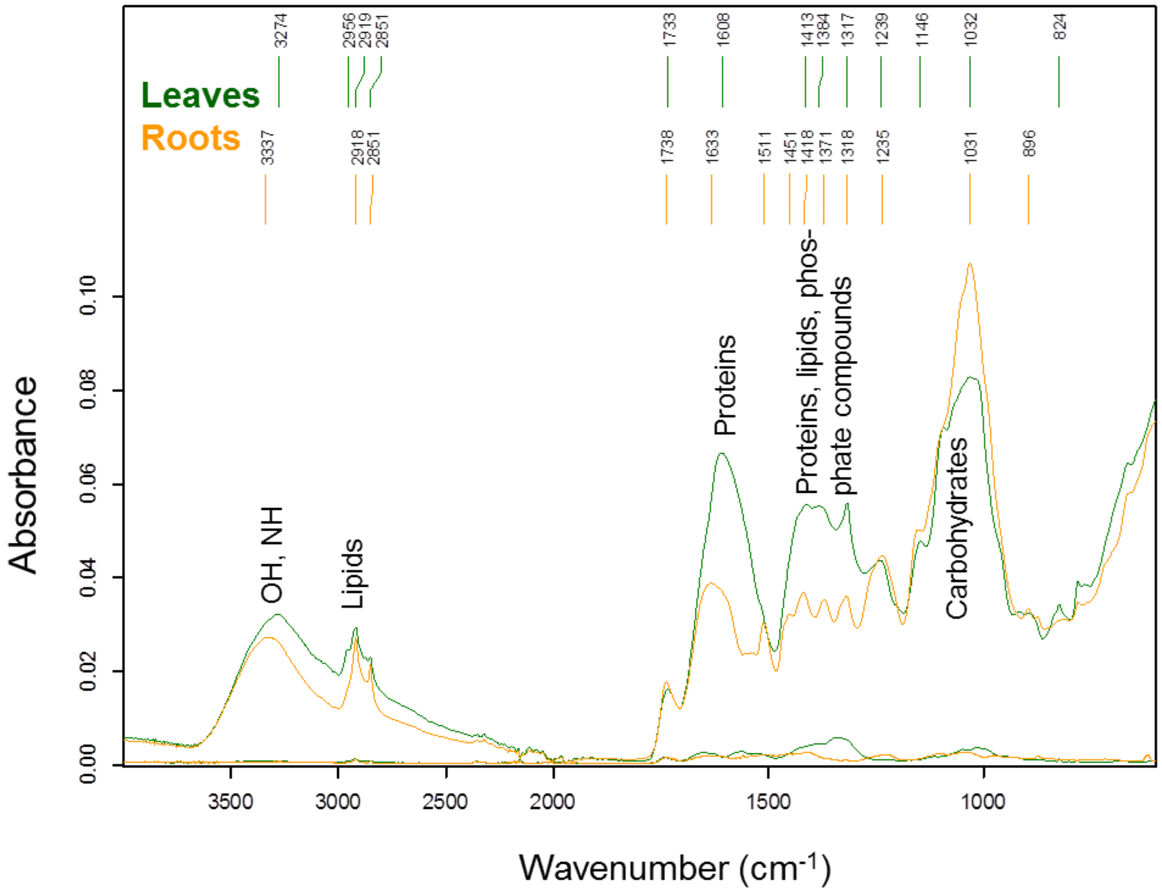


Figure S5. ATR-FTIR spectra of *Nicotiana attenuata* roots and leaves, mean and standard deviation spectra of 80 samples (after averaging of triplicate measurements of each sample, vector normalization, offset correction). Absorbance maxima in wavenumber cm^{-1} of leaves and roots given in the first and second row. Assignments of spectral wavenumber ranges to tentatively dominating chemical components were summarized from Naumann (2000) and Wilson et al. (2000) and stated above or below the spectra. A detailed overview of primary and secondary plant metabolites and their characteristic spectral absorbance features is given in Schulz and Baranska (2007).

Chapter 5

General discussion

In this thesis, I have investigated and presented the changes in different resistance and tolerance traits of the plant in response to earlier transient AG herbivory, as well as the potential influence of such changes on the performance of spatially and temporally separated BG herbivores. I have studied the potential priming response of the plants due to sequential AG and BG herbivory within a single generation and across progeny generation. I have also examined the significance of identity of both plant and herbivore for the outcome of such plant-mediated interaction between herbivores. In chapter 2, I have shown how the genetic variation of a plant species affects its interaction with AG and BG herbivores. In chapter 3, I have shown the importance of the feeding guilds of AG herbivores for the interaction of plants with subsequent BG nematodes. In chapter 4, I have presented the short- and long-term impact of AG herbivory on plants and BG herbivores. I have also elucidated the transgenerational effects of herbivory in parental plants on progeny plants in terms of resistance and tolerance against herbivores.

Herbivore-induced changes in plant traits

Systemically induced defense compounds are one of the most significant mediators of AG and BG herbivore interactions. Several secondary compounds such as PIs, glucosinolates, and nicotine are documented to be induced in the root tissue due to AG herbivory (Baldwin 1989; Soler et al. 2007; Yang et al. 2011). For example, foliar feeding by *Pieris brassicae* induced a higher level of indole glucosinolates in the root tissue of host plant *Brassica nigra*, which in turn significantly reduced the survival of the root-feeding herbivore *Delia radicum* and its parasitoid *Trybliographa rapae* (Soler et al. 2007). In another study, Yang et al. (2011) found that the foliar sap-feeding whitefly (*Bemisia tabaci*) induces the upregulation of the transcriptional expression of PI-related genes in the root of pepper (*Capsicum annuum*) plants. In tobacco plants, defensive alkaloid nicotine is biosynthesized in the root tissue as a response to AG herbivory; it is later translocated to the damaged site to deter herbivores (Baldwin 1989; Kaplan et al. 2008a). Therefore, I analyzed the root and leaf tissue of herbivore-treated plants for the induction of specific defense compounds or defense-regulatory phytohormones, depending upon the model plant, to see if earlier AG herbivory induced or primed the plant for enhanced defense in the root tissue.

Although a few previous studies have found the induction of defensive compounds such as polyphenol oxidases and PIs in the leaves of *S. dulcamara* plants in response to AG herbivory (Bronner et al. 1991; Viswanathan and Thaler 2004), in my study neither the foliar-feeding caterpillar *S. exigua* nor the root-feeding herbivore *Agriotes* spp. induced PIs in the root tissue of *S. dulcamara* plants (Chapter 2). While I expected to see an increase in PIs level as a priming response in the plant that received both herbivores sequentially, no such priming of defense responses was found. In tomato (Chapter 3), I measured the defense regulatory phytohormones JA and SA to estimate the induction of defense in leaf and root tissue following herbivory treatments. Nematode infestation, on its own or following caterpillar feeding, induced leaf JA, but not in plants previously treated with aphids. Root JA was reduced by caterpillar herbivory alone or followed by nematode herbivory. However, there was no interaction effect of the AG and BG herbivory treatments on either leaf or root JA content. On the other hand, caterpillars and nematodes, either alone or in combination, decreased the root SA content, while previous aphid feeding nullified the effect of nematodes on root SA. This result shows that the previous aphid herbivory modified the plant's response to nematodes. In tobacco (Chapter 4), I measured the concentration of the defensive alkaloid nicotine in the root tissue upon herbivory in the short-term experiment, but I found no effect of earlier caterpillar *M. sexta* feeding in root nicotine content; however, nematode herbivory induced nicotine in root tissue as a local response. Similar induction of nicotine in the root tissue of commercial tobacco (*N. tabacum*) plants due to nematode herbivory was reported in a study by Kaplan et al. (2008b). In my experiments, defensive compounds, namely PIs (in *S. dulcamara* plants), and nicotine (in *N. attenuata* plants) were measured two and three weeks after the removal of AG herbivores from the plant system respectively, which might be too long for the plants to stay induced due to earlier AG herbivory.

Along with resistance traits, changes in a plant's tolerance traits such as shoot and root biomass, and primary metabolites also regulate the outcome of plant-mediated herbivore interactions. Reduction in root and shoot biomass due to herbivory may hinder nutrient uptake and photosynthesis, resulting in poorer growth and thereby affecting the plant's interaction with subsequent herbivores. For example, Machado et al. (2013) reported the poor regrowth ability of the *N. attenuata* plant as a result of reduced non-structural carbohydrate levels in the roots of plants challenged with simulated *M. sexta* herbivory. Similarly, an increase in shoot or root biomass following herbivory as compensatory growth may also bring changes in subsequent plant-herbivore interaction. Such an increase or decrease in biomass changes the architectural features of the shoot or root tissue, which may or may not be preferable for subsequent herbivores (Ohgushi 2005).

In *S. dulcamara* plants (Chapter 2), earlier transient feeding by *S. exigua* caterpillar reduced shoot and root biomass only in the M4 genotype, indicating the importance of genotypes within a plant species for plant-herbivore interaction. In tomato plants (Chapter 3), earlier shoot herbivory by both chewing (*S. exigua*) and sucking (*M. persicae*) herbivores, when applied alone, reduced the shoot biomass but had no effect on root biomass. On the other hand, aphid-treated plants that were later treated with nematodes showed compensatory shoot growth, but there was no such compensatory shoot growth in caterpillar-treated plants following nematode infestations. This result suggests that the subsequent nematode herbivory had a positive effect on the plants' recovery from the loss of biomass due to earlier aphid herbivory. Such compensatory growth is regarded as a tolerance response of the plant, which is considered as a cost-effective means for the plant to deal with biotic or abiotic stresses compared to the induction of a resistance response (van der Meijden et al. 1988). In tobacco plants (Chapter 4), transient AG herbivory by *M. sexta* caterpillar reduced the shoot and root biomass in the short-term experiment, similar to the effect of AG herbivore *S. exigua* in *S. dulcamara* plants. Such negative effects of *M. sexta* caterpillar disappeared in the long-term experiment. Interestingly, nematodes decreased the shoot biomass in the long-term without any alteration in root biomass. On the other hand, *M. sexta* feeding tended to reduce shoot biomass, while nematodes reduced the root biomass in the transgenerational experiment.

Another major tolerance response of plants upon herbivory is the reallocation of plant nutrients (primary metabolites), which allows plants to shield their valuable resources from herbivores. Such diverged nutrients can later be used for compensatory growth (Creelman and Mullet 1997). Primary metabolites such as carbohydrates and proteins are key compounds for several physiological processes of plants; therefore, shifts in their concentrations may alter the nutritional as well as defensive quality of plant tissue, potentially influencing plant-herbivore interaction (Awmack and Leather 2002). For example, Huang et al. (2013) found that the leaf feeding by *Bikasha collaris* adults elevated root nitrogen concentration and thereby increased larval survival of the conspecific larvae in the root tissue of *Triadica sebifera* (Euphorbiaceae) plants. Two major elements, carbon (C) or nitrogen (N), are the largest constituents of primary and secondary metabolites; therefore, they are considered a proxy for the nutritional quality of plant tissue. Several studies have found the shift or acquisition of C or N as a response to herbivory to increase the plant's resistance or tolerance. Hence, I measured C and N concentration in leaf and root tissue to estimate the shifts in resource allocation and in nutritional status of leaf and root tissue following herbivory.

In the *S. dulcamara* plants (Chapter 2), earlier transient feeding by *S. exigua* caterpillars increased the N concentration in shoots of both genotypes (M4 and E9), while total protein content was increased in the root of M4 plants only. In tomato plants (Chapter 3), N concentration in both leaf and root tissue was increased by the sequential AG and BG herbivory by caterpillars (*S. exigua*) and nematodes (*M. incognita*); such sequential herbivory by aphid (*M. persicae*) and nematodes did not change the leaf or root N concentration. Caterpillar feeding reduced the root C, but subsequent nematode herbivory counteracted such alterations in C concentration. In tobacco plants (Chapter 4), caterpillar feeding increased the leaf C concentration, while nematode herbivory increased root C concentration but reduced the root N concentration in the short-term experiment. In my experiment, such alternation in C concentration was limited to local tissue (leaf or root) in response to respective AG or BG herbivory, while Kaplan et al. (2008a) showed the allocation of higher C in the root tissue due to AG caterpillar *M. sexta* feeding in *N. tabacum* plants. In the long-term experiment, nematodes increased both C and N concentration in roots without altering shoot C or N concentration. These results show only tissue-specific and independent responses of the herbivory on primary metabolites. A recent study by Schöning and Wurst (2016) showed similar increases in C and N concentration in the root of *N. attenuata* plants due to long-term nematode herbivory. Such increment of N in shoot and/or root tissue of tomato and tobacco plants indicates the uptake and acquisition of more N from the soil pool to meet the increased demand of N for compensatory growth or for N-based defense compounds such as nicotine and PIs. Earlier studies have shown increased nutrient availability in the rhizosphere of plants infested with nematodes or AG herbivores, which might be the reason for higher N in our nematode-treated plants. AG herbivores and nematodes are known to facilitate the microbial activities in the rhizosphere through root exudation or nutrient leaching, and such improved microbial activities increase nutrient availability by accelerating nutrient cycling and mineralization (Bardgett et al. 1998; Tu et al. 2003).

In the tomato experiment (chapter 3), I demonstrated the significance of the AG herbivore's feeding modes for the interaction with the plants and thereby for the indirect interaction with spatially and temporally separated BG herbivores. Most of the plant responses to herbivory (for instance, changes in shoot and root biomass, primary metabolites, and phytohormone content) were dependent on whether the damage was inflicted by a chewing or a sucking herbivore. For example, caterpillar feeding decreased the root SA, while aphid feeding did not. Plant responses dependent on herbivore identity were evidenced in several other studies; for example, Rodriguez-Saona et al. (2010) showed that the caterpillar *S. exigua* induced PI activity in tomato plants, while aphid (*M. euphorbiae*) herbivory did not. Plants are known for fine-tuning their response for maximum utilization of limited plant resources invested in defense and tolerance. Therefore, it might be a more efficient and effective strategy for the plants to respond with a precise defense tool that is effective against chewing or sucking herbivores, rather than inducing a broad-spectrum defense.

I conducted a long-term experiment on tobacco (Chapter 4) to elucidate the long-term impact of earlier transient AG herbivory on the fitness of both nematodes and plants. I examined seed characteristics to estimate the effect of long-term AG and BG herbivores on quantitative and qualitative seed traits that may affect the growth, development, and resistance of progeny plants. Foliar feeding caterpillar (*M. sexta*) reduced the total number of seed capsules but increased the total number of seeds per capsule, having no effect on total seed yield. This result signifies the long-term consequences of transient AG herbivory on plant performance. On the other hand, nematodes reduced both the number of capsules and the total seed yield but increased the individual seed mass. The plants' fitness was directly influenced by the herbivory because the plants needed to invest their resources in defense as well as in basic growth. Baldwin et al. (1998) reported a trade-off between the induction of defensive alkaloid nicotine and seed yield in *N. attenuata* plants. In my experiment, the *N. attenuata* plant also induced nicotine in the root tissue upon nematode herbivory, which might have resulted in reduced seed yield in the nematode-treated plants in the long run. Interestingly, caterpillar and nematode herbivory had an interaction effect on seed C/N ratio, which was increased in the plant that received both herbivores. Changes in seed quality and mass indicate seed provisioning by mother plants. Since the seed's nutritional reserves and size are seen to have a strong influence on seed germination and seedling vigor, these characteristics are considered an alternative strategy of the plant to insure success of progeny plants (Roach and Wulff 1987; Limami et al. 2002).

Plant-mediated interaction between AG and BG herbivores

Aboveground herbivory-induced changes in BG plant traits are expected to have an impact on the performance of BG herbivores. In my experiments, AG herbivores were added ahead of the BG herbivores on plants; thus, I expected to find negative effects of AG herbivores, because AG herbivores, when they feed first on the plant, are more likely to have adverse effects on the performance of BG herbivores (Johnson et al. 2012).

In the *S. dulcamara* plants (chapter 2), the AG caterpillar *S. exigua* was found to have no such effects on the performance of the root herbivore *Agriotes* spp. However, in the feeding bioassays, *Agriotes* larvae tended to prefer roots of M4 genotype, and their mass gain was found to be higher when fed with M4 root material compared to E9. This result shows that the genotype-dependent response of BG herbivore resulted from the difference in the nutritive quality of two genotypes, rather than from the effect of previous AG herbivory. A difference in plant quality between two genotypes was strongly supported by the results from Fourier Transform Infra-Red (FTIR) analysis, which was performed for the quick analysis of metabolic profiles of the plant tissues. Although AG feeding by *S. exigua* larvae had no impact on BG *Agriotes* spp. larvae in my experiment, previous AG herbivory by the beetle *Psylliodes affinis* was found to negatively influence the performance of subsequent conspecific and heterospecific (*Plagiometriona clavata*) AG herbivores (Viswanathan et al. 2005).

In tomato plants (chapter 3), earlier shoot herbivory by either chewing caterpillar (*S. exigua*) or sucking aphid (*M. persicae*) had no impact on overall performance of nematodes; however, numbers of smaller galls (<1 mm in size) per mg of root tissue were significantly reduced in the plant previously treated with aphids but not with caterpillars. The common *Mi-1* gene is known to regulate the defense of tomato plants against aphid and nematode herbivory. Further studies have shown that the SA signaling pathway is essential for *Mi-1*-mediated resistance against aphids and nematodes, indicating the inducibility of *Mi-1*-mediated defense (Rossi et al. 1998; Branch et al. 2004; Li et al. 2006). In the root tissue, nematode herbivory, either alone or following caterpillar herbivory, was found to suppress SA content. Such reduced SA content, which suggests lower resistance of plants, might therefore have contributed to the better performance of nematodes in the plants treated with nematodes alone and nematodes following caterpillar feeding. Along with the abovementioned defense-related biotic factors, abiotic factors, such as an abundance of soil nutrients, also shape the indirect interactions between AG and BG herbivores. For example, Kutiniok and Müller (2013) found the negative effects of aphids (*Brevicoryne brassicae*) on nematodes (*Heterodera schachtii*) only when there was high nitrate fertilization, whereas aphids had a facilitative effect on nematodes with low nitrate supply on *A. thaliana* plants. Therefore, the impact of AG herbivory on BG herbivores is highly context-dependent, and several indiscernible physiological processes in the plants following herbivory as well as abiotic factors in their environment may significantly alter the AG and BG herbivore interaction.

In the transgenerational experiment with wild tobacco plants (chapter 4), earlier transient AG herbivory by caterpillars (*M. sexta*) (in the same generation) facilitated the performance of the root-knot nematodes as indicated by higher number of galls on the roots of caterpillar-treated plants compared to plants treated with nematode only. A similar positive but mild effect of earlier caterpillar herbivory was found in the long-term experiment, where there was a significantly higher number of bigger galls (>2 mm in size) and a tendency toward a higher number of total galls in the root of caterpillar-treated plants. In addition to growth, I also found the tendency toward a positive effect of earlier AG herbivory on nematode reproduction, indicated by a higher total number of egg masses in the root of caterpillar-treated plants. A similar facilitating effect of caterpillar feeding on nematodes was reported in commercial tobacco (*N. tabacum*) by Kaplan et al. (2008a), where authors found AG caterpillars (*M. sexta* and *Trichoplusia ni*) improved the performance of nematodes (*M. incognita*). Contrastingly, there was no such effect of caterpillar herbivory in the short-term experiment. The same procedure was followed to perform the transgenerational experiment in the next season. Such difference could be due to the difference in seed source: seeds from naïve plants were grown for the short-term experiment, while seeds from the herbivore-treated plants in long-term experiment were grown for the transgenerational experiment. Another reason could be associated with abiotic factors in the greenhouses as these experiments were carried out in two different greenhouses. Although the greenhouse conditions (temperature and photoperiod) were kept identical in both experiments, other abiotic conditions such as shading, light quality, and humidity may have contributed to the difference in plant response against nematodes. The effect of a specific greenhouse can also be seen in the shoot biomass of the plants, which more than doubled in size in the transgenerational experiment compared to the short-term experiment of the same duration.

Priming and transgenerational priming response

As a priming response, plants with earlier AG herbivory experience are expected to defend or tolerate BG herbivores more efficiently and effectively, as earlier encounters with herbivores modify or prepare the plant to perform better against recurring herbivores (Hilker et al. 2015). In my experiment, I expected to demonstrate such priming responses resulting in poor performance of BG herbivores.

There was no hint of priming response in the *S. dulcamara* experiment (Chapter 2) while there were some interactions effects of AG and BG herbivory on some of the response of tomato plants (Chapter 3). For example, nematodes alone suppressed the SA content in the root tissue, while previous aphid herbivory modified the plant’s response to nematodes. As a result, root SA content in plants treated with aphids followed by nematodes was similar to control plants. This result indicates the modified response of plants previously challenged with AG herbivores upon subsequent BG herbivory and can be referred to as priming response. However, such a modified response did not influence the performance of BG herbivores. In tobacco (Chapter 4), I found what could be labeled as negative priming, where the BG herbivores performed better on the plants with previous experience of AG herbivory. The only interaction between AG and BG herbivores was found in the C/N ratio of the seeds, which was increased in the plants treated with sequential AG and BG herbivory.

I have elucidated an interesting transgenerational priming response in the *N. attenuata* plants: Sequential herbivory by caterpillars (*M. sexta*) and nematodes in the parental plants increased the resistance of progeny plants against conspecific caterpillars. The mass gain of caterpillars feeding on progeny plants was significantly reduced due to sequential herbivory treatments in parental plants. Similar results were shown by Agrawal et al. (1999), who challenged parental wild radish (*Raphanus raphanistrum*) plants with caterpillars of the cabbage white butterfly (*Pieris rapae*) and observed the significant reduction in mass gain of conspecific caterpillars in their progeny plants. In my experiment, in addition to the increase in resistance of progeny plants, nematode herbivory in the parental plants led to an increase in the root biomass of the progeny plants. Herbivore-induced changes in the quantitative and qualitative seed traits in the parental generation might have contributed to such transgenerational priming responses, as the modification of nutritive or defensive quality of the seed by the mother plant is known for such a response (Agrawal et al. 1999, Holeski et al. 2012). The increased root biomass of the progeny plants due to herbivory in the parental generation indicated the transgenerational priming of a tolerance response, as roots play significant role in resource uptake and may therefore increase the plants' fitness. In a study with same plant species (*N. attenuata*), it was shown that the induced status and N availability in parental plants have positive effect on the progeny plant to deal with similar environmental stresses as its parents, as evidenced by improved germination rate and fitness of progeny plants (van Dam and Baldwin 2001).

From an evolutionary perspective, the priming of plant defense is most likely to evolve when the next herbivory event is predictable. For instance, the flea beetles *Psylliodes affinis* and *Epitrix pubescens* specialize on *S. dulcamara* plants and have a shoot-feeding adult stage and a root-feeding larval stage (Calf and van Dam 2012); in such plant-insect interaction linked with the herbivore's phenological progression, the arrival of the larvae is predictable, thus the priming of plant defense would most likely be advantageous for the plant. However, recent studies examining plant-mediated interactions between conspecific larvae and adults sharing the same host plant showed no evidence for the priming of plant defense. Instead, scientists have reported that AG adults facilitated BG larvae via host plant manipulation (Erwin et al. 2014; Huang et al. 2014). In a study on rice, previous AG feeding by adult rice water weevils (*Lissorhoptrus oryzophilus*) led to a stronger induction of jasmonic acid in roots by conspecific root-feeding larvae, but with additive negative effects for root growth and no effects on larval performance (Cosme et al. 2016). So, thus far, there is no evidence for a priming of host plant defense by AG against BG herbivore of the same or different species.

Conclusions

There is a growing support for the notion that the terrestrial ecosystem consists of both AG and BG subsystems, and the interaction between these two subsystems is being acknowledged for its strong role in ecosystem functioning and community structure. A wide range of mutualistic, facilitative, and antagonistic feedback occurs between AG and BG herbivores by way of the plant; therefore, visible AG and invisible BG plant parts that were previously studied separately have now been accepted as indispensable drivers of indirect interaction between AG and BG biota. In the last two decades, enormous effort has been invested in the study of plant-mediated interaction between AG and BG herbivores. The majority of these studies focused only on the spatial aspect of AG and BG realms, while very few studies extended their work to include both spatial and temporal aspects. Through sequential AG and BG herbivory treatments, I have demonstrated the plant-mediated interaction between spatially as well as temporally separated AG and BG herbivores, focusing in an AG to BG direction. In addition, I have investigated plants' priming and transgenerational priming response due to dual herbivory events. Using different model plants and herbivores, I revealed the importance of different biotic factors, such as plant genotype or the feeding guild of herbivores, in the outcome of AG and BG herbivore interaction.

Considering the significant role of AG and BG biota in ecosystems, the interaction between them should be taken into account in further ecological studies to understand the extent of different players that influence plant and herbivore biodiversity. Further studies of the impact of AG and BG herbivory and priming response on plant fitness may reveal the ecological and evolutionary relevance of such plant responses. Field studies should be emphasized to validate the findings of controlled greenhouse experiments, as field studies provide more realistic scenarios.

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Contribution to the manuscripts

1. Kafle D, Krämer A, Naumann A, Wurst S. 2014. Genetic variation of the host plant species matters for interactions with above- and belowground herbivores. *Insects* 53:651-667.

My contributions: DK and SW conceived and designed the experiments. DK implemented the experiments, collected and analyzed data, and wrote the manuscript. AK and AN conducted the FTIR spectroscopy and analysis. All authors reviewed the manuscript.

2. Kafle D, Hänel A, Lortzing T, Steppuhn A, Wurst S. Sequential above- and belowground herbivory modifies plant responses depending on herbivore identity.

My contributions: DK and SW conceived and designed the experiments. DK and AH implemented the experiments and collected data. TL and DK carried out the phytohormone measurements. DK analyzed the data. DK wrote the manuscript and submitted to BMC Ecology. All authors reviewed and the manuscript.

3. Kafle D, Naumann A, Wurst S. Herbivory in the parental generation affects root traits and herbivore performance on progeny plants.

My contributions: DK and SW conceived and designed the experiments. DK implemented the experiments, collected and analyzed data, and wrote the manuscript and submitted to Oikos. All authors reviewed the manuscript.

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Curriculum vitae

For reasons of data protection,

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