Friedrich-Schiller-Universität Jena

Chemisch-Geowissenschaftliche Fakultät

Max-Planck-Institut für chemische Ökologie

Frass-induced defense response in poplar

Masterarbeit zur Erlangung des akademischen Grades eines
Master of Science im Studiengang Chemische Biologie

Vorgelegt von Elisabeth Mindt

Matrikelnummer: 174311

Erstgutachterin: Dr. Sybille Unsicker

Zweitgutachter: Prof. Dr. Christoph Steinbeck

Jena, den 20. November 2019

Table of Contents

1. List of abbreviations

3-QA	3-caffeoyl-quinic acid
AA	Amino acid
AIC	Akaike information criterion
ANOVA	Analysis of variance
C/N	Carbon/nitrogen ratio
C. populi	*Chrysomela populi*
DAD	Diode array detector
DMADP	Dimethylallyl diphosphate
DMNT	4,8-dimethylnona-1,3,7-triene
ESI	Electrospray ionization
FID	Flame ionization detector
FW	Fresh weight
GC-MS	Gas chromatography-mass spectrometry
GLS	Generalized least squares
GLV	Green leaf volatile
H/DAMP	Herbivory/damage-associated molecular pattern
HCC	Hydroxycyclohexanone carboxylic acid
HPLC	High performance liquid chromatography
IDP	Isopentenyl diphosphate
IS	Internal standard
JA	Jasmonic acid
JA-Ile	Jasmonoyl-L-isoleucine
L. dispar	*Lymantria dispar*
M/PAMP	Microbe/pathogen-associated molecular pattern
MAPK	Mitogen-activated protein kinase
MEP	2-C-methyl-erythritol-4-phosphate
MRM	Multiple reaction monitoring
MS/MS	Tandem mass spectrometry
PET	Polyethylene terephthalate
P. nigra	*Populus nigra*
PVC	Polyvinyl chloride
RP	Reversed phase
Rpm	Revolutions per minute
R_T	Retention time
SA	Salicylic acid
SE	Standard error

TIC	Total ion current
UV	Ultraviolet
VOC	Volatile organic compound

2. List of figures

3. List of tables

4. Abstract

4.1 English abstract

Forests not only play a role for the environment as hosts to many organisms or producing oxygen, but also have economical value for example as timber product providers. Accomplishing these tasks becomes harder as forests face impacts of climatic change leading to drought or increased occurrence of insect or pathogen mass outbreaks. Research in plant ecological interactions with herbivores has mainly focused on herbaceous plants and the direct interaction between feeding herbivore and plant. During insect outbreaks, large amounts of insect frass accumulate at the forest floor and its potential as defense-inducing agent has not been thoroughly studied yet. Understanding ecosystem processes following a mass outbreak of herbivores might aid forest conservation. Hence in this study, the response of young black poplar trees towards frass application of the generalist *Lymantria dispar* or the specialist insect *Chrysomela populi* and subsequent herbivory by the generalist defoliator *Lymantria dispar* was investigated in root and leaf tissue. The frass application did not lead to drastic changes in morphological parameters of the poplar saplings and therefore fertilizer-like qualities of the frass are improbable. The analysis focused on defense activation-associated signals and compounds like phytohormones, volatile organic compounds and phenolics. For the phytohormones, jasmonates were not influenced by frass application alone but showed the typical increase in concentration due to herbivory. Salicylic acid levels were impacted by frass in root tissue showing an increase upon specialist insect frass application. The major VOC classes were not affected by the frass treatment but showed the well-known increase in emission upon herbivory. However, for a group of unidentified volatiles an emission-suppressing behavior of *Chrysomela populi* frass was observed. Phenolics were inducible in leaves and roots following frass application, but the response was compound-specific. It can be concluded that even though the plant definitely perceives a signal from the applied insect frass, the response towards generalist *versus* specialist frass varied in a compound- and tissue-dependent manner and ranged from increase to no change or decreased compound levels. This demonstrates the complexity of plant-frass interactions and warrants further study.

4.2 German abstract

Wälder spielen nicht nur eine große Rolle für die Umwelt, als Heimat zahlloser Organismen oder Sauerstoffproduzenten, sondern sind auch von wirtschaftlichem Nutzen beispielsweise als Ressource für Holzprodukte. Die Erfüllung dieser Aufgaben wird für Wälder immer schwieriger in einer Zeit, die geprägt vom Klimawandel zu gehäufter Wasserknappheit oder Pathogen- und Insektenmassenausbrüchen führt. In der ökologischen Forschung der Pflanzen-Herbivorwechselwirkung stehen seit langem krautige Pflanzen mit dem Schwerpunkt auf der direkten Interaktion zwischen Pflanze und Herbivor im Fokus. Während eines Insektenmassenausbruchs sammeln sich große Mengen Insektenkot auf dem Waldboden an und das Potenzial des Insektenkots als Verteidigungsverursachendes Agenz wurde bis jetzt noch nicht gründlich studiert. Das Verständnis von Ökosystemprozessen nach Insektenmassenausbrüchen kann zur Erhaltung von Wäldern beitragen. Daher wurde in dieser Studie die Antwort junger Schwarzpappeln auf Insektenkotapplikation des Generalisten *Lymantria dispar* und des Spezialisten *Chrysomela populi* und anschließende Herbivorie durch *Lymantria dispar* in Wurzel- und Blattgewebe untersucht. Der Insektenkoteintrag führte zu keinen drastischen morphologischen Veränderungen der Pappeln, was gegen eine Düngerähnliche Wirkung des Insektenkots spricht. Die Untersuchung war auf verteidigungsassoziierte Signale und Moleküle wie Phytohormone, Duftemission und phenolische Stoffe fokussiert. Bei den Phytohormonen wurden die Jasmonatkonzentrationen nicht durch alleinige Insektenkotapplikation beeinflusst, zeigten aber die typische durch Herbivorie hervorgerufene Induktion. Salicylsäurelevel in den Pappelwurzeln zeigten eine Konzentrationserhöhung nach Insektenkotapplikation des Spezialisten. Die Emission der Hauptduftstoffgruppen wurde nicht durch Insektenkoteintrag beeinflusst, zeigte aber die gut charakterisierte Emissionserhöhung durch Herbivorie. Jedoch wurde für eine Gruppe nicht-identifizierter volatiler Stoffe eine Emissionsunterdrückende Wirkung von *Chrysomela populi* Kot beobachtet. Phenolische Stoffe waren nach Insektenkoteintrag sowohl in Blättern als auch in Pappelwurzeln induziert, allerdings war die Auswirkung des Insektenkoteintrages Stoffabhängig. Es kann geschlussfolgert werden, dass obwohl die Schwarzpappel definitiv ein Signal aus dem applizierten Kot aufnimmt, die Antwort gegenüber Insektengeneralist und -spezialistenkot Stoff- und Gewebespezifisch variiert und Erhöhung, keine Veränderung oder verringerte Stoffkonzentrationen nach sich zieht. Dies zeigt die Komplexität der Pflanzen-Insektenkot Wechselwirkung und bedarf weiteren Untersuchungen.

5. Introduction

Plants are the primary organic matter producers in terrestrial ecosystems and hence constitute the basis of complex food webs. 30 % of the Earth's terrestrial surface is covered by forests. They fulfill important tasks in emission reduction, oxygen production, provision of timber products and provide scenic landscapes for human recreation. In addition, they are hosts to and interact with many organisms, which leads to beneficial as well as negative effects for the tree. A well-studied beneficial interaction is the mutualistic symbiosis with mycorrhizal fungi that help plants to assimilate nitrogen from the surrounding soil and in exchange receive carbohydrates from the plant. In contrast, herbivore attack is an example for a negative interaction of the plant with its surroundings. Over the course of their life, plants have to react and adapt to various modifications in their biotic and abiotic environment. Biotic factors include herbivore or pathogen attack, while light and temperature or water availability are abiotic influences. Establishing either beneficial interactions or defense against abiotic and biotic stress demands reliable recognition patterns and effective response machineries. The involved signal pathways for both events are said to overlap and therefore need to be finely tuned in order to result in an advantageous response from the plant (Dicke et al., 2009; Erb et al., 2012; Eyles et al., 2010; Holopainen, 2011; Philippe and Bohlmann, 2007; Pieterse and Dicke, 2007).

Regarding biotic threats to a plant, initially the attacker needs to be recognized and if possible identified to formulate species-specific defense responses. Recognition is modulated *via* so-called microbe/pathogen-associated molecular patterns (M/PAMPs), or in case of herbivory: herbivory/damage-associated molecular patterns (H/DAMPs), respectively. Both harm-indicating molecular patterns are perceived by pattern recognition receptors on the host's cell surface. This usually leads to a signal cascade resulting in a first, basic resistance of the plant. However, herbivores and pathogens can evade activation of these signaling pathways by releasing effector molecules into the host tissue, which suppress the recognition reaction. Known examples of such effector molecules are herbivore- or pathogen-produced plant hormones, mimics thereof or endosymbiotic substances (Erb et al., 2012). Plants in turn have evolved to notice these effector molecules and induce defense reactions through their recognition. This co-evolution gives a good indication of the defense/attack response race between plants and their biotic stressors. Concerning herbivores, elicitor molecules were found in oral secretions, ovipositional fluid or frass. These elicitors were shown to increase the defense response level and are said to be differentiable for several species, allowing for a species-specific defense response of the plant (Basu et al., 2018; Derksen et al., 2013; Erb et al., 2012; Pieterse and Dicke, 2007; Ray et al., 2015; Ray et al., 2016b, 2016b; War et al., 2012).

Due to the sessile lifestyle of plants, they evolved multiple defense mechanisms to ward off attackers. They are divided into constitutive and induced defenses and further can be sectioned into structural or chemical defense. Constitutive defenses are present at all times, e.g. structural features like thorns or waxes, whereas toxic compounds such as phytoanticipins belong to constitutive chemical defenses. These constitutively expressed defenses are also termed direct defenses since they directly target the attacker and act as deterrents, repellants, anti-digestive or toxic agents (Frost et al., 2008; Ray et al., 2016a; Venkatesan, 2015; War et al., 2012). Inducible defenses are synthesized *de novo* upon plant attack and hence alter the plant's chemical phenotypic traits. Examples are the toxic phytoalexins as chemical and callose deposition or lignification as structural response. They target herbivore fitness or increase structural support following wounding, respectively. Inducible defenses are considered an energy-efficient strategy, as the defenses are only produced when an attacker is present. An interesting concept for long-living plant species is the phenomenon of delayed induced resistance, where the upregulated defense state post herbivory expands over a longer timeframe such as a subsequent growing season (Boeckler et al., 2013; Caarls et al., 2015; Dicke et al., 2009; Dixon et al., 2002; Eyles et al., 2010; Frost et al., 2008; Frost and Hunter, 2008; Lämke and Unsicker, 2018; Lawrence et al., 2006; Philippe and Bohlmann, 2007; Pieterse and Dicke, 2007; Ray et al., 2016b; War et al., 2012).

Regarding plant ecological interactions for example with pathogens or herbivores, research mainly focused on herbaceous plants. However, over the course of their long lifespan trees might be exposed to constant herbivore or pathogen pressure. Therefore it would be advantageous for trees to formulate flexible defense mechanisms that can act against a wide range of attackers (Eyles et al., 2010; Philippe and Bohlmann, 2007). In order to adapt to continuously changing abiotic and biotic environments, trees need to allocate nutrients, energy resources and defense mechanisms differently than herbaceous, annual species. Elucidating the complex molecular mechanisms behind the long life of trees as well as their resource allocation make them challenging study organisms (Eyles et al., 2010; Irmisch et al., 2014; Lämke and Unsicker, 2018; McCormick et al., 2014a; Philippe and Bohlmann, 2007; Tuskan et al., 2006).

5.1 *Populus nigra* as model organism

Poplars are utilized in large-scale plantation forestry due to their fast reproductive maturity and growth. Further, many species can reproduce asexually or are wind-pollinated. *Populus* species also pose favorable candidates as renewable energy resources. *Populus nigra* (*P. nigra*) is native to Europe, northern Africa and Asia and its natural habitat comprises lowland floodplain forests with sandy soil. It is a dioecious species, meaning that one tree only ever develops male or female flowers. Poplar is known to be preyed upon by a large

number of insect species, over 500 in its native habitat, which include shoot feeders, defoliators or stem borers. The availability of the complete genome sequence of *Populus trichocarpa* (Tuskan et al., 2006) has provided genetic tools to uncover and understand the metabolic pathways and plant-insect or -pathogen interactions of species of this woody perennial, making poplar a favorable model species (Boeckler et al., 2011, 2013; Debeljak et al., 2015; Irmisch et al., 2014; McCormick et al., 2014a; Philippe and Bohlmann, 2007; Pieterse and Dicke, 2007; Shuxin et al., 2017). Philippe and Bohlmann (2007) state that even though poplar trees can cope with severe defoliation events, when unfavorable climatic conditions are paired with recurring defoliation a large-scale dying of a poplar forest can occur. Additionally, there is a sharp decrease of riparian forest abundance. In order to sustain these economically and ecologically important forests and plantations, an environmentally friendly, sustainable pest management is crucial. Incorporation of to be elucidated poplar-pathogen or -insect interactions as well as poplar resistance and defense mechanisms could help in this endeavor (Debeljak et al., 2015; Eyles et al., 2010; Philippe and Bohlmann, 2007; Tuskan et al., 2006; War et al., 2012).

5.2 Herbivorous insects of black poplar

As described above, poplars are attacked by many generalist and specialist insect herbivores, such as the gypsy moth *Lymantria dispar* (*L. dispar*) or the poplar leaf beetle *Chrysomela populi* (*C. populi*), respectively. Generalist insects such as *L. dispar* utilize a wide range of plants from different plant families as feeding hosts (polyphagous). In contrast, specialist insects like *C. populi* have a more narrow host spectrum which is often confined to one plant family or even only consists of a few species (Ali and Agrawal, 2012; Jankovic and Petrovskii, 2013; Müller et al., 2015).

Lymantria dispar is a polyphagous insect native to Europe and Asia and currently uses around 300 tree species as hosts. It is known to cause mass outbreaks in its native as well as its introduced habitat of the United States of America. In the United States of America, outbreaks are recurrent every 8-10 years and last up to 3 years, where even complete defoliation was observed. The generalist herbivore usually produces one generation per year, however it is suggested that increasing temperatures could lead to multiple generations within one growing season (Vanhanen et al., 2007). The insect undergoes the developmental stages egg, several larval instars, pupa and adult moth. Due to the high increase in biomass during the larval stage, it is the most dangerous phase with respect to defoliation. As the female adult is regarded as mostly flightless, it is of importance for the female to find an adequate host during the larval stage, which will also provide good nutrition for its offspring. With regard to lowered nutritional value of a host tree, the gypsy moth can counteract in several ways. It could switch to another host or feed more to compensate for the lowered nutrient intake. Induced secondary metabolites from the plant could be dealt

with by faster metabolism of ingested material or upregulation of digestion or detoxification enzymes (Barbehenn et al., 2013; Boeckler et al., 2016; Jankovic and Petrovskii, 2013; Lazarevi´c et al., 2002; Li et al., 2002; Lovett and Ruesink, 1995; Madritch et al., 2007; McCormick et al., 2016; Philippe and Bohlmann, 2007).

The poplar leaf beetle is a specialist insect species that can lead to detrimental biomass losses in poplar plantations and forests. It undergoes the life stages egg, several larval stages, pupa and adult beetle and can produce multiple generations per growing season (GOMI et al., 2008). *C. populi* preferentially attacks young leaves rich in salicinoids, a group of defense-associated phenolics found in poplar species. In contrast to a generalist feeder, salicinoid compounds rather attract than deter *C. populi* for which feeding, oviposition events and performance were increased (Ali and Agrawal, 2012; Boeckler et al., 2011; Burse et al., 2009; GOMI et al., 2008; Hilker and Fatouros, 2016; Jankovic and Petrovskii, 2013; Madritch et al., 2007; Müller et al., 2015). Preferential feeding on salicinoid-rich leaves is due to the fact that after uptake the beetle not only evades being negatively affected but uses the salicinoids for its own defense. This phenomenon is called sequestration and in this case works by converting plant-derived salicin into salicylaldehyde. Salicylaldehyde acts as feeding deterrent for beetle enemies and can be used in different life stages of the beetle, for example excretion from glandular reservoirs upon attack in the larval stadium or secretion as protection of oviposition sites from an adult female. The concept of energy balance is interesting for salicinoid sequestration of *C. populi*, as the cleavage of salicinoids results in free glucose moieties, which maybe even outweigh the energetic input into sequestration (Ali and Agrawal, 2012; Boeckler et al., 2011; Burse et al., 2009; Müller et al., 2015; Pauls et al., 2016).

Ali and Agrawal (2012) state that the level of specialization could correspond to the degree of adaptation towards plant defense. This means that a polyphagous herbivore is less likely to develop species-specific avoidance mechanisms of plant defense in comparison to herbivores with only one (monophagous) or a few (oligophagous) hosts from within the same plant family. Adaptations include manipulation of or tolerance to plant defense. Hence, it would be beneficial for the insects if generalists manipulated defense mechanisms by targeting signal pathways that are conserved over many plant families and showed tolerance to an array of plant defenses. Even though specialists exhibit increased tolerance to plant defense as compared to generalists, they are not immune to strong induction levels of toxic secondary plant metabolites. Thus, it is believed that while specialists might only need to reduce the level of induced plant defense, it would be beneficial for generalists to suppress defense induction altogether. Generally, specialist and generalist insects might need to be defended against in different ways by the plant (Ali and Agrawal, 2012; Erb et

al., 2012). Ali and Agrawal (2012) state that it is unclear whether indirect defenses might be harder to adapt to and hence more efficient with regard to specialists than direct defenses.

5.3 Impact of insect frass on the forest ecosystem

Forests face increasing events of water scarcity, pollution or insect damage. The fitness and health of forests or plantations can be detrimentally affected by both insect and pathogen mass outbreaks. Global warming could lead to an increase in occurrence of these incidences (Holopainen, 2011; Shuxin et al., 2017). Literature indicates the devastating effects to biomass in large-scale defoliation events (Christenson et al., 2002; Jankovic and Petrovskii, 2013; Philippe and Bohlmann, 2007). Mass outbreak defoliation levels most likely result in changes of tree chemistry, increasing leaf litter accumulation on the forest floor, alteration of the forest composition by propagation of more resistant trees, nutrient cycling and increased nutrient availability in soil. Not only the growth of the tree itself but the speed and overall quantity of leaf regrowth after a defoliation event can be affected (Christenson et al., 2002; Jankovic and Petrovskii, 2013; Lovett and Ruesink, 1995; Shuxin et al., 2017).

The main focus of research lays on the direct interaction between feeding herbivore and plant. However especially during insect outbreaks, the herbivores have supplemental means of altering a forest ecosystem, such as frass and leaf litter input. Therefore, insects can affect the timing and quality of nutrient input into the forest floor. By that, soil nutrient cycling and availability might be changed which can have consequences for other ecosystem players, such as soil microbes. Nonetheless not only nutrient cycling in the soil but also within the tree changes, because herbivore-defoliation occurs before nutrients, especially nitrogen, can be resorbed as carried out prior to natural senescence (Christenson et al., 2002; Frost and Hunter, 2007, 2008; Hillstrom et al., 2010; Lovett and Ruesink, 1995; Madritch et al., 2007).

Most plant-herbivore defense induction experiments result from testing the impact of oral secretions or herbivore feeding on the plant, whilst insect frass has been largely overlooked as possible inducer of defense mechanisms (Ray et al., 2015; Ray et al., 2016b). Also, even though the role of frass deposition on N-cycling within a forest ecosystem has received more attention (Frost and Hunter, 2008; Hillstrom et al., 2010; Kagata and Ohgushi, 2012b; Lovett and Ruesink, 1995; Madritch et al., 2007), the underlying plant processes have not been thoroughly studied yet. Frass deposition as well as leaf litter from re-flushed leaves result in two large nutrient inputs within the same growing season. The effect of these events onto the tree chemistry has not yet been elucidated (Madritch et al., 2007). This however is of importance to understand ecosystem changes and processes for example following a mass outbreak of insect herbivores and might help in forest conservation.

Insect frass was shown to accumulate at the forest floor in outbreak situations and poses a nitrogen-rich nutrient source as well as containing readily degradable carbon contents (Christenson et al., 2002; Frost and Hunter, 2008; Madritch et al., 2007). The frass can easily be washed out due to rainfall or be mobilized due to other abiotic effects caused by the removal of the tree canopy, e.g. soil water movement or increased temperature, and enter the soil. Other processes such as volatilization could lead to nitrogen loss. After frass deposition, soil microbe activity and soil respiration are enhanced. The microbe activity seems to be more dependent on quantity instead of quality of the applied insect feces, but decomposition is faster in N-rich compared to N-low frass (Christenson et al., 2002; Hillstrom et al., 2010; Kagata and Ohgushi, 2012b; Lovett and Ruesink, 1995). Microbial frass decomposition could decrease leaching loss for high frass input levels due to arresting the mobile nitrogen by creating a soil sink. However, nitrogen uptake by microbes decreases the recyclable N-amount for the tree. Hillstrom et al. (2010) as well as Kagata and Ohgushi (2012b) suggest that the immobilized nitrogen is located near the soil surface (0-30 cm) and at least initially is not accessible for tree re-uptake (Frost and Hunter, 2007, 2008; Hillstrom et al., 2010; Kagata and Ohgushi, 2012b; Lovett and Ruesink, 1995; Madritch et al., 2007). This stands in contrast to suggestions of fertilizer-like activity of frass on trees (Frost and Hunter, 2008). Overall, nitrogen from frass is thought to re-enter the soil N-cycle faster and during severe defoliation might also exceed the N-input compared to leaf litter. Frass deposition is termed a fast cycle effect, occurring within the same season as the original outbreak (Frost and Hunter, 2007, 2008; Hillstrom et al., 2010; Kagata and Ohgushi, 2012b). The N-cycle as well as resource allocation of a defoliated forest is dependent on biotic and abiotic conditions such as topography and climate as well as the prevalent animal, microbial and plant community (Christenson et al., 2002). Of course not only the surroundings, but also the tree species for example due to secondary metabolites can influence degree of damage, a measure of frass quantity, and herbivore digestion efficiency, contributing to frass quality. These factors result in different re-inputs into the cycle and affect the decomposition of leaf litter as well as soil nutrient cycling.

Frass chemistry is dependent on the chemical composition of its origin leaf material, the metabolism of the defoliating herbivore and general nutrient availability as well as on microbes present in the insect gut or within the frass itself. (Hillstrom et al., 2010; Kagata and Ohgushi, 2012b; Madritch et al., 2007; Ray et al., 2016b). Hence, Ray et al. (2016b) consider frass-induced defense responses of plants as host-herbivore system-specific. They investigated frass-induced anti-pathogen and -herbivore defenses in plant systems where the insect feces accumulate at the wound site, e.g. maize and cabbage, and systems where no frass accumulation at the wound site occurs, such as on rice or tomato leaves. For non-accumulating systems, frass application was demonstrated to switch on herbivore defense responses. Some tested herbivore-plant systems, for example rice, even displayed

suppression of pathogen defense pathways (Ray et al., 2016b). Further, they observed tissue specificity for frass-induced defenses: insects feeding on frass-induced tomato leaves showed decreased growth whereas insects feeding on frass-induced tomato fruit led to increased growth compared to controls. With respect to the frass accumulation in close proximity to feeding sites, the data of an earlier experiment illustrating pathogen defense gene activation in correspondence with better performance of herbivores on frass-treated plants (Ray et al., 2015), could not be replicated. This might correlate to a time dependent switch between anti-herbivore *versus* anti-pathogen defenses (Ray et al., 2016b). Nevertheless, Ray et al. (2015) observed that an active frass protein could be correlated with herbivory-associated defense gene upregulation.

5.4 Defense mechanisms of black poplar

Insect frass is hypothesized to contain elicitors that could evoke plant defense (Basu et al., 2018). However, the specific plant response towards frass and the mechanism of signal perception, transduction and defense molecule production are still unknown. Regarding herbivore feeding or pathogen infection, defense machineries of black poplar are established and include phytohormone upregulation and signaling, emission of volatile compounds as well as the abundance of phenolics (Boeckler et al., 2013; Philippe and Bohlmann, 2007; War et al., 2012).

For the induction of defense chemicals after herbivore or pathogen attack, the plant cue needs to be generated locally at the wound- or entrance site and afterwards has to be transmitted to the respective plant organs responsible for secondary metabolite biosynthesis or allocation. Herbivore-damaged leaf tissue and interaction with elicitors from their oral secretions is hypothesized to lead to Ca^{2+}-mediated triggering of the mitogen-activated protein kinase (MAPK) cascade. The MAP kinases are the regulators for phytohormone upregulation upon herbivory (Ali and Agrawal, 2012; Basu et al., 2018; Dicke et al., 2009; Erb et al., 2012; Eyles et al., 2010; Frost et al., 2008; Venkatesan, 2015).

5.4.1 Phytohormones

Plant hormones translate changes within the plant's biotic and abiotic environment into endogenous signals and hence are responsible for the trade-off between development and defense. This compound group is structurally unrelated and comprises growth-, development- or stress-related hormones such as auxin and cytokinin, nitric oxide or abscisic acid as well as the defense-associated hormones ethylene, salicylic acid (SA) and jasmonic acid (JA) (Boeckler et al., 2013; Derksen et al., 2013; Dicke et al., 2009; Dixon et al., 2002; Venkatesan, 2015; War et al., 2012).

Here, the focus is laid on the defense induction-associated phytohormones JA and SA. JA regulates plant response subsequent to necrotrophic pathogen or chewing herbivore attack, while SA-mediated defense responses are activated upon biotrophic pathogens or (phloem) sucking insects (Ali and Agrawal, 2012; Boeckler et al., 2013; Caarls et al., 2015; Cheong and Choi, 2007; Dicke et al., 2009; Erb et al., 2012; Li et al., 2002; Pieterse and Dicke, 2007; Ray et al., 2015; Venkatesan, 2015). JA induction is correlated with herbivore damage and subsequent anti-herbivore defenses, i.e. Irmisch et al. (2014) measured increased JA and jasmonoyl-L-isoleucine (JA-Ile) conjugate levels after herbivory in damaged leaves in contrast to undamaged controls. Jasmonic acid is generated *via* the octadecanoid pathway and in many plant systems its induction leads to conversion into its bioactive form JA-Ile, which is coupled to subsequent transcriptional activation of JA-associated defense molecules (Caarls et al., 2015; Cheong and Choi, 2007; Derksen et al., 2013; Lawrence et al., 2006; Venkatesan, 2015; War et al., 2012). An example of JA-mediated defense is the production and emission of volatile compounds after herbivore infestation (Irmisch et al., 2014; Venkatesan, 2015).

Salicylic acid is likely derived from the phenylpropanoid pathway. This pathway is the biosynthetic origin of many defense or structural support-associated plant compounds and consequently important for plant stress reactions. The substrate of the phenylpropanoid pathway is L-phenylalanine. Nonetheless, literature suggests several routes for SA formation in plants, and it was argued that the induced pathway depends on the formulated response, such as defense gene activation or interaction with other hormone signals (Dixon et al., 2002; Dixon and Paiva, 1995; Fraser and Chapple, 2011; Venkatesan, 2015; Vogt, 2010). Due to induced concentrations of SA within the plant, activation of transcription factors leads to SA-mediated gene expression (Caarls et al., 2015; Derksen et al., 2013; War et al., 2012).

The JA and SA pathways are discussed to exhibit crosstalk. This means that their simultaneous induction leads to interactions between their biosynthetic pathways. The hormone crosstalk is suggested to aid the plant in prioritizing its actions against multiple attackers instead of a ‘first come, first served’ mechanism. Despite this, plant attackers have been reported to exploit this regulatory network by inducing signals that will suppress defense responses of the plant (Ali and Agrawal, 2012; Dicke et al., 2009; Pieterse and Dicke, 2007). Literature provides examples of antagonistic, synergistic and no interaction for JA and SA. The phytohormone crosstalk is believed to be regulated at a transcriptional level but the exact mechanisms remain elusive (Caarls et al., 2015; Cheong and Choi, 2007; Derksen et al., 2013; Dicke et al., 2009; Eberl et al., 2018; Pieterse and Dicke, 2007; Ray et al., 2015; Venkatesan, 2015; War et al., 2012).

Jasmonate induction, here comprising JA and JA-Ile, has been linked to tree volatile production. McCormick et al. (2014b) found that while application of SA to detached poplar leaves did not have an effect on the emission of volatiles, JA application induced all main classes (described below). Hence, it was hypothesized that jasmonates are involved in the signaling cascade resulting in volatile production and emission following herbivore damage. The emission of certain volatile molecules nearly immediately after feeding damage or mechanical wounding is the fastest defense response of a tree (Erb et al., 2012; Irmisch et al., 2014; McCormick et al., 2014b; McCormick et al., 2014a; Venkatesan, 2015).

5.4.2 Volatile organic compounds (VOCs)

VOCs are molecules with a low boiling point/high vapor pressure, which allows the tree to release them into its surroundings after conversion from their liquid to gaseous state. Location of release are usually membranes on epidermal tissue (Venkatesan, 2015). McCormick et al. (2014b) found that VOCs are presumably synthesized *de novo* at the release site from damaged and, in a systemic response, undamaged leaves instead of being transported from damaged to adjacent undamaged tissue. Volatile production and release can be either constitutive or induced due to changes in biotic or abiotic factors in the tree's environment. VOCs fulfill roles as pollinator attractants, in plant-insect or plant-pathogen, as well as plant-plant interactions and can be emitted from roots, leaves, flowers and fruits (Eyles et al., 2010; Frost et al., 2008; Irmisch et al., 2014; McCormick et al., 2016; Müller et al., 2015; Philippe and Bohlmann, 2007; Pieterse and Dicke, 2007; Unsicker et al., 2009; Venkatesan, 2015; War et al., 2012).

In case of herbivory, emission patterns change over the course of feeding, for example during day and night. The time lapse until emission after onset, as well as remaining emission after offset of herbivory can differ between compounds (McCormick et al., 2014a). Even though the composition of the emitted volatile blend might stay nearly constant in response to a plant stressor, the concentration of individual compounds might change drastically. Moreover, the production of novel compounds that are non-existent within the volatile blend of an unchallenged plant were described in literature (Holopainen, 2011; Lämke and Unsicker, 2018; McCormick et al., 2014a; War et al., 2012).
Induced volatile production and emission can have direct and indirect effects. Examples for direct defenses are hindrance of pathogen development or the deterrence of herbivores from both feeding and oviposition. Attracting herbivore enemies and parasitoids to either the herbivore itself or oviposition sites is an indirect defense mechanism (Hilker and Fatouros, 2016). Thereby, the volatiles are thought to be highly conclusive with regard to identity of attacker and host, herbivore age and abundance. Further indirect defenses include response induction in contiguous, undamaged plant parts of the same individual as

well as priming of neighboring trees for impending herbivory. In a primed state the tree does not immediately synthesize defense compounds due to the received priming chemical, however it is able to formulate a faster defense response upon herbivory as compared to an unprimed tree. The metabolic costs of priming are lower compared to constitutively expressed defenses, buffer the time delay of induced defenses and hence can result in a higher level of plant resistance. It is suggested that plants react to biotic stress with a mixture of induced defenses and priming. As beneficial volatiles may seem for the tree, VOCs can also be exploited by herbivores to localize an adequate host leading to increased susceptibility (Dicke et al., 2009; Eyles et al., 2010; Frost et al., 2008; Hilker and Fatouros, 2016; Holopainen, 2011; Irmisch et al., 2014; McCormick et al., 2014b; McCormick et al., 2014a; Philippe and Bohlmann, 2007; Pieterse and Dicke, 2007; Unsicker et al., 2009; Venkatesan, 2015; War et al., 2012).

Typical VOCs released from poplar species can be categorized into the following chemical classes: terpenoids, green leaf volatiles (GLVs), aromatic and nitrogenous compounds, esters, alcohols, aldehydes, ketones, alkanes and alkenes (Holopainen, 2011; Irmisch et al., 2014; Maffei, 2010; McCormick et al., 2014a; Müller et al., 2015; Unsicker et al., 2009).

Terpenes are the most diverse and abundant compounds found in a *P. nigra* volatile blend. They are divided into mono- (C_{10}), sesqui- (C_{15}), diterpenes (C_{20}) and so forth depending on the number of C-atoms in their carbon skeleton. The degree of volatility decreases with increasing number of C-atoms. Terpenes are biosynthesized from the isoprenoid (C_5) unit isomers isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP) *via* the 2-C-methyl-erythritol-4-phospate (MEP) as well as the mevalonate pathway. The huge structural variety of terpenes is gained downstream *via* terpene synthase enzymes due to carbocation formation and follow-up reactions, e.g. cyclization (Irmisch et al., 2014; McCormick et al., 2014b; Venkatesan, 2015). Terpene synthases are important enzymes in terpene formation, their activity and upregulation aid understanding the internal plant processes after attack from a herbivore, such as *de novo* synthesis or allocation of volatiles in damaged and undamaged adjacent tissue. Exemplarily, it has been demonstrated that the expression level of terpene synthases increases after herbivore attack, which is in concordance to VOC emission increase. Most terpenes start being emitted a few hours after herbivory onset and exhibit prolonged emission in a light dependent manner after herbivory is stopped, which resulted in their association with induced defense mechanisms (Irmisch et al., 2014; McCormick et al., 2014a; McCormick et al., 2016; Unsicker et al., 2009).

GLVs are compounds with a six-carbon chain skeleton that comprise the typical smell of damaged leaves or freshly cut grass. They are derived from polyunsaturated fatty acids *via* a side branch of the octadecanoid pathway (Holopainen, 2011; Maffei, 2010; Scala et al.,

2013; Unsicker et al., 2009; Venkatesan, 2015; War et al., 2012). Green leaf volatiles are induced upon mechanical damage regardless of biotic or abiotic origin and were found to have an important role in priming. Since their emission starts early on after herbivore infestation and soon declines after herbivory is stopped, they are regarded as promising markers for herbivore presence and could be exploited by herbivore enemies (Lawrence et al., 2006; McCormick et al., 2014b; McCormick et al., 2016; Venkatesan, 2015; War et al., 2012).

Aromatic volatiles are derived from the phenylpropanoid pathway. The structural feature common for all aromatic VOCs is an aromatic ring within the molecule (Dixon et al., 2002; Holopainen, 2011; Maffei, 2010; Venkatesan, 2015; Vogt, 2010). Amino acid-derived volatile compounds are probably produced from 2-keto acids through decarboxylation, esterification or reduction reactions (McCormick et al., 2014b; Venkatesan, 2015). Even though they do not contribute high emission levels to the volatile blend of a herbivore-challenged poplar, nitrogenous compounds have strong attractive effects on herbivore enemies. Close to no emission in undamaged trees make them a reliable sign of herbivore presence (McCormick et al., 2014b).

5.4.3 Phenolics

Poplar phenolics with a known or hypothesized role in tree defense include salicinoids, phenolic acids like coumaric or ferulic acid; flavan-3-ols such as catechin, epicatechin and gallocatechin; flavonoids; oligomer-derivatives of flavan-3-ols (condensed tannins) and cell structure-associated compounds such as lignin precursors. All these compounds originate from different branches of the phenylpropanoid pathway (Boeckler et al., 2011, 2013; Philippe and Bohlmann, 2007; Vogt, 2010).

Salicinoids are secondary metabolites of the Salicaceae that have an important role in plant-pathogen and plant-herbivore interaction functioning as toxins or feeding deterrents. They are a subclass of phenolic glycosides and classified as constitutive as well as inducible defense. The building blocks of salicinoids are comprised of salicyl alcohol, which is bound to the anomeric C-atom of a β-D-glucopyranose unit. The name of this compound group was inferred from its structurally simplest member salicin, which also functions as scaffold of all higher-order salicinoids. Higher-order salicinoids can be derived from salicin *via* one or several esterifications of either the alcohol or sugar hydroxyl groups with different organic acids (Boeckler et al., 2011, 2013; Boeckler et al., 2016; Chedgy et al., 2015; Lämke and Unsicker, 2018; Philippe and Bohlmann, 2007). So far, the salicinoid biosynthetic pathway in poplar is not completely elucidated (Boeckler et al., 2011). Nonetheless, literature suggests that the salicyl alcohol moiety of salicinoids as well as the hydroxycyclohexanone

carboxylic acid (HCC) moiety found in salicortin result from the phenylpropanoid pathway (Babst et al., 2010; Boeckler et al., 2011, 2013; Chedgy et al., 2015). Babst et al. (2010) suggested that salicin might not be an immediate precursor of the higher-order salicinoid salicortin.

Salicinoids are abundant in roots and shoots of poplar and their concentration level is dependent on different factors such as genotype, developmental stage or season. Salicinoid concentration can vary in a tissue-dependent manner or due to abiotic and biotic stress. Thereby, the biggest effect is correlated to the genotype of the tree. Regarding ontogenetic variation, the youngest tissue shows the highest salicinoid content and these levels slowly decrease during maturation, thus young tissue is protected better against herbivore damage. This might result from the longer lifetime and hence higher carbohydrate production that is expected of younger tissue compared to older foliage. In this context, both *de novo* synthesis as well as translocation from older tissue is discussed (Boeckler et al., 2011, 2013; Chedgy et al., 2015; Massad et al., 2014; Philippe and Bohlmann, 2007).

Salicinoids as found in the plant are non-toxic, but sugar cleavage and further reactions lead to a toxic aglycone. Activation towards the aglycone can be due to induction of plant enzyme activity, such as β-glucosidases or spontaneous reactions after wounding, but salicinoids can also be activated inside the digestive system of the herbivore. Thereby, plant or insect β-glucosidases could target the glycosidic bond between salicylic alcohol and sugar. In case of plant-derived activation, enzyme and target molecule are compartmentalized to only induce the reaction upon combining both after tissue disruption through wounding. For complex salicinoids, esterases could additionally cleave off the bound organic acids (Babst et al., 2010; Boeckler et al., 2011, 2013; Boeckler et al., 2016; Burse et al., 2009; Philippe and Bohlmann, 2007; Vassão et al., 2018). Salicinoids or rather their degradation products were implied to act as feeding deterrents and to reduce fitness of the generalist insect *L. dispar*, e.g. increasing developmental time and decreasing survival rate, larval and pupal weight or the number of eggs produced by females. Toxicity is thereby conferred by HCC, saligenin and catechol or downstream metabolites such as *o*-quinones. Quinones are electrophiles and can undergo reactions with nucleophilic substituents of biomolecules, hampering their function (Boeckler et al., 2011; Boeckler et al., 2016; Chedgy et al., 2015).

Figure 1 (Fig. 1) shows a schematic overview of plant-pathogen as well as plant-insect interactions and subsequent plant responses. The recognition, signaling and response mechanism of a poplar tree towards frass, accumulating at the foot of the tree due to herbivory, have not been thoroughly studied yet and remain to be elucidated.

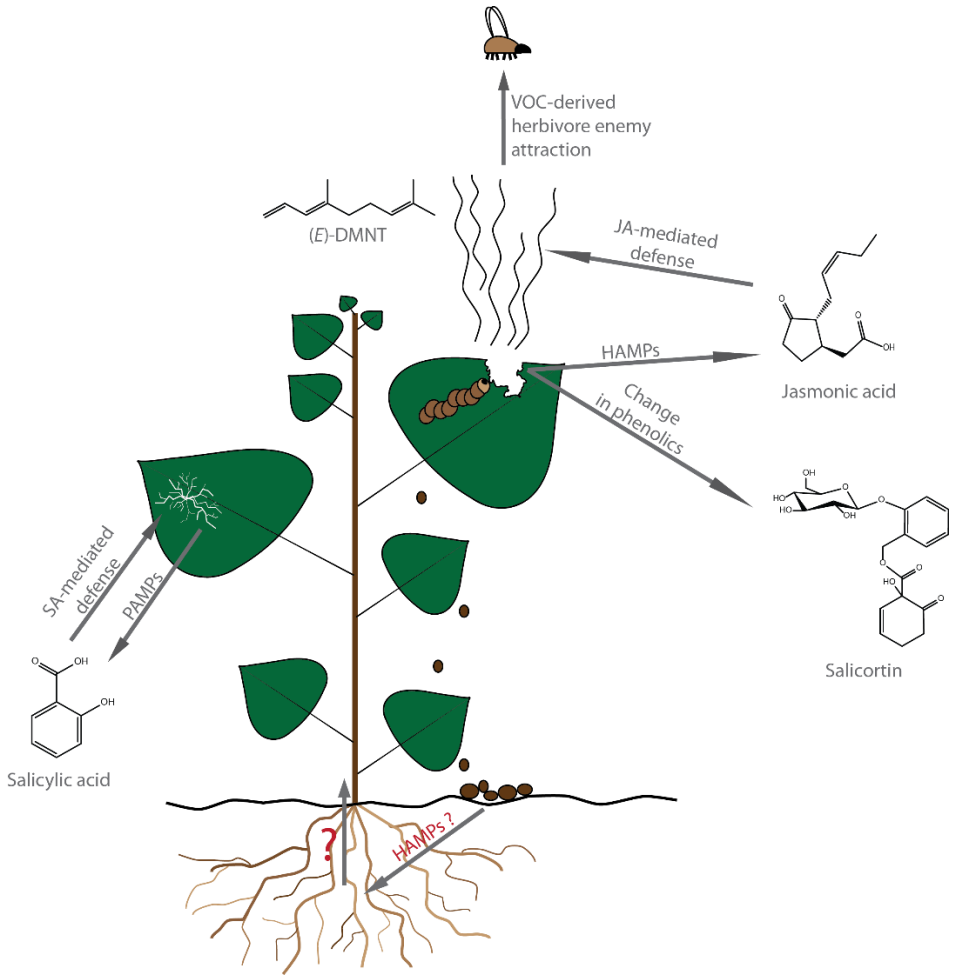

Figure 1. Schematic overview of plant-insect and plant-pathogen interactions (Adobe® Illustrator® CS5). After recognition of a biotrophic pathogen or a herbivore *via* pathogen-associated molecular patterns (PAMPs) or herbivory-associated molecular patterns (HAMPs), respectively, the poplar can respond with upregulation of defense compounds. Biotrophic pathogens or caterpillar herbivory leads to salicylic acid (SA)- or jasmonic acid (JA)-mediated defense responses of the tree, accordingly. JA-mediated defense includes the emission of volatile organic compounds (VOCs) such as the terpene (*E*)-4,8-dimethylnona-1,3,7-triene (DMNT). The VOCs can for example deter the herbivore from further feeding or attract herbivore enemies. Next to volatile emission, caterpillar herbivory can also lead to a concentration change in phenolic compounds, for example salicinoids like salicortin, which play a role in plant defense. The elicitors from frass, which accumulates at the foot of the tree due to herbivore feeding, the interaction between poplar and frass as well as the resultant tree response remain to be elucidated.

In this study, the response of young *P. nigra* trees to frass application of the generalist *L. dispar* or the specialist insect *C. populi* and subsequent herbivory by the generalist defoliator *L. dispar* was investigated. The general research questions were a) whether the poplars could recognize frass application and b) if this would lead to defense activation. Therefore, the investigated hypotheses were that recognition of insect frass application occurs in poplar saplings. Further, if recognition occurred, it was hypothesized that the poplar trees would respond differently to frass application from a specialist as compared to a generalist. This is due to the fact that specialists are more adapted to their host plant and might be able to evade certain defense mechanisms of the plant. Hence, a lowered response to *C. populi* frass than towards *L. dispar* frass was anticipated. By investigating

the defense mechanisms typically induced by herbivory, the hypothesis that frass application triggers a similar defense machinery was tested.

6. Material and Methods

6.1 Plants, Insects and Frass

The experiments were conducted on three-month-old f65 *P. nigra* trees, which were obtained from stem cuttings of a female f65 genotype growing in Isserstedt, Germany. The cuttings were reared in a greenhouse at 21 – 23 °C or 19 – 21 °C during day and night, respectively, 50 – 60 % relative humidity and 14 light hours (summer conditions) in mineral wool using a *Nicotiana*-hydroponic solution (constituents appendix Table 1 (A1)) for two months. Apparent algae on the mineral wool and roots were removed at regular intervals and before transferring the poplars into polyvinyl chloride (PVC) containers for the experiment. The cuttings were transferred into amber-colored 200 mL PVC containers (Kautex, VWR^{TM} International, Darmstadt, Germany), encased ¾ in light-impenetrable cloth (FloraSelf Unkrautvlies, Bornheim, Germany) 7 or 12 days before starting the preliminary or main experiment, respectively. A 5 mL pipette tip was cut off at the 2.5 mL marking and inserted into the container next to the plant for watering (Fig. 3). The plants were watered daily between 8 and 9 AM with a poplar hydroponic solution (Table A2). The cuttings were placed beneath the artificial light sources (Valoya, Helsinki, Finland) one day after being transferred into the 200 mL containers.

The young trees showed early-stage signs of a mildew infection and therefore the fungal mycelium was removed daily from the leaves using a wet cotton cloth. Further, the plants were treated with UV light (254 and 365 nm, Herolab, Wiesloch, Germany) once per day, since it has been implied to increase the resistance towards leaf pathogens (Demkura and Ballaré, 2012). 16 hours prior to the herbivory treatment of the main experiment, the plants were transferred to another greenhouse cabin with the following conditions: day/night temperature of 19 – 21 °C/ 17 – 19 °C, 45 – 60 % humidity and 14 hours of light. The poplar saplings were kept under these conditions throughout the herbivory treatment, volatile collection as well as the sampling of plant tissue.

Gypsy moth caterpillar eggs were kindly provided by Dr. Nadel (US Department of Agriculture, Buzzards Bay, USA) and reared on an artificial diet (MP Biomedicals LLC, France) until reaching the wanted developmental stage. The herbivory treatment of the frass induction experiment was carried out using *L. dispar* of developmental instar stages L3 and L4.

Poplar leaf beetle eggs were collected in the field in the surroundings of Jena, Germany and were reared on black poplar. New biodiversity is introduced to the established breed from newly collected field-*C. populi* each summer.

The insect frass was obtained from *L. dispar* caterpillars of different developmental larval stages or adult *C. populi*, respectively, feeding on a mixture of different female poplar genotypes. The air-dried frass for each species was collected in October 2018 and from 22nd March 2019 – 22nd May 2019. Dried leaf material, caterpillar exuvia or beetle eggs were removed as best as possible and the frass from the different sampling time points was pooled. The frass was kept at -20 °C until processing.
Lymantria dispar frass had a green, light brown to yellow color and consisted of small pellets of round to cylindrical shape with a size of up to 3 mm. Its' smell was reminiscent of the forest floor. The poplar leaf beetle's frass was even smaller/finer compared to the caterpillar frass. It showed an oval shape with a color range from bright to dark brown or black. The contained salicylaldehyde carries a prominent, sour odor (Fig. 2B and E).

6.2 Preparation of frass solution for application to black poplar saplings

Due to the total amount of available frass, 1 g ground frass (analytical balance PG1003-S, Mettler Toledo, Gießen, Germany) per species was dissolved in 50 mL tap water to prepare frass solutions for both the preliminary and main experiment. Before grinding, the frass of both species differed in color and shape, while after grinding both consisted of a green to yellow powder. The *C. populi* frass still had a slightly brighter color as compared to *L. dispar* (Fig 2B and E). The frass-water mixture was vortexed for 1 minute, then shaken with 150 revolutions per minute (rpm) at 25 °C for 30 minutes (Novotron AI82 K incubation shaker, Bottmingen, Suisse). Afterwards, the remaining unsolved frass particles were spun down at 200 rpm for 2 minutes at 20 °C [Avanti™ J-20 XP centrifuge with JS-4.3 rotor (Beckman Coulter ™, Brea, USA)] and the supernatant was transferred to a new 50 mL vial (SARSTEDT, Nümbrecht, Germany). The frass solutions were stored at -20 °C. On each frass application day, the solutions were thawed in a hand-warm water bath and frozen again after administration. As can be seen in Fig. 2, both frass solutions showed a brown color, however the *L. dispar* frass solution (Fig. 2C) had a darker hue compared to the *C. populi* solution (Fig. 2F). Tap water was used to lower possible contaminations such as pollen and further latent variables from rainwater.

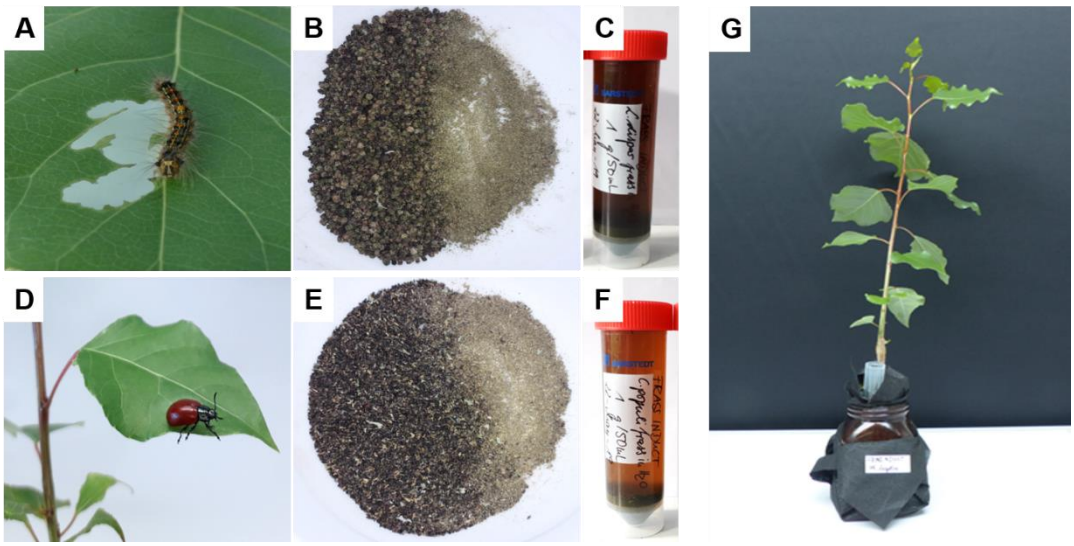

Figure 2. Experimental chain from the feeding insect to the frass solution for *Lymantria dispar* (A-C) and *Chrysomela populi* (D-F), respectively. Frass of both insect species having fed on black poplar (A, D) was collected, air-dried and ground. In solution, the *Lymantria dispar* frass had a darker brown hue than the *Chrysomela populi* frass after a shaking time of 30 minutes and subsequent centrifugation (C, F). The experimental set up for the frass induction experiment is viewed in G.

6.3 Frass treatment of black poplar saplings in hydroponic solution

The plants were evenly distributed into three blocks each containing four replicates of a treatment. Within one block, the treatments were randomly distributed. The treatments comprised controls, *L. dispar* frass-treated and *C. populi* frass-treated trees. These 3 frass treatments were additionally divided into a herbivory or non-herbivory treatment. 12 individual plants served as biological replicates for each treatment. The duration of frass application amounted to 6 days. The controls received 3.5 mL (Labmate 5 mL pipette, Abimed HTL, Warsaw, Poland) of tap water from the greenhouse whereas the respective solution was added to the insect frass treatments (Fig. 3). Then all poplar hydroponic PVC containers were filled up to 200 mL with hydroponic solution (seripettor®, BRAND, Wertheim, Germany). Frass administration and watering of the trees was carried out between 8:30 and 9 AM. The trays holding the poplar saplings were rotated clockwise every second day within one block to establish homogeneous abiotic conditions. The poplars were watered with hydroponic solution on the herbivory and sampling day in order to rule out drought stress signaling within the trees.

6.4 Herbivory treatment of black poplar saplings in hydroponic solution

The poplar saplings were encased in 61 x 30 cm polyethylene terephthalate (PET) bags (Toppits® Bratschlauch, Minden, Germany) and the shoots of herbivory treatment plants were infested with a constant amount of L3/L4 gypsy moth caterpillars for each block (ratio of L3/L4 for block I to III: 12/4, 7/7, 11/5) (Fig. 3). All treatments were encased in PET bags to level out their effect on the plant, such as reduced photosynthesis (Frost and Hunter, 2007). In order to reduce transpiration of the plants, silica and charcoal-filtered air was

pushed into the system at 0.8 L/min *via* Teflon tubing and air was allowed to leave the system through a small Teflon tube. After 24 hours of herbivory, the VOCs of all 24 plants per block were collected.

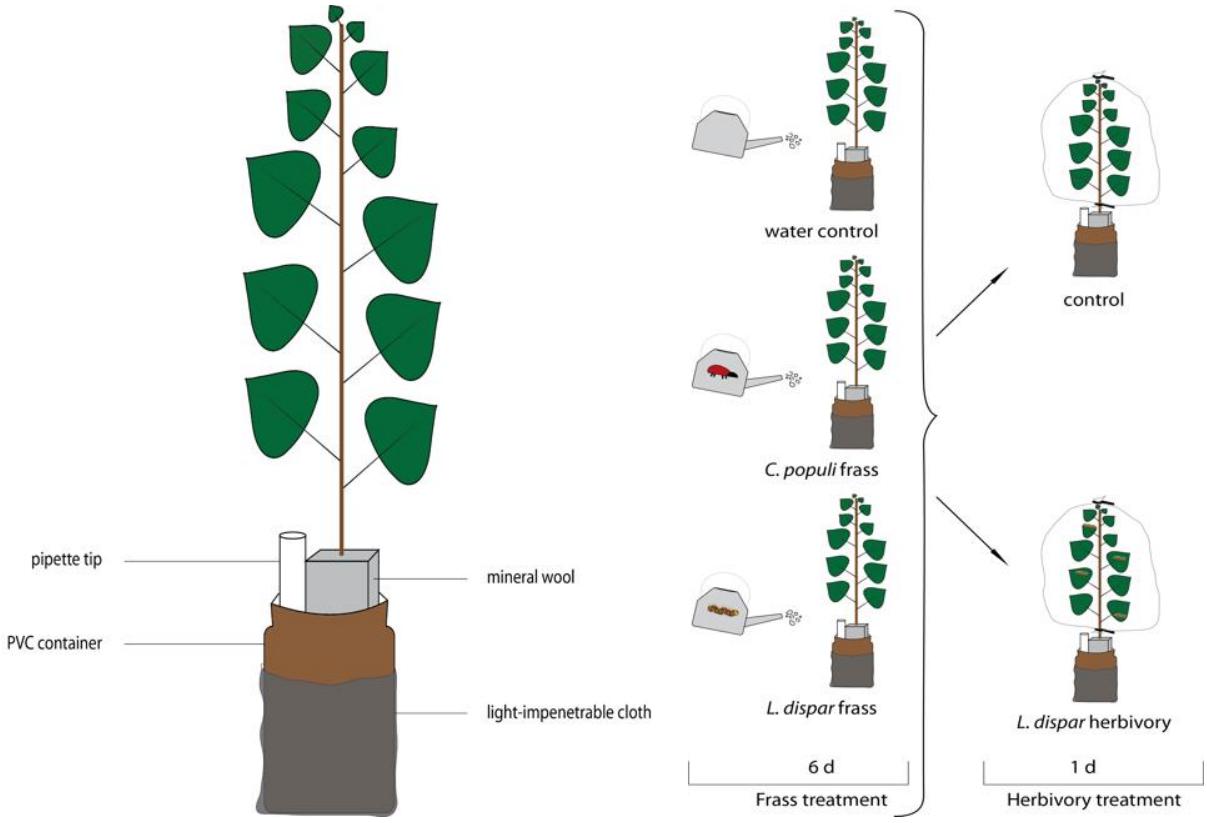

Figure 3. Schematic illustration of the set up (left-hand side) and experimental design of the frass induction study (right-hand side) (Adobe® Illustrator® CS5). The poplars were divided into three groups and subjected to the different frass solutions or tap water as control for six days. Each treatment was split into two groups of additional herbivory or no herbivory, respectively. *Lymantria dispar* caterpillars fed on the herbivory-treated poplars for one day.

6.5 Volatile collection, elution and measurement

The VOC collection was carried out utilizing a push-pull system for 3 hours, where silica and charcoal-filtered air was pumped into the bottom of the PET bags at 0.8 L/min [flowmeters (Brooks instrument, Hatfield, USA)] *via* Teflon tubing. After having passed the whole system, the incoming air was pulled out again at 0.6 L/min over filters containing a PoroPak volatile trap (Analytical Research Systems, Inc., Gainesville, USA). The VOC emission measurements only result from tree shoots since the set up hindered measurement of volatiles from root or hydroponic solution. Regarding herbivory-treated trees, the caterpillars were still present during the volatile collection (Fig. 4). Afterwards, the filters were sampled and stored at 4 °C until extraction. In order to distinguish the caterpillar scent from the tree volatiles, caterpillars of the *Lymantria dispar* frass-treated trees were transferred into a new 30 x 30.5 cm PET bag. 4 caterpillar scent replicates were measured for each block. Their VOCs were collected for 2 hours in the same manner as stated above with an air inflow and outflow of 0.6 L/min and 0.4 L/min, respectively.

The filters were eluted two times with 100 µL [500 µL syringe (Hamilton, Reno, USA)] dichloromethane (GC ultra grade, Carl Roth, Karlsruhe, Germany) containing 10 ng μL^{-1} nonyl acetate (Sigma Aldrich, St. Louis, USA) as internal standard (IS). Extracts were stored at -20 °C until analysis.

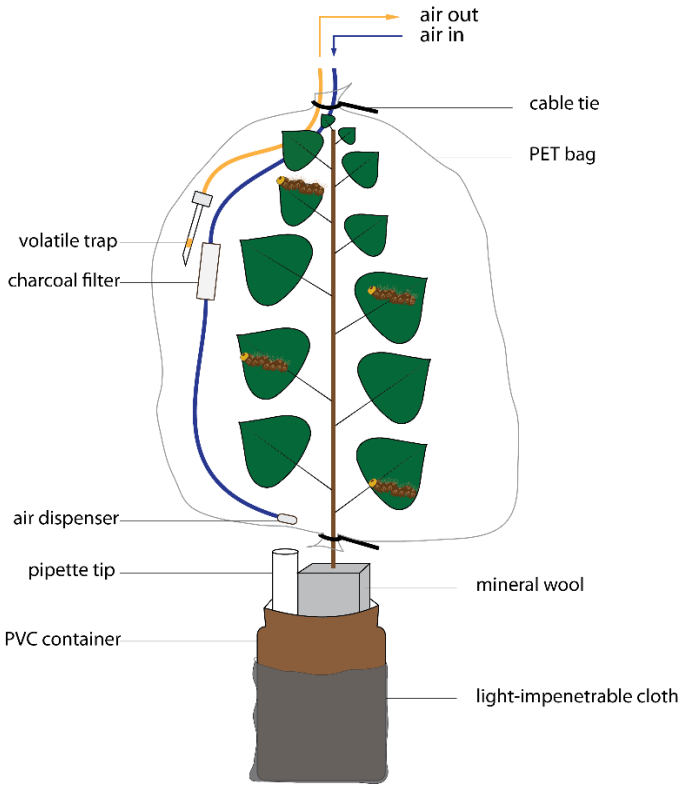

Figure 4. Schematic illustration of the volatile collection apparatus (Adobe® Illustrator® CS5).

Qualitative and quantitative measurements were conducted on a gas chromatograph coupled to a mass spectrometer (GC-MS) [6890N Network GC System (Agilent Technologies); mass selective detector 5973 inert (Agilent Technologies, Santa Clara, USA)], or a flame ionization detector (GC-FID) (Agilent Technologies, Santa Clara, USA) respectively. Both instruments contained a DB5-MS chromatographic column (30 m x 0.25 mm x 0.25 µm, Agilent Technologies, Santa Clara, USA) and He or H_2 were used as carrier gas for the GC-MS or GC-FID, correspondingly. The instrument parameters as well as the computing of the mass spectra and chromatograms was carried out according to Eberl et al. (2018). Compounds were identified by matching sample MS spectra to references of the Wiley and National Institute of Standards and Technology libraries and for the FID by matching retention times (R_T) and peak patterns as compared to the MS. The analyte concentration was calculated *via*:

$$c_{analyte} = \frac{area_{analyte} \text{ x relative response factor x } c_{IS}}{area_{IS} \text{ x leaf fresh weight x duration of VOC collection}} \qquad \text{equation 1}$$

Thereby the relative response factor was either measured using authentic standards, or estimated according to the effective carbon number concept (Scanlon and Willis, 1985).

6.6 Plant tissue sampling and extraction

After the VOC collection, the leaves were photographically documented to investigate the leaf area loss due to herbivory utilizing Adobe® Photoshop® CS5 (Version 12.0 x64). The roots were freed from mineral wool and accumulated algae and washed thoroughly. Subsequent to recording the fresh weight of roots and leaves (balance LabStyle602, Mettler Toledo, Gießen, Germany), the plant tissue was flash frozen in liquid nitrogen. Leaf sampling began at the first fully developed leaf and the oldest leaves were not sampled due to their different chemical constitution (Boeckler et al., 2011). However, when herbivore-infested plants showed herbivory within the youngest or oldest leaves, they were sampled as well. The plant material was stored at -80 °C until processing.

For extraction, the leaf and root material was lyophilized (Alpha 1-4 LD plus, Christ, Osterode am Harz, Germany), equipped with 5 steel balls of 2-4 mm diameter (ASKUBAL, Korntal-Münchingen, Germany) and homogenized in a Skandex S-7 paint shaker (Fluid Management, Satorius AG, Göttingen, Germany). For each sample, 10 mg ground plant material (analytical balance XP26, Mettler Toledo, Gießen, Germany) were extracted with 1 mL 100 % methanol (gradient grade for LC, Merck, Darmstadt, Germany) containing the IS for phytohormones: [40 ng mL^{-1} D_6-salicylic acid (Santa Cruz Biotechnology, Dallas, USA), 40 ng mL^{-1} D_6-jasmonic acid (HPC Standards GmbH, Cunnersdorf, Germany), 40 ng mL^{-1} D_6-ABA (Santa Cruz Biotechnology, Dallas, USA), 8 ng mL^{-1} $^{13}C_6$-jasmonoyl-isoleucine (synthesis as stated in Kramell et al. (1988))]; phenolics: 0.8 mg mL^{-1} phenyl-β-glucopyranoside (Sigma Aldrich, St. Louis, USA), phenolic acids [10 ng mL^{-1} syringic acid (Sigma Aldrich, St. Louis, USA), 10 ng mL^{-1} triflouro-methyl-cinnamic acid (Alfa Aesar, Haverhill, USA)] and the amino acids [10 µg mL^{-1} for each ^{13}C, ^{15}N-labeled amino acid (instead of tryptophan, labeled phenylalanine was used) (Isotec®, Miamisburg, USA)]. The samples were shaken for 30 minutes at 240 rpm (HS250 basic, IKA® Labortechnik, Steifen im Breisgau, Germany) and centrifuged at 3200 rpm for 5 minutes [centrifuge 5810 (Eppendorf, Hamburg, Germany)].The supernatant was used for analysis. For the sugar analysis, external standards of the respective analytes were used in a concentration range of 0.0625, 1.25, 2.5, 5, 10 and 20 µg mL^{-1}.

6.7 Analysis of phytohormones, phenolic acids and amino acids

The phytohormone, phenolic acids and amino acid (AA) measurements were conducted on a high performance liquid chromatograph coupled to a tandem mass spectrometer (HPLC-MS/MS) system [HPLC 1260 Infinity II (Agilent Technologies, Santa Clara, USA) - QTrap® 6500^+ (AB Sciex, Waltham, Massachusetts, USA)] in multiple reaction monitoring (MRM) mode. The analytes were separated on a reversed phase (RP) ZORBAX Eclipse XDB-C18 column (1.8 µm, 4.6 mm x 50 mm, Agilent Technologies, Santa Clara, USA). The mobile phase consisted of 0.05 % formic acid (Fisher Scientific, Waltham, Massachusetts, USA) in

MilliQ® water (Synthesis A10 Millipore Water, Merck, Darmstadt, Germany) as solvent A and acetonitrile (HPLC LC-MS grade, VWR International, Darmstadt, Germany) as solvent B. B was used in a gradient starting at 5 % (0.00 – 6.00 min), increased to 37.4 % (6.00 – 6.02 min) and further to 100 % (6.02 – 7.00 min), held shortly at 100 % (7.00 – 7.02 min) and was then decreased again to the 5 % starting level (7.02 – 9.5 min). The injection volume and mobile eluent flowrate amounted to 1 µL or 1.1 mL min^{-1}, respectively. Negative ionization mode was used at an ionization energy of -4500 eV for the electrospray ionization (ESI) source with a depolarization potential of -30. Other MS source parameters include a curtain gas of 40, electrospray and drying gas of 60, with the temperature of the drying gas being set to 700 °C. The measured precursor and quantifier ions for phytohormones and phenolic acids can be viewed in Table A4. Peak integration was carried out utilizing the software Analyst 1.6.3 (AB Sciex, Waltham, Massachusetts, USA).

Listed below are the LC and MS parameters, which differed among the phytohormone and AA analysis. The LC eluents were MilliQ® water as solvent A and acetonitrile as solvent B. B was subjected to a gradient, starting with 3 % (0.00 – 2.70 min), subsequent increase to 100 % (2.70 – 3.00 min) and held at 100 % (3.00 – 3.10 min) before decreasing B to starting levels of 3 % (3.100 – 6.00 min). MS parameters for the AA analysis include positive ionization mode with an ionization energy of 5500 eV, depolarization potential of 20, a curtain gas of 40 whereas electrospray and drying gas amounted to 70, with a drying gas temperature of 650 °C. The investigated mass fragments are stated in Table A5. Peak integration was carried out using the software Analyst 1.6.3 (AB Sciex, Waltham, Massachusetts, USA).
The sample concentration for phytohormones, phenolic acids and AAs was normalized according to equation 2:

$$c_{analyte} = \frac{\text{peak area}_{analyte} \text{ x relative response factor x } c_{IS} \text{ x dilution factor}}{\text{peak area}_{IS} \text{ x sample weight x extraction volume}} \qquad \text{equation 2}$$

6.8 Analysis of sugars and phenolics

Sugar contents of the samples were measured on a HPLC-MS/MS system [HPLC 1200 Series (Agilent Technologies, Santa Clara, USA) –triple quadrupole API 3200 (MDS Sciex, Applied Biosystems, Waltham, Massachusetts, USA)] in MRM mode. For chromatographic separation, an apHera™ NH_2 hilic HPLC column fitted with a hilic pre-column was used (5 µm, 150 mm x 4.6 mm, Supelco analytical, Altmann Analytic, Munich, Germany). The injection volume amounted to 5 µL and the mobile phase flowrate was set to 1 mL min^{-1}. The eluents were MilliQ® water and acetonitrile as solvent A and B, respectively. B underwent a gradient from 80 % (0.00 – 0.50 min), to 55 % (0.50 – 13.00 min), was afterwards increased again to 80 % (13.00 – 14.00 min) and held at this final concentration

for 4 minutes. The ESI-MS source was used in negative ionization mode at -4500 eV. The curtain gas was set to 20, electrospray and drying gas amounted to 50 or 60, respectively, with a temperature of 600 °C for the drying gas. The investigated mass fragments are stated in Table A6. Analyte concentrations were deduced from the external standard curves and normalization carried out with equation 2. Peak integration was carried out using the software Analyst 1.5.1 (AB Sciex, Waltham, Massachusetts, USA).

Targeted salicinoids and flavonoids were separated on a HPLC-ultraviolet light (UV) system [HPLC 1100 Series (Hewlett Packard, Berlin, Germany)] and detected *via* a diode array detector (DAD). A nucleodur sphinx RP column (5 µm, 250 mm x 4.6 mm, Macherey-Nagel, Düren, Germany) was used to separate the analytes. The mobile phase consisted of MilliQ® water and acetonitrile as solvent A and B, respectively. B was used in a gradient and increased from 14 to 58 % (0.0 – 22.0 min), was then increased to 100 % (22.0 – 22.1 min), held at 100 % (22.1 – 25.0 min) and subsequently decreased to initial levels of 14 % (25.0 – 25.1 min) and kept at this concentration (25.1 – 30.0 min). The flowrate amounted to 1 mL min^{-1}. The measured UV range lay within 190 and 360 nm. The analyte absorption spectra were matched to their corresponding standard. Normalization was carried out as stated in equation 2.

6.9 Carbon and nitrogen content analysis of insect frass

In order to investigate the realistic fertilization properties of the *C. populi* and *L. dispar* frass, their carbon and nitrogen content (C/N) was analyzed using a VarioEL CNS elemental analyzer (Elementar, Langenselbold, Germany). Thus, the samples were homogenized, dried over night at 65 °C (Heraeus function line, Kendro Laboratory Products, Hanau, Germany) and cooled down to room temperature in a desiccator (Duran, Wertheim, Germany) filled with cobalt(II) chloride-dyed silica gel (Carl Roth, Karlsruhe, Germany). For the measurement of the insect frass C/N ratio, three technical replicates were analyzed and their mean value was computed.

6.10 Statistical analysis

Initial data visualization was carried out in IBM® SPSS® Version 25 (IBM Corp., Armonk, USA). Statistical testing was carried out with R version 3.6.1 (R Core Team, 2019).

As the labels of a few leaf samples were lost during the extraction procedure, these samples were not incorporated into the results. The data was evaluated for normal distribution and heteroscedasticity. When referring to data, means ± standard errors (SE) will be given. Generalized least squares (GLS) models, allowing for heterogeneity of variance in the raw data, were fitted to the dataset in the manner of backward model selection as presented by Zuur et al. (2009). The Akaike Information Criterion (AIC) was utilized for analysis of

goodness of fit of the tested models. Analysis of Variance (ANOVA) tests were performed on the final model and *F*- and *P*-values are stated (Crawley, 2007; Zuur et al., 2009).

7. Results and discussion

7.1 Preliminary experiments to optimize the experimental design

In order to apply frass resulting from complete defoliation of one poplar sapling to the trees in the main study, a first experiment aimed at identifying the corresponding frass amount. 20 plants were set up as mentioned in the material and methods section, containing 4 controls and 8 plants per insect species which were sacrificed to 6 adult *C. populi* or a mixture of 8 L2/L3 *L. dispar* caterpillars, respectively. All plants were encapsulated in PET bags and equipped with air in- and outlet. Due to time constraints, the experiment was stopped before complete defoliation. The herbivory damage was concluded by averaging it for one plant and using the rule of three to estimate the frass accumulation in case of 100 % herbivory (equation 3 and Table A3). The caterpillars and beetles used in the experiment were starved for 16 hours to obtain the frass within the insects' system. Calculation for the average frass accumulation from one whole sapling:

$$\text{frass mass for one sapling} = \frac{(\text{averaged frass amount from all saplings x 100 \% damage})}{\text{averaged leaf damage on all saplings}} \qquad \text{equation 3}$$

Applying equation 3 and adjusting it for the increased height of the main experiment plants, an average frass amount of 0.354 g for *C. populi* and 0.524 g for *L. dispar* was expected to result from one defoliated poplar sapling. The final concentration of the frass solution was set to 20 mg mL^{-1} and was prepared in tap water. Poplar saplings were treated daily with 3.5 mL of the solution over a 6-day period in the frass induction experiment. Hence, 0.42 g frass were administered for the whole treatment period. In case of *L. dispar* a frass amount equivalent to roughly 80 % herbivory and regarding the poplar leaf beetle, roughly 119 % herbivory of a neighboring poplar sapling were applied. Setting the weight as constant parameter for the frass solutions of both insect species facilitates their comparison when elucidating the plant chemistry as well as in an untargeted analysis due to equal mass concentration.

A shaking time series of poplar defense compounds with both *L. dispar* and *C. populi* frass solutions (20 mg mL^{-1}) was conducted to determine whether shaking time had an effect on solvated compounds. No standards were added to the water extracts, absolute abundance was compared *via* peak areas in counts. The used markers were salicin, salicylic acid, jasmonic acid, caffeic and coumaric acid as measured with HPLC-MS/MS [HPLC 1260 Infinity II (Agilent Technologies, Santa Clara, USA) - QTrap® 6500^{+} (AB Sciex, Waltham, Massachusetts, USA)]. The 30-minute shaking treatment posed the best compromise between abundance of investigated compounds and preparation time (data not shown) and hence was used for the main experiment.

Previous observations demonstrated that the greenhouse provides excellent growth conditions for algae within transparent PVC containers. As the algae can induce unwanted reactions in the poplar, which might mask or interfere with the response to the frass treatment, the reduction of algal growth in the used system was desired. It is known that algae growth is closely linked to light intensity and temperature (Carvalho and Malcata, 2003). To test whether algal growth could be slowed down due to reduced light intensity, 3 replicates of 4 different treatments were tested: transparent PVC containers as control, amber-translucent PVC containers, transparent PVC containers coated in black acryl paint and transparent containers covered with a black light-impenetrable cloth (Fig. 5A, C, E, G). Within three days, strong algal growth was observed within the controls (Fig. 5A and B). In contrast, for the acryl paint, cloth and amber-translucent container treatments no algae were visible on the roots or container walls (Fig. 5D, F, H). However, the hydroponic solution of all treatments contained some small, brown algae particles. Hence, the amber-translucent PVC containers were utilized for the frass induction experiment. Furthermore, it was observed that the roots in the paint and cloth treatments were better developed than in both translucent PVC container treatments. This seems reasonable, as the darkened treatments more closely resemble the natural environment of poplar saplings growing in sandy riverbank soil (Debeljak et al., 2015). This resulted in the experimental set up as described in the methods section, the light impenetrable cloth was used to compromise between beneficial root development and algal growth hindrance *versus* filling level visibility. The mineral wool showed algal growth for all treatments depending on light availability and therefore was encapsulated in light-impenetrable cloth. For the main experiment, the roots looked healthy and well developed. The optimized set up aided in reducing the algae amount at the root tissue in the frass induction experiment.

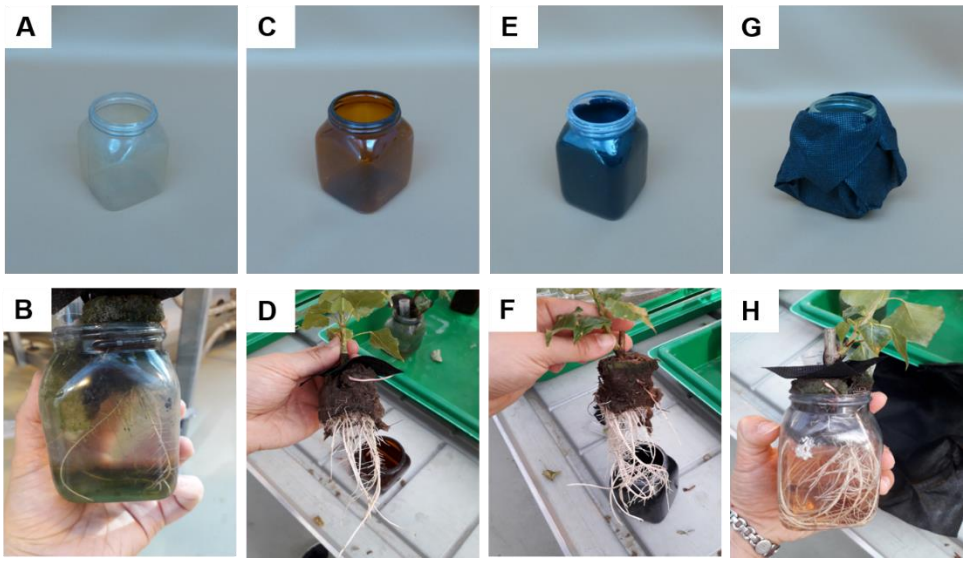

Figure 5. Preliminary experiment to reduce algal growth in the PVC containers. Algal infestation and root development compared for the transparent controls (A-B), amber-translucent (C-D), cloth (E-F) and paint treatment (G-H).

7.2 Frass induction experiment

7.2.1 Morphological parameters of poplars in the frass induction experiment

Major and Constabel (2007) suggest that above- or belowground herbivory could result in enhancement of resistance traits in both shoots and roots and hence root and leaf tissue was investigated in this study. Also, the first site of attack was frass application to the root tissue and changes in root development or chemistry could aid the understanding of subsequent plant processes. Observations of the general plant parameters, such as fresh weight (FW), height increase or leaf area loss due to herbivory are visualized in Fig. 6 and Table A8.

The overall leaf FW amounted to 5.31 ± 0.10 g (Table A8) and was affected by blocks and the frass as well as herbivory treatment (Fig. 6 and Table 1). Regarding the frass treatment alone, a significant difference in mean leaf FW was obtained where *C. populi* frass-treated trees showed lower values compared to control and *L. dispar* frass-treated trees. This could be an indication that a signal of the *C. populi* frass either hindered growth due to limitations or too high concentrations of nutrients, it is also possible that the plant started to invest energy into defense instead of growth (Kagata and Ohgushi, 2012b). These hypotheses need to be tested by looking at the chemical constitution of the plants. For additional caterpillar herbivory on top of the frass treatment, a similar outcome to the sole frass treatment was observed with lower leaf FW for *C. populi* frass-treated trees compared to control and *L. dispar* frass-treated poplars (Fig. 6). As expected, caterpillar herbivory led to decreased leaf FW compared to undamaged trees. The blocks differed significantly in their leaf FW (Table 1) with higher values for blocks II and III compared to block I. This probably results from the longer growing period of blocks II and III (2 or 4 days, respectively) in contrast to block I.

For the treatments combining frass and caterpillar herbivory, the leaf area loss from herbivore feeding amounted to 15.12 ± 0.97 % (Table A8). Even though a trend for higher leaf area loss on *L. dispar* frass-treated plants compared to *C. populi* frass-treated plants or controls was found it was non-significant (Fig. 6 and Table 1). On average, 12.80 ± 0.38 leaves were damaged by caterpillars and no differences between the treatments or blocks were observed (Table 1). This demonstrates that caterpillar herbivory was distributed evenly throughout the poplar shoot instead of excessive feeding on a few leaves, which could potentially lead to a local instead of a systemic response and was undesired in this experiment. The frass treatment did not seem to have induced a signal that made the tree impalatable or resulting in a bad taste for the caterpillars. Indications of this would have been minimalistic feeding on many leaves as well as increased mortality of caterpillars.

The overall height increase of the poplars during the frass treatment period amounted to 9.95 ± 0.30 cm (Table A8). As can be obtained from Fig. 6 and Table 1, the poplar height

increase did not differ strongly between the frass treatments alone or in combination with caterpillar herbivory. This corresponds to Frost and Hunter (2008) who did not observe changes in the tree growth rate, measured as stem width increase, upon frass treatment of red oak. This is in contrast to literature stating fertilizer-like quality of deposited frass (Frost and Hunter, 2008). Possibly the duration of the frass treatment was too short to induce growth, which due to cycling of taken-up nutrients within the tree might need longer to be induced. For the frass induction experiment, caterpillar herbivory could not have had any effect, because the caterpillar herbivory treatment was carried out after plant growth measurements. The blocks differed significantly in their height increases. This is due to the fact that initial plant height was measured at the same day for all trees and trees from block II and III were grown 2 or 4 days longer than trees from block I.

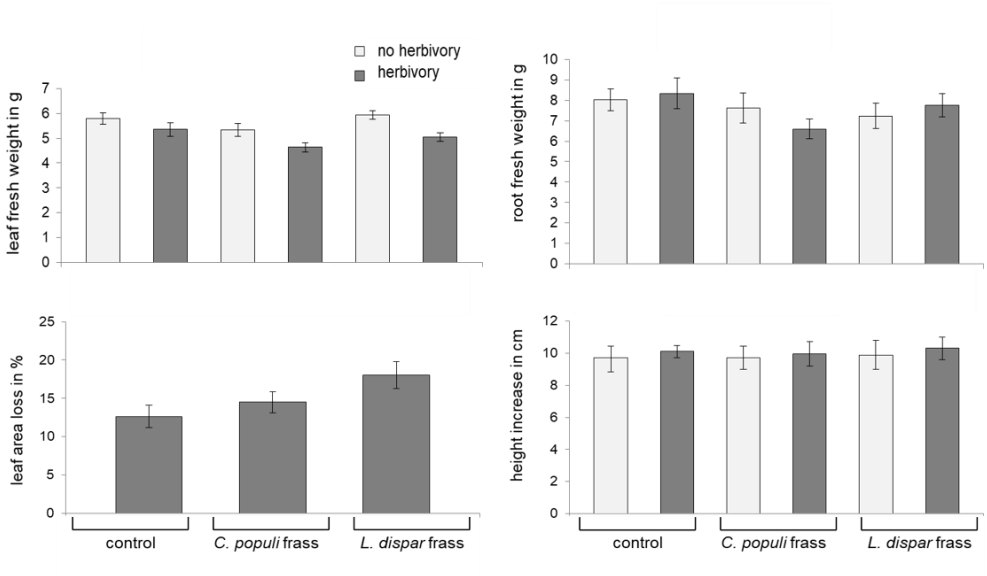

Figure 6. Leaf and root biomasses as well as leaf area loss and height increase of the poplar trees over the course of the experiment. Poplars were either treated with water (control), *Chrysomela populi* frass or *Lymantria dispar* frass and additionally subjected to a caterpillar herbivory or non-herbivory treatment (color code). The data is given as mean ± SE (n= 9-12 for leaves and n= 12 for roots).

The averaged root FW amounted to 7.59 ± 0.26 g (Table A8) and was affected by the blocks. Leaf caterpillar herbivory led to a slight but non-significant decrease in root FW of *C. populi* frass-treated plants. Significant differences in root FW were found between blocks (Fig. 6 and Table 1) where block II and III showed higher FWs, which could result from their longer growth (2-4 days) with respect to block I. This result, just as for the height increase, could mean that frass application of two insect species with differing specialization degree does not influence root growth, or that the reaction thereof has a longer induction period than the one measured in this experiment. The phenomenon could be subject of a follow-up study of frass influence on poplar trees. It is striking that while leaf FW differed between frass treatments, no such effect was found in the roots, the immediate site of application. No signs of tissue necrosis or other negative influences of the applied frass on the trees

were observed during the frass induction experiment which is in concordance with literature (Frost and Hunter, 2008).

Table 1. Results from GLS models for leaf and root fresh weight (FW), leaf area loss, height increase and number of herbivory-damaged leaves (n= 9-12 for leaves and n= 12 for roots) of the frass induction experiment.

	Frass		Herbivory		Block	
	F	*P*	*F*	*P*	*F*	*P*
Leaf FW	**6.18**	**<0.01**	**20.93**	**<0.001**	**19.77**	**<0.001**
Leaf area loss	2.86	0.07	/	/	0.97	0.39
Height increase	0.32	0.73	1.37	0.25	**106.15**	**<0.001**
Number of damaged leaves	0.36	0.70	/	/	0.54	0.59
Root FW	2.30	0.11	0.26	0.61	**27.14**	**<0.001**

7.2.2 Phytohormones

Since phytohormones regulate the trade-off between plant development and defense, and are associated to be the signals that lead to plant defense against a biotic attacker, they can give important hints concerning defense response activation within the plant (Erb et al., 2012; Pieterse and Dicke, 2007). Jasmonates and SA as defense signaling-associated compounds are presented in Fig. 7 for leaf and root tissue.

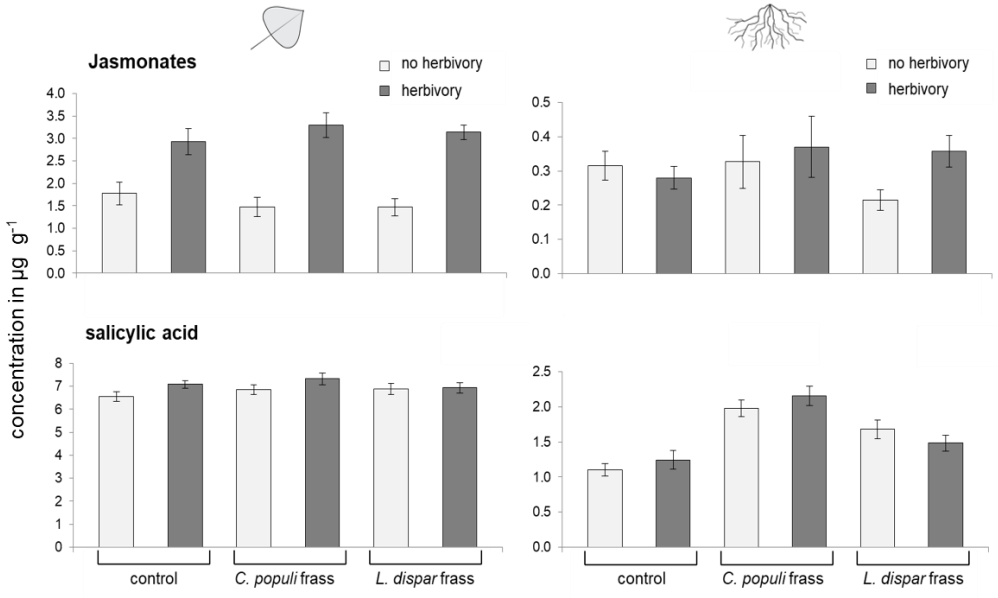

Figure 7. Defense pathway activation-associated molecules salicylic acid and jasmonates, comprising JA and JA-Ile, for leaf and root tissue of poplar trees. Plant treatment contained control, *Chrysomela populi* or *Lymantria dispar* frass application and an additional herbivory treatment (color code). The abundance is given as mean ± SE (n= 10-12 for leaves and n= 12 for roots).

The overall jasmonate concentration was 2.39 ± 0.14 µg g^{-1} (Table A8) and was affected by blocks and the herbivory treatment. A trend for decreased jasmonate levels in the frass-treated compared to control plants was obtained, but not significant. Additional caterpillar herbivory led to a statistically significant increase in jasmonate concentration for the frass

treatments and control trees with highest levels expressed in *C. populi* frass-treated plants (Fig. 7 and Table 2). Induction of jasmonate levels was expected upon caterpillar herbivory, as it is known from literature that plant defenses are activated *via* jasmonate signaling upon mechanical wounding or insect herbivory (see further hydroxyl- and carboxylated jasmonates Tables A8 and A9) (McCormick et al., 2014b). The blocks also had significant differences in jasmonate levels with block II showing lower levels compared to block I and III in samples from the frass and additional herbivory treatment (Table 2). This could be a result of the higher amount of L4 instars in block II, as herbivore age is known to play a role in the magnitude of induction of defense compounds (McCormick et al., 2014a).
The averaged leaf SA level amounted to 6.49 ± 0.09 µg g^{-1} (Table A8) and was not affected by blocks, the frass or herbivory treatment. Caterpillar herbivory led to a slight but marginally insignificant increase in leaf SA levels for control and *C. populi* frass-treated plants (Table 2). No effect of herbivory was observed for *L. dispar* frass-treated plants which is in concordance with results from feeding studies on old-growth poplar (Boeckler et al., 2013). Also, Frost and Hunter (2008) neither observed an increase nor decrease in shoot SA concentration due to frass deposition during the same growing season.

In the root tissue, jasmonate levels on average amounted to 0.31 ± 0.02 µg g^{-1} (Table A8) and were affected by blocks and the herbivory treatment. Jasmonate concentrations are lower in root compared to leaf tissue. As observed in leaves, additional caterpillar herbivory led to a significant difference for the frass treatments (see further hydroxyl- and carboxylated jasmonates Tables A8 and A9). However as can be seen in Fig. 7, the patterns differ between root and leaf tissue: whereas in leaves all frass treatments displayed a high increase in jasmonates upon herbivory, caterpillar herbivory has no influence on control and *C. populi* frass-treated plants but results in increased jasmonate abundance for *L. dispar* frass-treated plants in roots (Table 2). The interaction between frass and herbivory was tested to be significant (F= 6.59, P< 0.01).
Overall SA root levels amounted to 1.61 ± 0.07 µg g^{-1} (Table A8) and were affected by blocks and the frass treatment. The SA concentration was lower in roots than in leaves. Concerning the frass treatment, SA levels were significantly increased for both frass types with highest levels for *C. populi* frass-treated trees. The blocks also had significantly different SA levels, with block I tendentially showing higher SA root levels compared to block II and III (Table 2).

The concentration increase response of leaf jasmonate levels towards caterpillar herbivory was anticipated and demonstrated that the herbivory treatment activated the herbivory-associated defense signaling pathway *via* JA within the poplars (McCormick et al., 2014b). In addition, it can be seen that the signal is formed locally in the damaged leaf tissue and

does not respond as quickly in other plant parts such as roots. The root SA levels pose an intriguing outcome. SA-associated defense signaling is correlated with defense activation against biotrophic pathogens and sucking herbivores (Caarls et al., 2015; Pieterse and Dicke, 2007). Even though the plants showed early stages of mildew infection, no differences in leaf SA levels were found for the frass treatments. Hence, mildew infection does not explain indifferent leaf SA levels and increased root SA levels in *C. populi* and *L. dispar* frass-treated compared to control plants. It is known that *C. populi* frass contains salicylaldehyde and *L. dispar* frass contains salicin (Boeckler et al., 2016). As these compounds as well as SA are derived from the phenylpropanoid pathway, maybe a conversion or breakdown reaction of salicylaldehyde or salicin to SA occurred. It remains unclear whether the elevated SA levels in roots result from *de novo* synthesis or due to taking up SA as breakdown product, or converting salicylaldehyde or salicin to SA within the roots. This could be investigated by monitoring the biosynthetically involved enzymes, as far as known, for SA. Also, SA might only be adhered to the roots instead of being taken up by them, however the roots were thoroughly washed before the harvest. Finding an active transporter for phenylpropanoid products in the root membranes could help elucidating whether adherence or incorporation took place.

The jasmonic and salicylic acid pathways are discussed to exhibit crosstalk which can lead to antagonistic or synergistic effects (Caarls et al., 2015; Cheong and Choi, 2007; Derksen et al., 2013; Dicke et al., 2009; Eberl et al., 2018; Pieterse and Dicke, 2007; Ray et al., 2015; Venkatesan, 2015; War et al., 2012). SA-JA antagonism would be probable when increase of one of the compounds led to decrease of the other phytohormone partner, whereas synergism would entail the same response of both phytohormones with a total effect exceeding the sum of the individual compounds. Neither synergism nor antagonism was observed for SA and jasmonates for the frass induction experiment, e.g. increased SA root levels did not lead to a typical antagonistic effect for jasmonates in root tissue. This might indicate that phytohormone crosstalk is dependent on the plant attacker in poplars or that SA was merely adhered to the roots and therefore could not interact with the jasmonate biosynthetic pathway within the roots (Caarls et al., 2015; Eberl et al., 2018; Pieterse and Dicke, 2007).

Table 2. Results of GLS models for the phytohormones SA and the jasmonates JA and JA-Ile in leaves and roots (n= 10-12 for leaves and n= 12 for roots).

	Frass		Herbivory		Block	
	F	*P*	*F*	*P*	*F*	*P*
Leaf SA	0.87	0.43	3.73	0.06	3.07	0.055
Root SA	**25.52**	**<0.001**	0.15	0.70	**3.90**	**0.03**
Leaf jasmonates	0.56	0.57	**103.42**	**<0.001**	**39.19**	**<0.001**
Root jasmonates	0.78	0.46	**75.62**	**<0.001**	**9.51**	**<0.001**

7.2.3 Volatiles

Altogether, the headspace of *P. nigra* saplings contained 57 VOCs of which 29 could be identified (Table A7). The volatile blend consisted of monoterpenes, green leaf volatiles, aromatic and nitrogenous compounds, sesquiterpenes and the homoterpene 4,8-dimethylnona-1,3,7-triene (DMNT). Terpenes were the most abundant volatile class in this experiment. As described in the methods section, compound identification was carried out *via* matching the mass spectra of the samples to references in a spectral library and to authentic standards. The comparison of standard to sample MS spectra is presented in Fig. A1 and shows selected compounds as annotated by the spectral library regarding the volatile classes GLVs, homo-, mono-, sesquiterpenes and aromatic compounds in the sample and the standard. The library successfully matched the investigated samples to the reference standards and therefore poses a reliable tool in compound assignment. Unidentified compounds termed 'unknown' had a too low concentration for appropriate matching to the spectral library or compound class identification (Table A7). Fig. 8 displays two typical GC-MS total ion current (TIC) chromatograms of the headspace of an untreated *P. nigra* sapling (control) and a plant that underwent *C. populi* frass- and a subsequent caterpillar herbivory treatment.

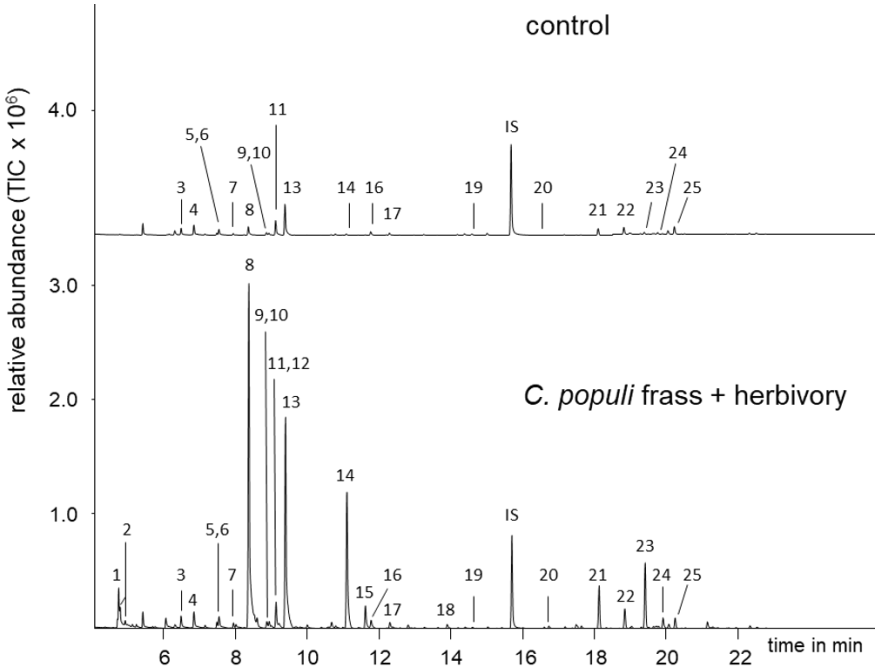

Figure 8. GC-MS total ion current chromatograms of a non-herbivore induced control (above) *versus* a *Chrysomela populi* frass and herbivore-induced sample (below). The indicated numbers correspond to the following relevant identified volatiles: (*Z*)-3-hexenol (1); 2-methylbutyraldoxime (2); α-pinene (3); camphene (4); sabinene (5); β-pinene (6); β-myrcene (7); (*Z*)-3-hexen-1-ol acetate (8); limonene (9); 1.8 cineole (10); (*Z*)-β-ocimene (11); salicylaldehyde (12); (*E*)-β-ocimene (13); (*E*)-DMNT (14); benzyl cyanide (15): camphor (16); borneol (17); (*Z*)-3-hexenyl-isobutyrate (18); ethylacetophenone (19); IS nonyl acetate; eugenol (20); β-caryophyllene (21); α-humulene (22); germacrene D (23); (*E*),(*E*)-α-farnesene (24); δ-cadinene (25).

It can be seen that the concentration of some compounds was drastically increased in the headspace of a plant receiving both a frass and herbivory treatment, e.g. (*Z*)-3-hexen-1-ol

acetate (8), (*E*)-β-ocimene (13) or (*E*)-DMNT (14). Moreover, new compounds that cannot be detected in the control plant arise due to the treatments, for example 2-methylbutyraldoxime (2) (Fig. 8). The major volatile compound classes stayed the same, but a qualitative and quantitative change in the emitted volatile blend was observed due to the frass and herbivory treatment, which is in concordance with literature (McCormick et al., 2014b; McCormick et al., 2014a).

Emission patterns of the major VOC classes for the frass induction experiment are depicted in Fig. 9. All major VOC classes were affected by the herbivory treatment, which led to a strong and significant induction of volatile emission for frass-treated as well as control trees. This effect of caterpillar herbivory on VOC emission is well-described in literature (Irmisch et al., 2014; McCormick et al., 2014b; McCormick et al., 2016; Venkatesan, 2015). The frass treatment did not affect volatile emission of the major VOC classes (Table 3).
On average, GLVs showed an emission of 119.51 ± 18.84 ng h^{-1} g^{-1} FW (Table A7). Caterpillar herbivory resulted in up to 40-fold increase in GLV emission. Moreover, the VOC class contains compounds that are only present in herbivore-treated trees, which makes them good biomarkers for herbivore presence (McCormick et al., 2016). Overall monoterpene emission amounted to 164.53 ± 19.45 ng h^{-1} g^{-1} FW (Table A7). Caterpillar herbivory led to up to 8-fold emission increase and the VOC class contains compounds known to play a role in plant-plant communication, such as (*E*)-β-ocimene (Irmisch et al., 2014). The homoterpene (*E*)-DMNT on average was emitted at 69.95 ± 11.81 ng h^{-1} g^{-1} FW and had induced levels of up to 77-fold due to caterpillar herbivory (Table A7). Altogether, 70.97 ± 9.07 ng h^{-1} g^{-1} FW sesquiterpenes (Table A7) were emitted on average during the VOC collection and contained β-caryophyllene, a known attractant for herbivore enemies (Irmisch et al., 2014). For sesquiterpenes, the blocks were significantly different showing higher values for block I in frass treatments without herbivory, whereas block I and III displayed higher concentrations for additional caterpillar herbivory (Table 3 and Table A7). The terpene data corresponds to earlier findings on volatile emission from herbivory-damaged young poplars stating that acyclic monoterpenes, sesquiterpenes and DMNT demonstrate a many-fold emittance induction due to caterpillar feeding (Holopainen, 2011; Irmisch et al., 2014; McCormick et al., 2014b; McCormick et al., 2014a; McCormick et al., 2016). Overall emission of aromatic volatiles amounted to 24.57 ± 3.99 ng h^{-1} g^{-1} FW where caterpillar herbivory led to an emission increase of up to 92-fold (Table A7). Nitrogenous compound emission amounted to 44.73 ± 7.42 ng h^{-1} g^{-1} FW with emission induction of up to 114-fold due to caterpillar herbivory (Table A7). The blocks showed significantly different emission of nitrogenous compounds with higher values for block I in frass treatments without herbivory, whereas block I and III showed higher levels for additional caterpillar herbivory (Table 3 and Table A7). Previous studies in poplar reported the detected benzyl

cyanide, salicylaldehyde and aldoximes as important herbivory damage-indicating and herbivory-inducible molecules (Irmisch et al., 2014; McCormick et al., 2014b; McCormick et al., 2014a; McCormick et al., 2016). The differences in the blocks for sesquiterpenes and nitrogenous compounds could be due to caterpillar age, block II tendentially contained a higher percentage of L4 instar larvae compared to blocks I and III. Herbivore age and feeding intensity were found to result in different magnitudes of VOC emission with higher emittance due to younger instar *L. dispar* (McCormick et al., 2014a).

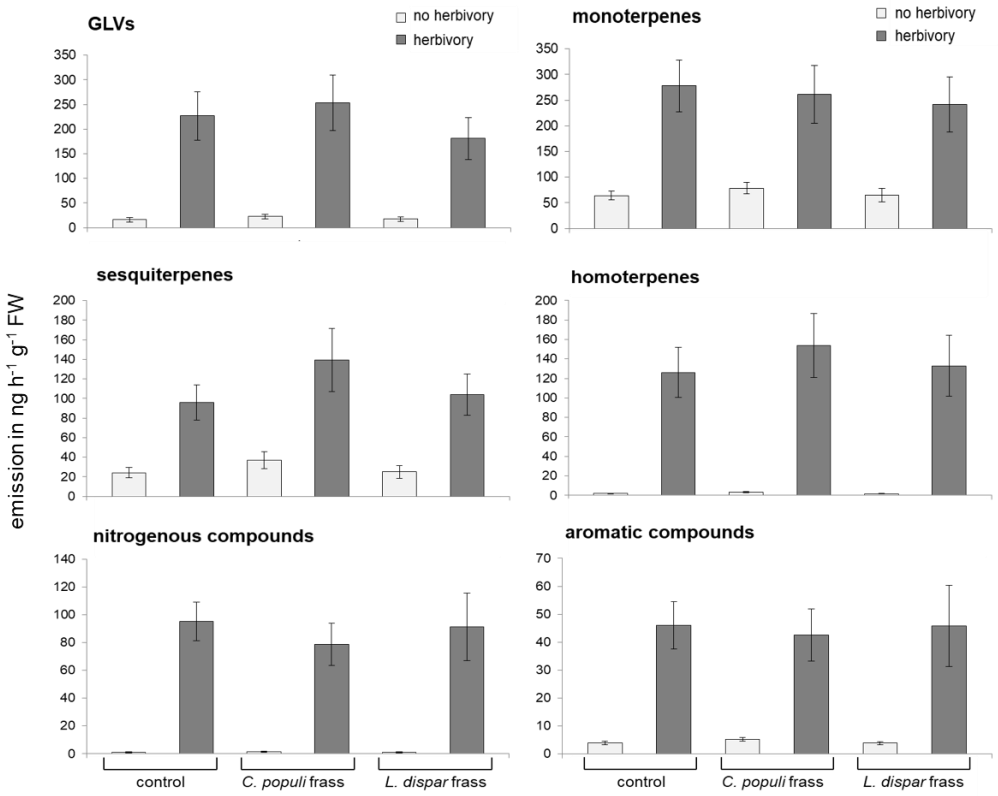

Figure 9. VOC emission of 3-month-old poplars after frass and herbivory treatment. The frass treatment comprised a water control, application of *Chrysomela populi* or *Lymantria dispar* frass, respectively. An additional caterpillar herbivory treatment is indicated with the color code. The abbreviation FW stands for fresh weight. The mean ± SE of the summed GLVs, monoterpenes, sesquiterpenes, homoterpenes, nitrogenous as well as aromatic compounds are displayed (n= 12). Detailed information about the specific compounds within each volatile class can be found in Table A7.

Caterpillar scent from the herbivory and *L. dispar* frass-treated trees was measured and revealed that mostly aromatic compounds like ethylacetophenone, salicylaldehyde, eugenol, (*Z*)-jasmone, camphene and some unidentified compounds were emitted by the caterpillars (Table A7). Considering the volatile data of the major VOC classes they pose good biomarkers for current herbivore feeding as all classes were induced upon herbivory. Identified compounds with background emission levels in absence and highly induced emittance in the presence of herbivores could be exploited by herbivore predators to locate their food source, aid in priming of neighboring plants for impending herbivory, but could also lead other herbivores to the host plant (McCormick et al., 2014b; McCormick et al., 2016; Unsicker et al., 2009; Venkatesan, 2015).

Some of the 28 unidentifiable compounds could be grouped according to their behavior in response to the treatments. All groups of unidentified compounds were affected by the herbivory treatment (Table 3). VOCs solely produced upon caterpillar herbivory regardless of frass treatment are represented by unknown 1 (Fig. 10) and further comprised the unknown compounds 2, 11, 15 and 18 (Table A7). These compounds with herbivory as significant factor could be useful biomarkers for herbivore enemies and precisely document herbivore abundance. However, the compounds could maybe be produced upon mechanical damage in general. Herbivory-induced unidentified compounds comprise unknown 4, 5, 7, 9, 10, 12, 14, 17, 20-22 and 28 (Fig. 10, Table 3 and Table A7), and as well could be suitable signals for predators of insect herbivores or priming other plants or plant parts for impending herbivory (Unsicker et al., 2009; Venkatesan, 2015). Unknown compounds 2, 3 and 6 (Table A7) display a fascinating trend for *C. populi* frass-treated samples. Whereas caterpillar herbivory led to a strong induction for control and *L. dispar* frass-treated poplars, no increased emission was found for *C. populi* frass-treated plants (Fig. 10 and Table 3). This could potentially mean that the specialist frass suppresses induction of these compounds. This phenomenon might be an adaptation strategy of *C. populi* towards its host plant to avoid induction of direct or indirect defenses (Ali and Agrawal, 2012). Due to the interesting defense-related implications of these compounds, it would be advantageous to isolate and structurally elucidate these molecules for example *via* fractionation and NMR analysis.

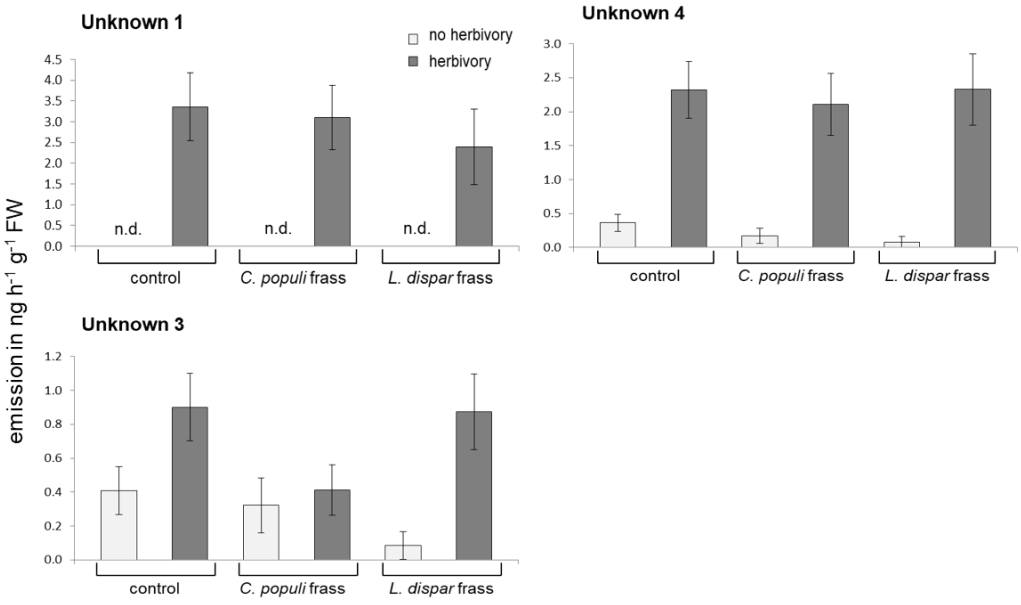

Figure 10. Unidentified VOC compounds displaying interesting trends. Unknown 1 is not abundant in undamaged plants, n.d. stands for not detected, and only observed for caterpillar herbivory samples. Unknown 4 is induced upon caterpillar feeding. Compound unknown 3 shows an interesting decreasing trend for the *Chrysomela populi* frass treatment with additional caterpillar herbivory. Mean values ± SE (n= 12) are given for control, *Chrysomela populi* or *Lymantria dispar* frass-treated plants. The herbivory treatment is indicated by the color code.

Table 3. Results of GLS models for the VOC groups GLVs, mono-, homo- and sesquiterpenes, aromatic and nitrogenous compounds and the single compounds unknown 1, 3 and 4 of the headspace of poplar undergoing the treatments control, *Chrysomela populi* or *Lymantria dispar* frass application and an additional caterpillar herbivory treatment (n= 12). Detailed information about the compounds in each volatile class is stated in Table A7.

	Frass		Herbivory		Block	
	F	*P*	*F*	*P*	*F*	*P*
GLVs	1.01	0.37	**48.29**	**<0.001**	0.40	0.68
Monoterpenes	0.51	0.60	**33.47**	**<0.001**	2.83	0.07
Sesquiterpenes	1.45	0.24	**30.29**	**<0.001**	**3.79**	**0.03**
Homoterpenes	2.74	0.07	**57.66**	**<0.001**	0.75	0.48
Aromatic compounds	1.36	0.27	**42.90**	**<0.001**	0.31	0.74
Nitrogenous compounds	0.39	0.68	**75.45**	**<0.001**	**12.93**	**<0.001**
Unknown 1	0.33	0.72	**34.21**	**<0.001**	0.18	0.84
Unknown 3	1.18	0.31	**9.63**	**<0.01**	0.82	0.45
Unknown 4	1.62	0.21	**65.65**	**<0.001**	1.39	0.26

7.2.4 Influence of insect frass application on free essential amino acids in poplar

Even though blocks, the frass and herbivory treatment also had a significant effect on free total sugar and amino acid levels (further information see Table A8 and Table A9), the focus is laid on essential amino acids here. The essential AA level defines the protein quality and with that nutritional value of a host plant and has a strong influence on herbivore growth and fitness. Essential AAs have to be taken up *via* the food and for leaf-feeding herbivores comprise histidine, lysine, arginine, methionine, phenylalanine, threonine, isoleucine, valine, leucine and tryptophan (Barbehenn et al., 2013; Ruuhola et al., 2003). Within the leaves, the average free essential AA concentration amounted to 48.75 ± 1.37 nmol g^{-1} (Table A8) and was affected by blocks, the frass as well as the herbivory treatment. The frass treatment alone showed a significant difference in mean leaf free essential AAs with higher values for *C. populi* frass-treated plants in comparison to controls or *L. dispar* frass-treated trees. Additional caterpillar herbivory led to a significant increase in concentration for the frass treatments and controls, where frass-treated trees showed a higher induction compared to controls (Fig. 11 and Table 4). The blocks resulted in significant differences in leaf mean essential AA levels with lower values for block II. One strategy of a plant to deter herbivores from feeding is to alter the chemical composition of the leaves reducing the nutritional value or creating a bad taste for the attacker. This in turn depends on the optimal intake need of the herbivore and the growth-limiting amino acids for the specific plant-herbivore system (Barbehenn et al., 2013; Frost and Hunter, 2008). Maybe raised essential amino acid contents lead to a bad leaf taste for the caterpillars. However, no deviation from normal feeding behavior was observed during the experiment. Drastic changes in amino acid composition levels could make the plant impalatable for the herbivore and might

explain the move from damaged leaves to undamaged foliage. Lowered nutritional value of the host plant could result in compensatory feeding and lead to ingestion of toxic levels of secondary metabolites (Ruuhola et al., 2003).

Phenylalanine is an essential amino acid and the substrate of the phenylpropanoid pathway known for the production of defense compounds in poplar. Average leaf abundance amounted to 527.11 ± 24.91 nmol g^{-1} (Table A8) and was affected by blocks and the frass treatment. The frass treatment alone resulted in a significant decrease of phenylalanine for *C. populi* and *L. dispar* frass-treated plants compared to controls. Also, a significant difference in phenylalanine levels between the blocks was obtained with higher phenylalanine values for block II in frass-treated undamaged plants and higher levels in block III for caterpillar-herbivory and frass-treated trees (Fig. 11 and Table 4). Further, the two-way interactions between frass and herbivory (F= 4.05, P= 0.03) or herbivory and block (F= 6.88, P< 0.01), respectively, were significant. Decreasing phenylalanine contents in the frass-treated samples could suggest that this precursor was used up more for defense compound production. This could be indicated by elevated concentration of defense products of the phenylpropanoid pathway and can be investigated by relating their induction profile to phenylalanine.

This phenomenon is even more clearly observed in the root tissue, where blocks, the frass as well as herbivory treatment affected phenylalanine levels (Table 4). For sole frass treatment, frass-treated poplars showed a significant decrease in phenylalanine levels compared to controls. Additional caterpillar herbivory had a significant effect on the phenylalanine concentration showing decreased levels in frass-treated plants compared to control trees. The blocks had a significant effect on root phenylalanine concentration where block II showed higher levels for non-damaged plants, whereas caterpillar herbivory led to higher values for block III. With an overall concentration of 465.04 ± 16.03 nmol g^{-1} (Table A8) the levels of phenylalanine did not vary strongly between roots and shoots (Fig. 11 and Table 4).

Regarding root free essential AA levels, the averaged amount was 7.44 ± 0.27 nmol g^{-1} (Table A8) and was affected by the frass treatment. The frass treatment resulted in a significant difference for free root essential AAs displaying reduced levels for *L. dispar* frass-treated plants in comparison to controls and *C. populi* frass-treated trees (Fig. 11 and Table 4). The different outcome regarding the caterpillar herbivory treatment in root compared to leaf tissue might suggest that the response is induced locally in the leaf and does not or at least not quickly affect root tissue levels of essential AAs. The concentration of essential amino acids in the roots is much lower compared to leaf levels.

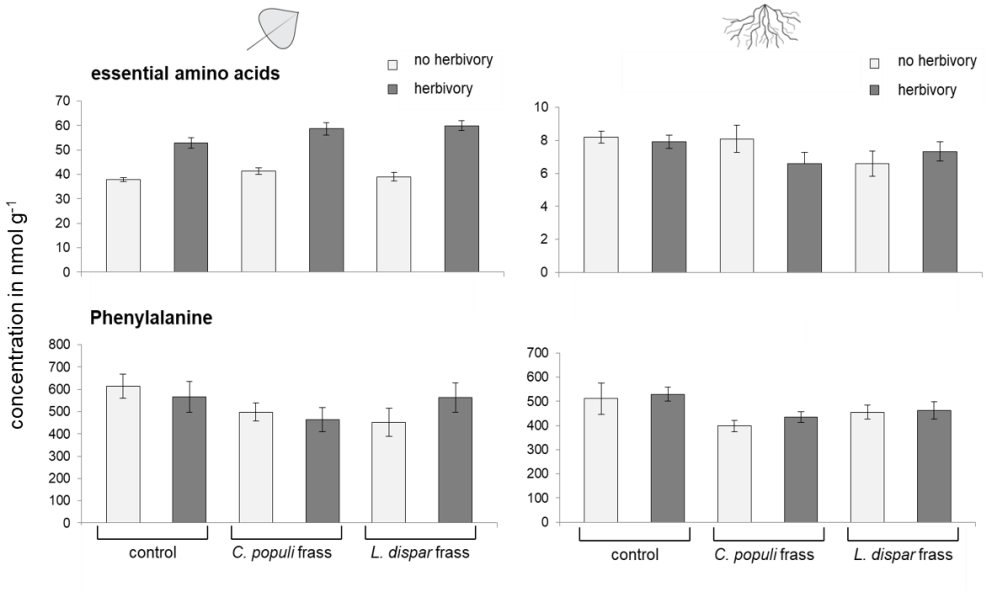

Figure 11. The free total essential amino acids comprising valine, threonine, phenylalanine, isoleucine and leucine or solely phenylalanine concentrations in leaf and root tissue of poplar saplings having been treated with water (control), *Chrysomela populi* or *Lymantria dispar* frass, respectively. The color code implies the herbivory treatment. The mean values ± SE are given (n= 10-12 for leaves and n= 12 for roots).

7.2.5 Role of phenolics in defense after insect frass application to poplar

With regard to phenolic secondary metabolites of black poplar, salicin, higher-order salicinoids and the flavan-3-ol catechin were investigated in leaf and root tissue and are presented in Fig. 12. Overall salicin levels in leaf tissue amounted to 7.81 ± 0.13 mg g^{-1} (Table A8) and were affected by blocks and the frass treatment. With regard to the frass treatment alone, a significant difference in mean salicin concentrations was obtained with higher values for *C. populi* or *L. dispar* frass-treated compared to control plants. The blocks had a significant influence on leaf salicin levels resulting in higher values for block III compared to I and II (Table 4). Higher-order salicinoids showed an average abundance of 135.42 ± 1.88 mg g^{-1} (Table A8) in leaves and were affected by blocks, the frass and herbivory treatment. The frass treatment resulted in significant differences in higher-order salicinoid concentration with higher levels for *C. populi* frass-treated trees compared to control trees. Additional caterpillar herbivory led to a significant change in salicinoid concentration, where levels for *C. populi* frass-treated plants were higher than for *L. dispar* or control trees (Fig. 12 and Table 4). The blocks showed a significant influence on higher-order salicinoid levels in the leaves where block I had higher concentrations than block II and III. The salicinoid content is known to be high in young leaf tissue to provide the best possible defense against attackers (Boeckler et al., 2011). A higher concentration of higher-order salicinoids is found in leaf tissue compared to salicin levels and the response pattern to the treatments varies between both substances. This might be an indication that salicin indeed is not a direct precursor of the higher-order salicinoids and that they are regulated independently of one another (Babst et al., 2010).

Average catechin leaf levels amounted to 0.40 ± 0.01 mg g^{-1} (Table A8) and were affected by blocks and the frass treatment. The frass treatment led to significant differences in catechin concentrations with higher values for frass-treated trees compared to controls. The blocks were significantly different with tendentially higher concentrations for block I (Fig. 12 and Table 4). Catechin levels in leaves are very low, which might be due to the fact that phenylalanine is a shared substrate for the flavan-3-ol and salicinoids that then enter different biosynthetic pathways. This could result in competition over the precursor or a trade-off between these compound groups. Hence, high constitutive levels of salicinoids in young leaf tissue could reduce the abundance and need for catechin regarding plant defense. Nonetheless, catechin was induced upon the frass treatment and also lowly concentrated compounds can lead to a better defense mechanism in the deterrence of herbivores.

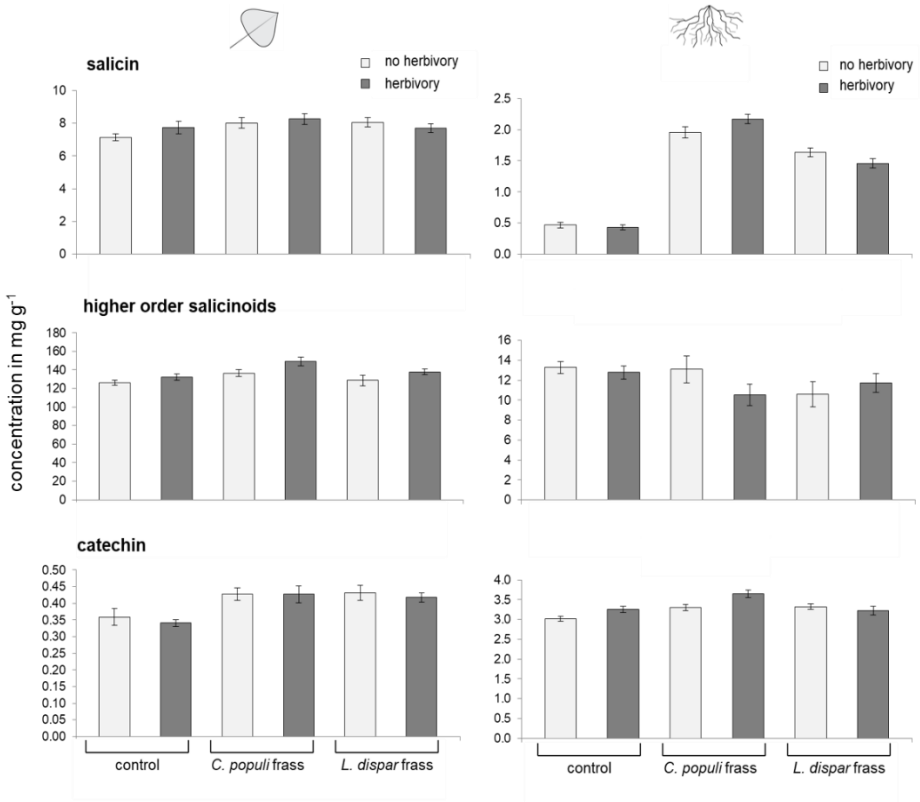

Figure 12. Salicin, higher-order salicinoids and catechin levels of poplar saplings for root and leaf tissue. Higher-order salicinoids comprise salicortin, homalosid D, tremulacin and 6'-*o*-benzoylsalicortin. The frass treatment was divided into control, *Chrysomela populi* and *Lymantria dispar* frass-treated plants. The caterpillar herbivory treatment is indicated with the color code. Concentration levels are stated as mean ± SE (n= 10-12 for leaves and n= 12 for roots, respectively).

All of the above-mentioned leaf phenolics result from the phenylpropanoid pathway and their induction goes in hand with a reduced concentration of phenylalanine, this supports the hypothesis that phenylalanine levels might decrease due to induced conversion into defense compounds.

The root salicin content amounted to 1.35 ± 0.08 mg g^{-1} (Table A8) and was affected by blocks and the frass treatment. Considerably lower concentration levels are found in roots compared with leaves (Fig. 12). As this also holds true for higher-order salicinoids, a possible explanation could be that salicinoids are either primarily stored or produced in the young leaves for their protection, leading to a tissue trade-off between leaves and roots. The frass application led to significant differences in root salicin contents, where higher mean values were found for *C. populi* frass-treated plants compared to control trees (Table 4). This is peculiar as it was hypothesized that the specialist might evade or lower the magnitude of defense induction to gain a fitness advantage. This however does not correlate with the obtained data. As *C. populi* is known to sequester salicinoids, defense level reduction might not be necessary to avoid toxicity as long as a certain threshold value is not exceeded (Ali and Agrawal, 2012). Induction in root salicin levels could result from preparing for defense against root herbivory due to the site of application of the frass. Another hypothesis is that the salicylaldehyde abundant in *C. populi* frass is converted to salicin before or after uptake by the root tissue as hypothesized for SA and finding several compounds with this pattern supports this hypothesis. As stated for SA, uptake is more likely than adherence onto the root tissue, however this needs to be tested. Even though salicin itself was not found in *C. populi* frass, it was previously described to be abundant in *L. dispar* frass and hypothesized to result from breakdown of higher-order salicinoids (Boeckler et al., 2016; Massad et al., 2014). Therefore, for *L. dispar* frass-treated plants the increase in salicin contents could be due to frass salicin uptake instead of induced production of this compound within root tissue. A biochemical approach of analyzing conversion and as far as known biosynthetic enzymes of salicin in root tissue could help to elucidate the origin of increased root salicin levels (Boeckler et al., 2016). The blocks were also significantly different with lower salicin values for block I as compared to II and III (Table 4).

Higher-order salicinoids amounted to 11.99 ± 0.44 mg g^{-1} (Table A8) and were affected by the frass treatment. The frass treatment led to a significant difference in higher-order salicinoid root levels with lower concentrations for *L. dispar* frass-treated trees (Fig. 12 and Table 4). Frass application resulted in an induction of higher-order salicinoid levels in leaves but a decrease is observed in roots. This could potentially be an indication of transport of higher-order salicinoids from roots to leaves. Even though signal transduction in poplar is regarded as basipetal (top to bottom) (Irmisch et al., 2014), Major and Constabel (2007) suggest bidirectional signaling. As obtained for leaves a different response of higher-order salicinoids *versus* salicin was observed for roots, which supports the hypothesis of their independent biosynthesis and regulation. However, this was not yet proven and the elucidation of the phenylpropanoid pathway will aid in elucidating this hypothesis (Babst et al., 2010).

Catechin roots levels amounted to 3.29 ± 0.04 mg g^{-1} (Table A8) and were affected by the frass and herbivory treatment. Compared to leaves the concentration was higher in root tissue, which goes in hand with a reduced salicinoid content and is probably due to competition for the same substrate as well as energy efficiency of already well-protected tissue (Boeckler et al., 2011, 2013). The frass treatment led to significant differences in root catechin levels, showing higher concentrations for *C. populi* or *L. dispar* frass-treated trees compared to controls. Additional caterpillar herbivory resulted in significant differences of catechin abundance, with higher values for *C. populi* frass-treated plants (Fig. 12 and Table 4). The two-way interaction between frass and herbivory was also significant (F= 6.60, P< 0.01). Boeckler et al. (2013) also observed no effect on flavan-3-ol levels in poplar leaves of old-growth trees after herbivory. Moreover, flavan-3-ols were higher concentrated in older tissue of undamaged trees leading to the conclusion that tissue type and developmental stage are important factors for flavan-3-ol levels.

Generally, debate is still ongoing about the inducibility of salicinoids which might be species, compound and system-specific (Boeckler et al., 2013; Massad et al., 2014), nonetheless salicinoid induction due to insect frass application was observed in this study. It would be intriguing to see whether intermediate-delayed induced resistance takes place in sapling leaves growing after a defoliation event. This defense mechanism could lead to higher constitutive defense compound levels such as salicinoids, or a reduced nutritional value for the herbivore in order to protect the new foliage better. It is possible that this phenomenon is not observed for the studied system as youngest tissue already has the highest salicinoid contents. There might be a threshold of usefulness for the induction of defense compounds with regard to the energy resource trade-off between defense and plant growth (Boeckler et al., 2011; Frost and Hunter, 2007).

Other investigated phenylpropanoid pathway-derived phenolics that are connected to plant defense comprise gallic, coumaric and caffeic acid. Further, caffeoyl-quinic acids were investigated which play a role in plant resistance concerning herbivore or microbial attack (Dixon et al., 2002; Dixon and Paiva, 1995). The phenolic acids were differently affected by the frass and herbivory treatment and focus was laid on gallic acid and 3-caffeoyl-quinic acid here (Fig. 13) (see Table A8 and A9 for further information on phenolic acids). The nomenclature of Clifford et al. (2003) and Clifford et al. (2008) was utilized for the caffeoyl-quinic acids.

In the leaf tissue, gallic acid showed an overall abundance of 0.28 ± 0.02 µg g^{-1} (Table A8) and was affected by blocks, the frass and herbivory treatment. The frass treatment had a significant impact on gallic acid leaf levels showing higher concentrations for *C. populi* or *L. dispar* frass-treated trees compared to controls. For additional caterpillar herbivory,

a significant increase in gallic acid levels was observed with higher levels for *C. populi* frass-treated trees compared to controls and *L. dispar* frass-treated plants. The blocks showed significant differences in gallic acid levels where block II had lower concentrations compared to block I and III. The two-way interactions of frass and block (F= 2.80, P= 0.04) and between herbivory and block (F= 9.36, P< 0.001) were significant (Fig. 13 and Table 4).
For the combined *cis*- and *trans*-3-caffeoyl-quinic acid (3-QA), no standard was available and hence no normalization possible. Since this compound displayed a peculiar outcome, it was included here and only patterns for the peak area divided by the dry weight are described. The abundance of 3-QA in poplar leaves was affected by blocks, the frass as well as herbivory treatment. The frass treatment led to significant differences in 3-QA concentration with strongly induced levels for *C. populi* or *L. dispar* frass-treated plants as compared to controls (520-740 fold). Additional caterpillar herbivory led to a significant difference between the frass treatments, where *C. populi* frass-treated plants had higher concentration levels as controls or *L. dispar* frass-treated trees. The blocks were significantly different in 3-QA levels with tendentially higher abundance in block I compared to block II and III. The frass-herbivory two-way interaction (F= 22.31, P<0.001) was also significant (Fig. 13 and Table 4).

Root levels for gallic acid amounted to 0.12 ± 0.004 µg g^{-1} (Table A8) and were affected by the frass and herbivory treatment. Regarding the frass treatment, a significant influence on gallic acid levels was observed with decreased concentrations for *C. populi* or *L. dispar* frass-treated plants when compared to control trees. Further caterpillar herbivory led to a significant difference in gallic acid concentration with increased levels for control plants but no effect for frass-treated samples compared to the sole frass treatment. This could indicate the suppressive behavior of both insect frasses on defense response activation after herbivory as hypothesized for the unidentified volatile compounds 2, 3 and 6. The astounding part for gallic acid is that whereas this suppressive behavior was only found for *C. populi* frass in the unknown volatiles 2, 3 and 6, it was observed for both specialist and generalist insect here (Fig. 13 and Table 4). The two-way interaction of frass and block was also significant (F= 2.57, P= 0.047).
For the caffeoyl-quinic acid, Fig. 13 displays that average root concentration are higher than leaf levels. Root 3-QA concentration was affected by frass and herbivory treatment. The same strong and significant induction as in leaves was found for *C. populi* or *L. dispar* frass-treated plants compared to controls (2,679-2,710 fold). Caterpillar herbivory significantly influenced 3-QA abundance, resulting in higher levels for *C. populi* frass-treated plants. Also, the two-way interaction between frass and herbivory was significant (F= 25.61, P<0.001) (Fig. 13 and Table 4). While the controls behave the same way in leaves and roots, leaf induction for *C. populi* frass-treated plants is higher for the sole frass treatment

compared to additional herbivory treatment and *vice versa* for the roots. A similar reversed trend was observed for *L. dispar* frass-treated plants with higher levels for caterpillar herbivory samples compared to sole frass treatment in leaves and *vice versa* in roots. It is fascinating that for this phenolic acid the frass treatment alone has the same magnitude of effect as herbivory on a control plant in roots and leaves, indicating how big an influence the frass treatment has on the plant and that it definitely perceives a signal from frass application. With that, the caffeoyl-quinic acids pose highly interesting biomarkers for plant-insect interaction and further work could focus on finding an appropriate standard for this compound class to be able to investigate them in a standardized manner. As literature states that increased caffeoyl-quinic acid levels due to artificially overexpressing the L-phenylalanine ammonia-lyase enzyme lowered plant resistance towards insects (Dixon et al., 2002), it remains unclear whether increased concentrations are merely a stress signal of the plant or mark active defensive properties for the investigated system. Moreover, the role of phenolics as precursors of lignin or salicinoids is discussed in literature. Given that the response reactivity of these phenolic intermediates of the phenylpropanoid pathway is higher than that of the end products they are important in the analysis of plant responses to biotic changes (Dixon et al., 2002; Dixon and Paiva, 1995; Fraser and Chapple, 2011).

As stated before, a decrease of the substrate phenylalanine that goes in hand with an increase in phenolic, defense-associated compounds could be linked and hypothesized to result from upregulated production of plant defense compounds from phenylalanine. A biochemical analysis concerning the activity of phenylpropanoid pathway enzymes could help elucidating this hypothesis.

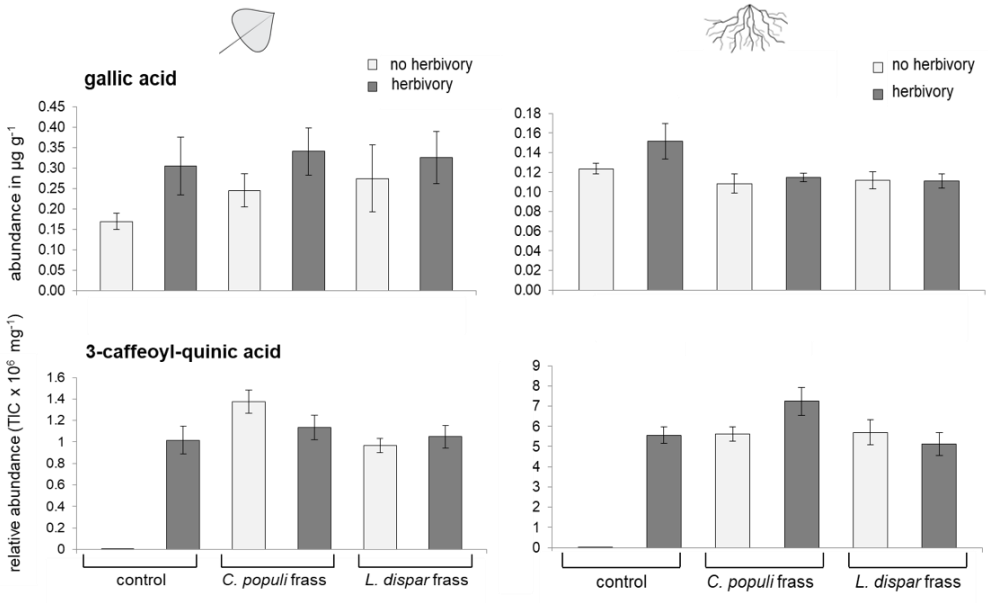

Figure 13. The investigated phenolic acids gallic acid and 3-caffeoyl-quinic acid. Displayed are the leaf and root tissue for control, *Chrysomela populi* or *Lymantria dispar* frass-treated trees. The caterpillar herbivory treatment is indicated by the color code. The mean values ± SE are given (n= 10-12 for leaves and n= 12 for roots).

Table 4. Results from GLS models for investigated compounds from primary and secondary metabolism of poplars with the treatments control, *Chrysomela populi* or *Lymantria dispar* frass application and an additional caterpillar herbivory treatment (n= 10-12 for leaves and n= 12 for roots). The abbreviation AA stands for amino acid.

	Frass		Herbivory		Block	
	F	*P*	*F*	*P*	*F*	*P*
Leaf catechin	**14.02**	**<0.001**	0.16	0.69	**10.11**	**<0.001**
Root catechin	**22.65**	**<0.001**	**4.67**	**0.04**	0.44	0.65
Leaf salicin	**3.23**	**0.048**	0.49	0.49	**11.08**	**<0.001**
Root salicin	**410.32**	**<0.001**	0.26	0.62	**8.28**	**<0.001**
Leaf higher-order salicinoids	**6.70**	**<0.01**	**8.97**	**<0.01**	**6.13**	**<0.01**
Root higher-order salicinoids	**16.29**	**<0.001**	0.01	0.92	3.04	0.06
Leaf free essential AAs	**4.39**	**0.02**	**133.26**	**<0.001**	**9.80**	**<0.001**
Root free essential AAs	**15.86**	**<0.001**	0.09	0.77	2.96	0.06
Leaf free phenylalanine	**4.82**	**0.02**	3.89	0.054	**9.21**	**<0.001**
Root free phenylalanine	**13.24**	**<0.001**	**5.06**	**0.03**	**8.97**	**<0.001**
Leaf gallic acid	**14.74**	**<0.001**	**13.17**	**<0.001**	**20.62**	**<0.001**
Root gallic acid	**7.59**	**<0.01**	**11.02**	**<0.01**	0.98	0.38
Leaf 3-caffeoyl-quinic acid	**250.49**	**<0.001**	**25.29**	**<0.001**	**3.91**	**0.03**
Root 3-caffeoyl-quinic acid	**255.36**	**<0.001**	**111.80**	**<0.001**	0.86	0.43

7.2.6 Insect frass

Dried eggs and finely crushed leaf particles could not be removed from the *C. populi* frass when preparing the frass solution. This could potentially have an influence on the investigated system, as oviposited insect eggs on leaves can induce defense responses within the plant. It is therefore hypothesized that the grinding step in the frass solution preparation will make the egg contents available for extraction. It is unclear whether the frass and egg mixture will lead to an additive defense induction within the plants, to no effect or whether partial masking of the frass treatment will take place (Ali and Agrawal, 2012; Hilker and Fatouros, 2016). Masking seems rather unlikely as the frass amount strongly outweighed the number of insect eggs and plant responses to the frass solution were detected. A synergistic effect cannot be ruled out. Nonetheless, application of the frass and egg material mixture represents natural conditions. Regarding the insect frass C/N ratio, *Chrysomela populi* frass had a carbon content of 45.57 % and a nitrogen level of 3.39 %. The gypsy moth caterpillar frass had a C/N ratio of 43.63/3.17 % (n= 3). Hence, the C/N ratio for the two investigated insect species is similar, *C. populi* frass has slightly higher values for both elements compared to *L. dispar* frass.

Concerning the frass application site Ray et al. (2016b) investigated several plant systems where frass either accumulated at the site of feeding or did not and found time- and system-dependent differences in the plant responses. For poplar, the frass is not accumulating in

proximity to the wounding site but rather at the foot of the tree, which could result in wash out, microbial immobilization and uptake by the roots (Christenson et al., 2002; Frost and Hunter, 2007; Kagata and Ohgushi, 2012a, 2012b). Frost and Hunter (2008) did not find modifications of herbivory-induced mechanisms due to frass application. This is in contrast to the obtained results of the frass induction experiment where combined frass and herbivory treatment could lead to a changed plant reaction and compound concentrations. The succession of events could be critical for the outcome, in the frass induction experiment the frass treatment was followed by a herbivory treatment, whereas the simultaneous frass and herbivory treatment for Frost and Hunter (2008) could potentially result in prioritizing one attack (Caarls et al., 2015; Cheong and Choi, 2007; Derksen et al., 2013). As stated in Frost and Hunter (2007) higher uptake than under natural conditions could have been observed in the frass induction experiment as the PVC containers resulted in an artificially high root density. This makes additional investigations under natural conditions necessary.

8. Conclusion

In this study, it was shown that frass application of a generalist or specialist insect to black poplar saplings leads to changes in plant chemistry. Furthermore, specific defense-related signaling pathways or the increase of defense-associated compounds were triggered. Whether the induction of defense compounds is mediated by *de novo* synthesis or re-allocation remains unclear and to be tested. The plant responses vary in a compound-, frass type- and tissue-dependent manner. Hence, the hypothesis that specialist and generalist frass results in different responses of the plant cannot be applied generally and is accepted for certain molecules only. This highlights the complexity of processes involved in plant defense against biotic attack and needs to be elucidated further. The hypothesis that *C. populi* as a well-adapted specialist insect induces lower levels of defense related compounds or signaling compared to a generalist defoliator (Ali and Agrawal, 2012) is rejected for the frass induction experiment as some defense-associated molecules even showed higher induction for *C. populi* frass-treated trees compared to *L. dispar* frass-treated poplars. Nonetheless, for an unidentified VOC group (unknown 2, 3 and 6) and gallic acid in root tissue an induction suppression upon *C. populi* frass application was found. With respect to defense activation, Lämke and Unsicker (2018) state that plant defenses are re-allocated as well as synthesized *de novo* upon plant attack. Allocation and biosynthetic mechanisms were not investigated in this study but analyzing biosynthetic enzymes of defense-associated compounds could aid to elucidate this question in future experiments. The experimental design with combined frass and herbivory treatment closely reflects natural conditions as a plant is usually challenged by multiple attackers (Unsicker et al., 2009). The results of Christenson et al. (2002) and Kagata and Ohgushi (2012b) imply that

the experimental environment of laboratory *versus* field site plays a huge role in the observed effects. This trade-off between field and lab experiments needs to be kept in mind for the applicability of laboratory experiments to real life scenarios and hence the data obtained from laboratory experiments should be re-evaluated under field conditions. The same poplar defense signaling machinery as for insect herbivory was triggered due to frass application, however a different response was formulated.

9. Outlook

At first, the frass induction experiment should be replicated with poplar saplings in sandy soil, where the frass is applied to the soil surface to simulate natural conditions (Frost and Hunter, 2008). Afterwards, field experiments could be conducted to account for abiotic and biotic interactions and possible soil sinks that reduce the availability and subsequent uptake of frass signals (Christenson et al., 2002; Kagata and Ohgushi, 2012b). Moreover, the extent of differential defense activation in older trees compared to the poplar saplings towards insect frass application could be investigated. Holopainen (2011) states that old-growth trees probably react differently to biotic stress as they already obtained a carbon reserve and hence can assign more resources into plant defense without detrimentally affecting plant growth. In order to elucidate whether *de novo* synthesis or resource allocation of defense compounds takes place after frass application, transcriptome analysis of important biosynthetically involved enzymes could be carried out as well as monitoring transcriptional activity of defense pathway-related genes and comparison to their end product concentration (Ray et al., 2016a). Additionally, it would be useful to elucidate the contents of the *C. populi* frass to be able to correlate induced compounds with the ones provided *via* frass application and to compare it to the frass contents of the generalist insect *L. dispar* which was elucidated by Boeckler et al. (2016). Bioactive fractionation could aid the search and identification of reliable frass defense-inducers in both frass types (Ray et al., 2015). Moreover, an untargeted HPLC-MS/MS analysis of both the generalist and specialist frass is envisaged. The frass could be extracted in the experimental medium tap water as well as with the methanol extraction buffer including IS. The untargeted analysis could expose differences in the frass contents or active fractions thereof and aid explaining the different or similar responses of poplar trees after frass application of either insect.

10. Acknowledgments

I would like to thank Dr. Sybille Unsicker and Prof. Dr. Christoph Steinbeck for their support and supervision throughout this project; Dr. Michael Reichelt for answering analytical questions; my group, especially Dr. Franziska Eberl and Beate Rothe for their time investment and help with lab-related questions. Another big thank you to the greenhouse team, especially Jana Zitzmann and Eva Rothe for their help in rearing the poplar cuttings; to Daniel Veit for helping with the construction of the experimental set up as well as to Melanie Werlich and Christiana Voy for their help during the plant harvest. Lastly, to everyone else who has supported me over the time course of my Master thesis, you know who you are, it means a lot to me, so thank you very much.

11. Appendix

Table A1. Constituents of a 5 L batch of the '*Nicotiana* hydro solution' used to rear the poplar cuttings.

Ingredient	Amount	Amount of substance
Deionized water	5 L	
$CaSO_4$ x 2 H_2O	0.646 g	3.75 mmol
$MgSO_4$ x 7 H_2O	0.616 g	2.50 mmol
K_2HPO_4	0.2395 g	1.38 mmol
KH_2PO_4	0.153 g	1.12 mmol
Micro nutrient solution	2.5 mL	
Fe-DTPA	2.5 mL	
1 M KNO_3	10 mL	
Micro nutrient solution (1 L):		
Deionized water	1 L	
H_3BO_3	2.533 g	0.041 mol
$MnSO_4$ x 2 H_2O	1.634 g	8.74 mmol
Na_2MoO_4 x 2 H_2O	0.151 g	0.63 mmol
$ZnSO_4$ x 7 H_2O	0.440 g	1.53 mmol
$CuSO_4$ x 5 H_2O	0.080 g	0.32 mmol
$CoCl_2$ x 6 H_2O	0.020 g	0.08 mmol
Fe-DTPA (1 L):		
Deionized water	1 L	
$FeSO_4$ x 7 H_2O	2.78 g	10 mmol
$C_{14}H_{23}N_3O_{10}$	3.93 g	10 mmol

Table A2. Constituents of a 5 L batch of the poplar hydroponic solution for the frass induction experiment.

Ingredient	Amount	Amount of substance
Deionized water	5 L	
$Ca(NO_3)_2$ x 4 H_2O	7.05 g	0.03 mol
Ferty® Basis 1 (0-14-38)	3.05 g	
$MgSO_4$ x 7 H_2O	1.36 g	5.52 mmol
Fe-DTPA	12.75 mL	
Fe-DTPA (1 L):		
Deionized water	1 L	
$FeSO_4$ x 7 H_2O	2.78 g	10 mmol
$C_{14}H_{23}N_3O_{10}$	3.93 g	10 mmol

Table A3. Raw data of the frass accumulation experiment that was used to optimize the experimental design.

ID	Herbivore species	Mean damage per tree	Mean damage per species	Total accumulated frass in g	Mean accumulated frass in g	Estimated frass one plant in g
1	*C. populi*	24%	35%	0.43	0.05	0.15
2	*C. populi*	43%				
3	*C. populi*	33%				
4	*C. populi*	66%				
5	*C. populi*	21%				
6	*C. populi*	27%				
7	*C. populi*	33%				
8	*C. populi*	32%				
13	*L. dispar*	63%	52%	0.96	0.12	0.23
14	*L. dispar*	46%				
15	*L. dispar*	37%				
16	*L. dispar*	49%				
17	*L. dispar*	51%				
18	*L. dispar*	40%				
19	*L. dispar*	38%				
20	*L. dispar*	63%				

Table A4. Precursor and quantifier ions for the phytohormone and phenolic acid LC-MS analysis. The ionization mode is indicated in round brackets. The abbreviation R_T stands for retention time.

Compound name	R_T in min	Precursor ion m/z	Quantifier m/z [collision energy]
SA	5.90	(-) 136.93	93.00 [22.0 V]
CA	6.70	(-) 147.00	102.80 [16.0 V]
Coumaric acid	4.70	(-) 163.00	118.90 [20.0 V]

Compound name	R_T in min	Precursor ion m/z	Quantifier m/z [collision energy]
Gallic acid	1.30	(-) 169.00	125.00 [18.0 V]
Caffeic acid	4.00	(-) 179.00	134.90 [22.0 V]
Ferulic acid	5.00	(-) 193.10	133.90 [22.0 V]
JA	7.20	(-) 209.07	59.00 [24.0 V]
OH-JA	4.40	(-) 225.10	59.00 [24.0 V]
ABA	6.00	(-) 263.00	153.20 [22.0 V]
JA-Ile	7.20	(-) 322.19	130.10 [30.0 V]
OH-JA-Ile	6.00	(-) 338.10	130.10 [30.0 V]
COOH-JA-Ile	5.65	(-) 352.10	130.10 [30.0 V]
Syringic acid	4.01	(-) 197.00	121.09 [24.0 V]
Trans-5-caffeoyl-quinic acid	3.20	(-) 353.13	190.88 [22.0 V]
Cis-5-caffeoyl-quinic acid	3.90	(-) 353.00	190.90 [22.0 V]
Cis-4-caffeoyl-quinic acid	3.20	(-) 353.11	173.10 [22.0 V]
Trans-4-caffeoyl-quinic acid	3.90	(-) 353.10	173.00 [22.0 V]
Cis-3-caffeoyl-quinic acid	3.20	(-) 353.02	179.10 [22.0 V]
Trans-3-caffeoyl-quinic acid	3.90	(-) 353.04	179.00 [22.0 V]
Coumaroyl-quinic1a	3.50	(-) 337.13	190.88 [22.0 V]
Coumaroyl-quinic1b	4.20	(-) 337.10	190.90 [22.0 V]
Coumaroyl-quinic2a	3.50	(-) 337.00	163.10 [22.0 V]
Coumaroyl-quinic2b	4.20	(-) 337.01	163.00 [22.0 V]
Coumaroyl-quinic3a	3.50	(-) 337.12	173.00 [22.0 V]
Coumaroyl-quinic3b	4.20	(-) 337.05	173.10 [22.0 V]
TriF-methyl-CA	7.30	(-) 215.06	171.06 [18.0 V]
D_4-SA	5.70	(-) 140.93	97.00 [22.0V]
D_6-ABA	6.04	(-) 269.00	159.20 [22.0 V]
D_6-JA	7.20	(-) 215.00	59.00 [24.0 V]
D_6-JA-Ile	7.30	(-) 328.19	130.10 [30.0 V]
327-D_6-JA-Ile	7.30	(-) 327.19	130.10 [30.0 V]
326-D_6-JA-Ile	7.30	(-) 326.19	130.10 [30.0 V]
214-D_6-JA	7.20	(-) 214.00	59.00 [24.0 V]
JA-$^{13}C_6$-Ile	7.30	(-) 328.19	136.10 [30.0 V]

Table A5. Precursor and quantifier ions for the amino acid LC-MS analysis. The ionization mode is indicated in round brackets. The abbreviation R_T indicates the retention time.

Compound name	R_T in min	Precursor ion m/z	Quantifier m/z [collision energy]
Alanine	0.40	(+) 90.10	44.10 [17.0 V]
Serine	0.40	(+) 106.00	60.10 [15.0 V]

Compound name	R_T in min	Precursor ion m/z	Quantifier m/z [collision energy]
Proline	0.60	(+) 116.10	70.00 [19.0 V]
Valine	0.60	(+) 118.10	72.20 [13.0 V]
Threonine	0.40	(+) 120.10	74.20 [13.0 V]
Isoleucine	0.90	(+) 132.20	86.10 [13.0 V]
Leucine	0.90	(+) 132.20	86.10 [13.0 V]
Aspartic acid	0.40	(+) 134.10	74.10 [19.0 V]
Glutamic acid	0.40	(+) 148.10	102.10 [15.0 V]
Methionine	0.70	(+) 150.20	104.10 [13.0 V]
Histidine	0.40	(+) 156.20	110.10 [17.0 V]
Phenylalanine	2.10	(+) 166.20	120.20 [17.0 V]
Arginine	0.40	(+) 175.10	70.10 [31.0 V]
Tyrosine	1.10	(+) 182.10	136.20 [17.0 V]
Asparagine	0.40	(+) 133.10	74.10 [21.0 V]
Glutamine	0.40	(+) 147.10	130.00 [13.0 V]
Tryptophan	2.80	(+) 205.20	188.10 [13.0 V]
Lysine	0.40	(+) 147.10	84.10 [23.0 V]
Glycine	0.40	(+) 76.00	76.00 [5.0 V]
Labeled alanine	0.40	(+) 94.10	47.10 [17.0 V]
Labeled serine	0.40	(+) 110.00	63.10 [15.0 V]
Labeled proline	0.60	(+) 122.10	75.00 [19. 0V]
Labeled valine	0.60	(+) 124.10	77.20 [15.0 V]
Labeled threonine	0.40	(+) 125.10	78.20 [13.0 V]
Labeled isoleucine	0.90	(+) 139.20	92.10 [13.0 V]
Labeled aspartic acid	0.40	(+) 139.10	77.10 [19.0 V]
Labeled glutamic acid	0.40	(+) 154.10	107.10 [15.0 V]
Labeled methionine	0.70	(+) 156.20	109.10 [13.0 V]
Labeled histidine	0.40	(+) 165.20	118.10 [17.0 V]
Labeled phenylalanine	2.10	(+) 176.20	129.20 [17.0 V]
Labeled arginine	0.40	(+) 185.10	75.10 [31.0 V]
Labeled tyrosine	1.10	(+) 192.10	145.20 [17.0 V]
Labeled glutamine	0.40	(+) 154.10	136.00 [13.0 V]
Labeled tryptophan	2.80	(+) 218.20	200.10 [13.0 V]
Labeled lysine	0.40	(+) 155.10	90.10 [23.0 V]
Labeled glycine	0.40	(+) 79.00	79.00 [5.0 V]
Tryptophan-146	2.80	(+) 205.10	146.10 [18.0 V]
Tryptophan-118	2.80	(+) 205.10	118.00 [25.0 V]
Glycine-30	0.40	(+) 76.00	30.00 [17.0 V]
Labeled glycine-32	0.40	(+) 79.00	32.00 [17.0 V]

Table A6. Precursor and quantifier ions for the sugar LC-MS analysis. The ionization mode is indicated in round brackets. The abbreviation R_T stands for retention time.

Compound name	R_T in min	Precursor ion m/z	Quantifier m/z [collision energy]
Glucose	6.60	(-) 178.80	89.00 [10.0 V]
Fructose	5.60	(-) 178.80	89.00 [12.0 V]
Disaccharides	8.00	(-) 340.90	59.00 [46.0 V]
Trisaccharides	10.50	(-) 503.10	179.00 [28.0 V]
Trisaccharides2	10.50	(-) 503.10	59.00 [68.0 V]
Tetrasaccharides	12.00	(-) 665.20	179.00 [48.0 V]
Tetrasaccharides2	12.00	(-) 665.20	383.00 [36.0 V]
Pentasaccharides	13.20	(-) 827.30	59.00 [124.0 V]
Pentasaccharides2	13.20	(-) 827.30	179.00 [58.0 V]

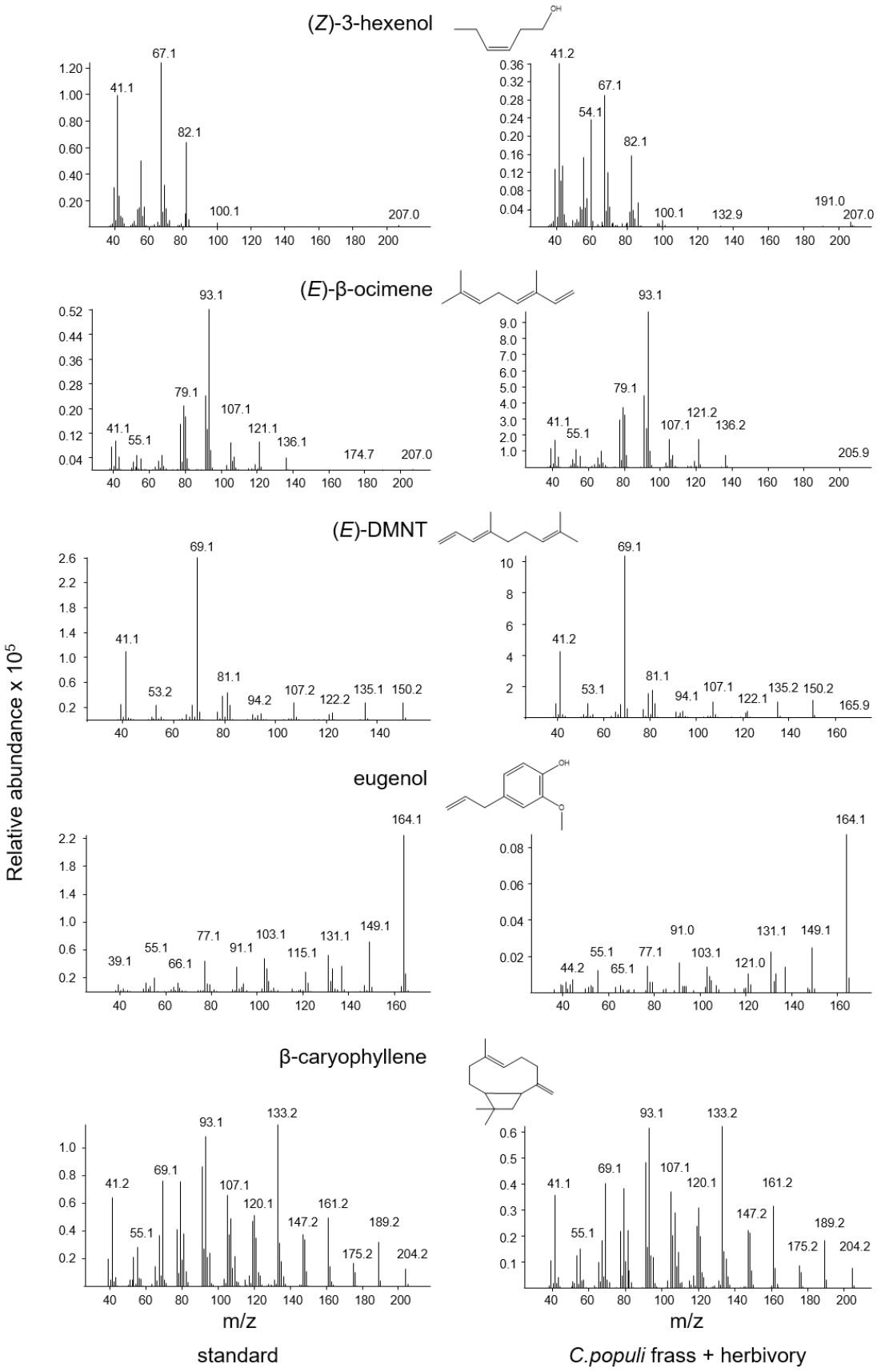

Figure A1. GC-MS spectra of a standard (left hand-side) and a *Chrysomela populi* frass and caterpillar herbivory-treated sample (right hand-side), respectively. The identified compounds comprise (*Z)*-3-hexenol [GLV], (*E*)-β-ocimene [monoterpene], (*E*)-DMNT [homoterpene], eugenol [aromatic compound], β-caryophyllene [sesquiterpene]. Volatile compound classes are indicated in square brackets.

Table A7. Volatile organic compound emission from *Populus nigra* after treatment with water (control), *Chrysomela populi* or *Lymantria dispar* frass, respectively. An additional caterpillar herbivory treatment is indicated by '+ herbivory'. Shoot volatile levels are given as mean ± SE (n= 12) in ng h^{-1} g^{-1} fresh weight.

	Control	**Control + herbivory**	***C. populi* frass**	***C. populi* frass + herbivory**	***L. dispar* frass**	***L. dispar* frass + herbivory**	**Caterpillars of *L. dispar* frass + herbivory**
GLVs	**16.01 ± 4.44**	**226.75 ± 49.91**	**22.76 ± 4.61**	**253.18 ± 57.84**	**17.15 ± 4.16**	**181.19 ± 43.93**	**0.00 ± 0.00**
(*Z*)-3-hexenol	0.60 ± 0.13	23.99 ± 4.81	0.74 ± 0.17	30.80 ± 6.31	0.51 ± 0.15	18.24 ± 5.15	0.00 ± 0.00
(*Z*)-3-hexen-1-ol acetate	15.41 ± 4.31	200.83 ± 44.68	22.02 ± 4.44	220.15 ± 50.98	16.64 ± 4.01	161.28 ± 38.29	0.00 ± 0.00
(*Z*)-3-hexenyl isobutyrate	0.00 ± 0.00	1.93 ± 0.42	0.00 ± 0.00	2.23 ± 0.55	0.00 ± 0.00	1.67 ± 0.49	0.00 ± 0.00
Monoterpenes	**63.80 ± 9.22**	**277.74 ± 52.63**	**78.48 ± 12.56**	**261.29 ± 59.11**	**64.85 ± 14.24**	**241.04 ± 58.56**	**0.59 ± 0.29**
α-pinene	4.20 ± 0.59	7.79 ± 1.29	4.19 ± 0.65	7.77 ± 2.02	4.01 ± 0.97	6.97 ± 1.57	0.00 ± 0.00
Camphene	6.69 ± 0.97	12.88 ± 2.05	6.19 ± 1.03	12.44 ± 3.22	6.25 ± 1.66	11.97 ± 2.83	0.59 ± 0.29
Sabinene	1.76 ± 0.26	4.27 ± 0.68	1.93 ± 0.28	5.54 ± 1.23	1.69 ± 0.42	4.34 ± 0.95	0.00 ± 0.00
β-pinene	4.70 ± 0.67	9.56 ± 1.58	4.47 ± 0.72	9.11 ± 2.46	4.40 ± 1.13	8.40 ± 1.90	0.00 ± 0.00
β-myrcene	2.25 ± 0.30	4.40 ± 0.66	2.67 ± 0.37	4.71 ± 1.01	2.15 ± 0.35	3.98 ± 0.82	0.00 ± 0.00
Limonene	2.12 ± 0.32	4.34 ± 0.74	2.19 ± 0.31	4.51 ± 1.14	1.98 ± 0.45	3.68 ± 0.86	0.00 ± 0.00
1.8 cineole	1.81 ± 0.32	4.58 ± 0.87	2.14 ± 0.35	6.89 ± 1.65	1.73 ± 0.40	4.61 ± 1.05	0.00 ± 0.00
(*Z*)-β-ocimene	9.49 ± 1.78	22.00 ± 3.75	11.75 ± 2.70	23.32 ± 5.44	9.99 ± 2.76	23.94 ± 5.02	0.00 ± 0.00
(*E*)-β-ocimene	24.47 ± 3.25	196.44 ± 39.27	34.98 ± 4.98	174.86 ± 38. 04	26.83 ± 5.04	163.50 ± 41.47	0.00 ± 0.00
Camphor	4.49 ± 0.51	7.19 ± 1.01	5.88 ± 0.78	8.15 ± 1.71	4.40 ± 0.74	6.28 ± 1.23	0.00 ± 0.00
Borneol	1.82 ± 0.25	4.29 ± 0.73	2.09 ± 0.39	3.99 ± 1.19	1.42 ± 0.32	3.37 ± 0.86	0.00 ± 0.00
Sesquiterpenes	**24.21 ± 5.55**	**96.04 ± 19.13**	**37.08 ± 9.27**	**139.37 ± 33.80**	**25.06 ± 6.96**	**104.06 ± 22.45**	**0.00 ± 0.00**
β-caryophyllene	7.53 ± 1.85	24.64 ± 4.73	11.38 ± 2.88	35.47 ± 8.62	7.97 ± 2.41	29.74 ± 6.09	0.00 ± 0.00
α-humulene	7.92 ± 2.10	14.28 ± 2.68	11.90 ± 3.27	21.49 ± 6.13	7.85 ± 2.40	18.96 ± 3.89	0.00 ± 0.00
Germacrene D	1.99 ± 0.42	37.16 ± 7.53	3.19 ± 0.71	60.49 ± 13.70	2.38 ± 0.73	38.26 ± 8.14	0.00 ± 0.00
(*E*),(*E*)-α-farnesene	1.28 ± 0.19	12.41 ± 2.79	2.66 ± 0.47	12.70 ± 3.13	1.31 ± 0.29	9.65 ± 2.59	0.00 ± 0.00
δ-cadinene	5.49 ± 0.99	7.55 ± 1.40	7.95 ± 1.94	9.22 ± 2.22	5.55 ± 1.13	7.45 ± 1.74	0.00 ± 0.00
Homoterpenes	**1.83 ± 0.25**	**126.10 ± 25.57**	**3.49 ± 0.70**	**153.66 ± 32.73**	**1.73 ± 0.33**	**132.88 ± 31.23**	**0.00 ± 0.00**
(*E*)-DMNT	1.83 ± 0.25	126.10 ± 25.57	3.49 ± 0.70	153.66 ± 32.73	1.73 ± 0.33	132.88 ± 31.23	0.00 ± 0.00
nitrogenous compounds	**1.06 ± 0.49**	**95.03 ± 14.44**	**1.50 ± 0.57**	**78.61 ± 15.93**	**0.94 ± 0.46**	**91.24 ± 24.98**	**0.00 ± 0.00**
2-Methylbutyraldoxime	0.73 ± 0.37	64.51 ± 8.84	0.93 ± 0.40	50.09 ± 9.49	0.53 ± 0.28	60.89 ± 14.49	0.00 ± 0.00
Benzyl cyanide	0.33 ± 0.12	30.52 ± 5.60	0.57 ± 0.17	28.52 ± 6.44	0.41 ± 0.18	30.35 ± 10.49	0.00 ± 0.00

	Control	**Control + herbivory**	***C. populi* frass**	***C. populi* frass + herbivory**	***L. dispar* frass**	***L. dispar* frass + herbivory**	**Caterpillars of *L. dispar* frass + herbivory**
Aromatic compounds	**3.89 ± 0.87**	**46.05 ± 9.53**	**5.30 ± 1.31**	**42.56 ± 9.73**	**3.84 ± 0.97**	**45.76 ± 15.16**	**16.17 ± 3.25**
Benzaldehyde	0.91 ± 0.15	2.05 ± 0.36	1.20 ± 0.17	2.52 ± 0.52	0.87 ± 0.17	2.88 ± 0.89	0.00 ± 0.00
Salicylaldehyde	0.34 ± 0.28	8.17 ± 2.73	0.59 ± 0.42	5.66 ± 1.60	0.28 ± 0.27	7.39 ± 2.69	2.19 ± 0.97
Benzyl cyanide	0.33 ± 0.12	30.52 ± 5.60	0.57 ± 0.17	28.52 ± 6.44	0.41 ± 0.18	30.35 ± 10.49	0.00 ± 0.00
Ethylacetophenone	1.77 ± 0.20	2.01 ± 0.21	1.66 ± 0.19	1.88 ± 0.16	1.65 ± 0.13	1.76 ± 0.18	13.44 ± 1.93
Eugenol	0.54 ± 0.12	3.30 ± 0.63	1.28 ± 0.36	3.98 ± 1.01	0.63 ± 0.22	3.38 ± 0.91	0.54 ± 0.35
Other compounds	**34.48 ± 6.73**	**72.62 ± 14.12**	**35.40 ± 9.59**	**68.38 ± 16.24**	**33.49 ± 7.81**	**75.15 ± 18.66**	**34.38 ± 6.78**
Buten-1-ol-acetate	17.00 ± 3.36	20.70 ± 3.84	19.53 ± 5.37	16.65 ± 3.73	15.77 ± 3.87	25.68 ± 5.21	0.00 ± 0.00
(*Z*)-jasmone	0.48 ± 0.10	2.11 ± 0.38	0.81 ± 0.15	3.41 ± 0.84	0.53 ± 0.10	2.41 ± 0.53	0.99 ± 0.34
Unknown 1	0.00 ± 0.00	3.36 ± 0.82	0.00 ± 0.00	3.10 ± 0.77	0.00 ± 0.00	2.39 ± 0.91	0.00 ± 0.00
Unknown 2	0.00 ± 0.00	7.16 ± 1.18	0.00 ± 0.00	3.82 ± 0.90	0.00 ± 0.00	6.32 ± 2.03	0.00 ± 0.00
Unknown 3	0.41 ± 0.14	0.90 ± 0.20	0.32 ± 0.16	0.41 ± 0.15	0.09 ± 0.08	0.87 ± 0.22	1.54 ± 0.53
Unknown 4	0.36 ± 0.13	2.32 ± 0.42	0.17 ± 0.11	2.11 ± 0.46	0.08 ± 0.08	2.33 ± 0.53	0.00 ± 0.00
Unknown 5	0.20 ± 0.07	2.31 ± 0.32	0.30 ± 0.12	2.18 ± 0.47	0.04 ± 0.03	2.58 ± 0.71	0.19 ± 0.18
Unknown 6	6.29 ± 0.98	6.30 ± 1.30	0.30 ± 0.12	3.69 ± 0.82	6.48 ± 1.43	6.58 ± 1.47	0.00 ± 0.00
Unknown 7	0.04 ± 0.04	2.81 ± 0.48	0.00 ± 0.00	4.08 ± 0.95	0.00 ± 0.00	2.53 ± 0.74	0.00 ± 0.00
Unknown 8	0.58 ± 0.08	0.72 ± 0.15	0.91 ± 0.20	0.86 ± 0.21	0.64 ± 0.11	0.89 ± 0.19	2.54 ± 0.69
Unknown 9	0.19 ± 0.08	1.69 ± 0.38	0.26 ± 0.10	1.96 ± 0.46	0.14 ± 0.07	1.46 ± 0.41	0.00 ± 0.00
Unknown 10	0.07 ± 0.05	0.61 ± 0.10	0.13 ± 0.07	0.63 ± 0.17	0.07 ± 0.05	0.46 ± 0.13	0.00 ± 0.00
Unknown 11	0.00 ± 0.00	2.52 ± 0.54	0.00 ± 0.00	2.10 ± 0.75	0.00 ± 0.00	2.18 ± 0.92	2.15 ± 0.95
Unknown 12	0.24 ± 0.10	1.22 ± 0.26	0.28 ± 0.13	1.12 ± 0.26	0.35 ± 0.15	1.20 ± 0.27	0.00 ± 0.00
Unknown 13	0.41 ± 0.09	0.58 ± 0.11	0.42 ± 0.09	0.56 ± 0.08	0.44 ± 0.06	0.45 ± 0.10	2.96 ± 0.65
Unknown 14	0.15 ± 0.06	0.43 ± 0.09	0.24 ± 0.09	0.55 ± 0.15	0.13 ± 0.06	0.41 ± 0.12	0.00 ± 0.00
Unknown 15	0.00 ± 0.00	0.21 ± 0.07	0.00 ± 0.00	0.50 ± 0.15	0.00 ± 0.00	0.29 ± 0.13	0.00 ± 0.00
Unknown 16	1.58 ± 0.14	1.91 ± 0.24	2.53 ± 0.44	2.26 ± 0.43	1.91 ± 0.20	2.14 ± 0.39	7.10 ± 0.90
Unknown 17	0.29 ± 0.09	1.05 ± 0.19	0.45 ± 0.13	1.46 ± 0.35	0.42 ± 0.13	1.16 ± 0.31	0.00 ± 0.00
Unknown 18	0.00 ± 0.00	2.45 ± 0.70	0.00 ± 0.00	2.31 ± 0.67	0.00 ± 0.00	1.92 ± 0.73	0.00 ± 0.00
Unknown 19	1.36 ± 0.20	1.51 ± 0.18	1.31 ± 0.16	1.47 ± 0.14	1.28 ± 0.15	1.37 ± 0.14	10.27 ± 1.54
Unknown 20	0.04 ± 0.04	1.05 ± 0.20	0.05 ± 0.05	1.72 ± 0.39	0.00 ± 0.00	1.14 ± 0.25	0.00 ± 0.00
Unknown 21	0.59 ± 0.10	1.52 ± 0.29	0.79 ± 0.23	2.27 ± 0.53	0.70 ± 0.16	1.60 ± 0.36	0.00 ± 0.00
Unknown 22	0.00 ± 0.00	0.49 ± 0.14	0.10 ± 0.07	0.84 ± 0.21	0.00 ± 0.00	0.57 ± 0.15	0.00 ± 0.00

	Control	**Control + herbivory**	***C. populi* frass**	***C. populi* frass + herbivory**	***L. dispar* frass**	***L. dispar* frass + herbivory**	**Caterpillars of *L. dispar* frass + herbivory**
Unknown 23	0.45 ± 0.08	0.51 ± 0.12	0.62 ± 0.12	0.58 ± 0.14	0.43 ± 0.11	0.29 ± 0.11	6.64 ± 1.00
Unknown 24	0.93 ± 0.19	1.65 ± 0.33	1.48 ± 0.42	2.31 ± 0.58	1.03 ± 0.23	1.67 ± 0.41	0.00 ± 0.00
Unknown 25	1.98 ± 0.35	2.37 ± 0.52	2.91 ± 0.72	2.96 ± 0.77	2.07 ± 0.44	2.31 ± 0.60	0.00 ± 0.00
Unknown 26	0.17 ± 0.08	0.43 ± 0.14	0.31 ± 0.16	0.54 ± 0.17	0.24 ± 0.09	0.48 ± 0.13	0.00 ± 0.00
Unknown 27	0.63 ± 0.14	0.89 ± 0.21	0.93 ± 0.27	0.98 ± 0.28	0.61 ± 0.17	0.83 ± 0.20	0.00 ± 0.00
Unknown 28	0.04 ± 0.04	0.84 ± 0.22	0.25 ± 0.11	0.95 ± 0.26	0.04 ± 0.04	0.64 ± 0.26	0.00 ± 0.00

Table A8. Morphological parameters of black poplar as well as the LC data for the frass induction experiment. The treatments comprise control, *Chrysomela populi* or *Lymantria dispar* frass-treated trees. An additional caterpillar herbivory treatment is indicated by '+ herbivory'. Values are stated as mean ± SE (n= 10-12 for leaves and n= 12 for roots). Indicated summed/total values were obtained from raw data.

	Control	**Control + herbivory**	***C. populi* frass**	***C. populi* frass + herbivory**	***L. dispar* frass**	***L. dispar* frass + herbivory**
Leaves						
Fresh weight[¥]	5.79 ± 0.23	5.35 ± 0.27	5.34 ± 0.26	4.63 ± 0.18	5.94 ± 0.18	5.04 ± 0.17
Leaf area loss[§]	0.00 ± 0.00	12.64 ± 1.45	0.00 ± 0.00	14.48 ± 1.37	0.00 ± 0.00	18.03 ± 1.76
Residual leaf area[‖]	20.25 ± 0.73	19.80 ± 1.05	19.93 ± 0.89	17.56 ± 0.94	20.96 ± 0.85	18.63 ± 0.72
Number of damaged leaves	0.00 ± 0.00	13.09 ± 0.57	0.00 ± 0.00	13.00 ± 0.66	0.00 ± 0.00	12.33 ± 0.70
Height increase[Δ]	9.70 ± 0.87	10.10 ± 0.39	9.70 ± 0.73	9.95 ± 0.76	9.89 ± 0.90	10.30 ± 0.70
Phytohormones[Θ]						
Abscisic acid	0.08 ± 0.00	0.26 ± 0.04	0.15 ± 0.04	0.33 ± 0.05	0.10 ± 0.01	0.28 ± 0.04
SA	6.54 ± 0.21	7.08 ± 0.17	6.86 ± 0.21	7.32 ± 0.25	6.87 ± 0.24	6.93 ± 0.23
Sum jasmonates	1.78 ± 0.26	2.93 ± 0.29	1.47 ± 0.22	3.29 ± 0.28	1.47 ± 0.19	3.14 ± 0.16
JA	1.71 ± 0.24	2.79 ± 0.27	1.42 ± 0.21	3.13 ± 0.26	1.43 ± 0.18	2.98 ± 0.15
JA-Ile	0.06 ± 0.01	0.14 ± 0.02	0.05 ± 0.01	0.16 ± 0.03	0.04 ± 0.01	0.16 ± 0.02

	Control	Control + herbivory	*C. populi* frass	*C. populi* frass + herbivory	*L. dispar* frass	*L. dispar* frass + herbivory
Sum hydroxyl- and carboxylated jasmonates	0.11 ± 0.01	2.14 ± 0.22	0.16 ± 0.02	2.72 ± 0.31	0.11 ± 0.01	3.06 ± 0.34
OH-JA	0.11 ± 0.01	2.03 ± 0.20	0.16 ± 0.02	2.59 ± 0.30	0.11 ± 0.01	2.92 ± 0.32
OH-JA-Ile	0.00 ± 0.00	0.09 ± 0.01	0.00 ± 0.00	0.11 ± 0.02	0.00 ± 0.00	0.11 ± 0.01
COOH-JA-Ile	0.00 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.03 ± 0.00
total AAs$^{\Phi}$	**49824.60 ± 3346.40**	**47060.99 ± 4640.69**	**48794.57 ± 3596.33**	**53468.18 ± 7097.24**	**57799.51 ± 10663.72**	**48319.19 ± 3549.69**
Alanine	5975.67 ± 218.28	6125.28 ± 485.31	5733.06 ± 358.65	5987.66 ± 411.86	6194.11 ± 591.56	5828.21 ± 176.07
Serine	2891.33 ± 229.44	2568.04 ± 248.39	2998.99 ± 302.99	2747.62 ± 304.63	3535.54 ± 655.08	2615.18 ± 189.17
Proline	497.26 ± 179.19	403.55 ± 54.39	276.46 ± 28.75	401.69 ± 59.48	312.99 ± 80.71	335.47 ± 30.90
Valine	509.48 ± 41.57	506.38 ± 52.39	406.62 ± 25.50	439.29 ± 44.02	404.80 ± 58.28	411.48 ± 28.13
Threonine	1606.87 ± 98.66	1448.53 ± 119.97	1486.09 ± 84.28	1232.60 ± 91.70	1618.94 ± 221.36	1200.81 ± 103.53
Isoleucine	566.27 ± 42.93	549.22 ± 49.24	399.87 ± 28.56	396.36 ± 30.71	412.10 ± 69.15	404.82 ± 33.44
Leucine	328.73 ± 25.92	362.27 ± 34.18	255.69 ± 15.70	266.63 ± 24.91	230.72 ± 25.02	308.94 ± 42.00
Glutamic acid	30419.60 ± 1529.50	26410.74 ± 1884.11	28397.23 ± 1300.42	26937.51 ± 1633.48	30407.94 ± 2694.43	25699.08 ± 1096.60
Phenylalanine	614.69 ± 54.80	565.20 ± 69.24	498.17 ± 40.47	464.05 ± 52.86	452.18 ± 62.54	563.96 ± 65.66
Tyrosine	312.19 ± 32.40	283.14 ± 37.11	292.73 ± 36.94	243.83 ± 23.63	248.55 ± 35.02	258.77 ± 16.16
Asparagine	436.26 ± 73.78	577.44 ± 133.83	704.35 ± 153.13	1111.20 ± 392.16	1383.37 ± 671.90	1069.70 ± 285.04
Glutamine	5666.25 ± 819.93	7261.20 ± 1472.53	7345.31 ± 1220.94	13239.74 ± 4027.80	12598.27 ± 5498.67	9622.77 ± 1482.99
total sugars$^{\Psi}$	**36.60 ± 0.68**	**34.09 ± 1.02**	**36.75 ± 1.41**	**33.93 ± 1.00**	**37.53 ± 1.02**	**33.53 ± 0.56**
Glucose	2.74 ± 0.27	2.43 ± 0.18	3.13 ± 0.49	3.09 ± 0.33	3.52 ± 0.51	2.64 ± 0.23
Fructose	1.15 ± 0.08	1.19 ± 0.11	1.13 ± 0.14	1.04 ± 0.11	1.16 ± 0.10	1.17 ± 0.07
Sucrose	31.55 ± 0.51	29.25 ± 0.82	31.26 ± 0.83	28.51 ± 0.61	31.62 ± 0.51	28.59 ± 0.37
Trisaccharides	0.88 ± 0.03	0.97 ± 0.05	0.91 ± 0.06	1.03 ± 0.07	0.94 ± 0.06	0.91 ± 0.05
Tetrasaccharides	0.27 ± 0.02	0.25 ± 0.02	0.31 ± 0.04	0.26 ± 0.02	0.30 ± 0.02	0.22 ± 0.02

	Control	Control + herbivory	*C. populi* frass	*C. populi* frass + herbivory	*L. dispar* frass	*L. dispar* frass + herbivory
Flavan-3-ols‡						
Catechin	0.36 ± 0.02	0.34 ± 0.01	0.43 ± 0.02	0.43 ± 0.03	0.43 ± 0.02	0.42 ± 0.01
Flavonoids‡						
Rutin	1.93 ± 0.12	2.00 ± 0.10	2.15 ± 0.10	2.27 ± 0.12	2.08 ± 0.16	2.34 ± 0.10
Salicinoids‡						
Salicin	7.14 ± 0.21	7.73 ± 0.38	8.01 ± 0.32	8.25 ± 0.33	8.06 ± 0.30	7.69 ± 0.28
Sum higher-order salicinoids	126.26 ± 2.62	132.36 ± 3.51	136.32 ± 3.70	149.11 ± 4.71	128.59 ± 5.89	137.80 ± 3.19
Salicortin	78.08 ± 1.45	80.52 ± 1.90	82.90 ± 1.94	89.96 ± 2.46	78.89 ± 3.17	83.34 ± 1.75
Homalosid D	37.86 ± 0.80	40.27 ± 1.10	41.31 ± 1.28	44.19 ± 1.54	38.98 ± 1.71	41.94 ± 1.04
Tremulacin	0.45 ± 0.06	0.65 ± 0.07	0.51 ± 0.06	0.62 ± 0.06	0.44 ± 0.04	0.60 ± 0.06
6'-o-benzoylsalicortin	9.87 ± 0.45	10.93 ± 0.61	11.61 ± 0.63	14.34 ± 0.78	10.27 ± 1.04	11.92 ± 0.57
Phenolic acids						
Coumaric acid$^{\ominus}$	2.34 ± 0.11	2.90 ± 1.58	2.49 ± 0.18	3.21 ± 0.20	2.23 ± 0.15	3.21 ± 0.19
Caffeic acid$^{\ominus}$	16.27 ± 0.94	15.40 ± 0.75	18.32 ± 1.13	18.38 ± 1.11	17.72 ± 1.14	18.32 ± 0.74
Gallic acid$^{\ominus}$	0.17 ± 0.02	0.31 ± 0.07	0.25 ± 0.04	0.34 ± 0.06	0.27 ± 0.08	0.33 ± 0.06
trans-5-caffeoyl- quinic acid$^{\int}$	483018 ± 139452	644257 ± 141972	505109 ± 135100	499899± 161031	566469 ± 159391	549156 ± 151130
cis-5-caffeoylquinic acid$^{\int}$	278995 ± 88131	435580 ± 119120	296905 ± 87177	314346 ± 115409	353092 ± 104964	335008 ± 99091
cis-4-caffeoyl-quinic acid$^{\int}$	5588 ± 357	6077 ± 553	6890 ± 557	7874 ± 453	7029 ± 412	7866 ± 815
trans-4-caffeoyl-quinic acid$^{\int}$	26374 ± 1829	27227 ± 1537	30860 ± 1903	34091 ± 1633	30351 ± 1840	34167 ± 1468
Sum 3-QA$^{\int}$	1858 ± 45	1016538 ± 127589	1375808 ± 106067	1134896 ± 112095	964665 ± 66950	1047603 ± 104109

	Control	**Control + herbivory**	***C. populi* frass**	***C. populi* frass + herbivory**	***L. dispar* frass**	***L. dispar* frass + herbivory**
trans-3-caffeoyl-quinic acid	1208 ± 44	856589 ± 110782	1133481 ± 85260	944273 ± 98406	811213 ± 60027	866725 ± 87495
cis-3-caffeoyl-quinic acid	650 ± 20	159949 ± 17405	242327 ± 30102	190623 ± 18068	153452 ± 7427	180878 ± 18993
roots						
Fresh weight[¥]	8.02 ± 0.52	8.34 ± 0.75	7.61 ± 0.73	6.60 ± 0.48	7.23 ± 0.62	7.76 ± 0.56
Phytohormones[⊖]						
Abscisic acid	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.00
SA	1.10 ± 0.09	1.24 ± 0.13	1.98 ± 0.12	2.16 ± 0.14	1.68 ± 0.13	1.48 ± 0.11
Sum jasmonates	0.31 ± 0.04	0.28 ± 0.03	0.33 ± 0.08	0.37 ± 0.09	0.21 ± 0.03	0.36 ± 0.05
JA	0.30 ± 0.04	0.26 ± 0.03	0.30 ± 0.07	0.34 ± 0.08	0.20 ± 0.03	0.34 ± 0.04
JA-Ile	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	0.02 ± 0.00
Sum hydroxyl- and carboxylated jasmonates	0.04 ± 0.01	0.62 ± 0.10	0.06 ± 0.01	0.82 ± 0.14	0.06 ± 0.02	0.79 ± 0.08
OH-JA	0.04 ± 0.01	0.60 ± 0.10	0.05 ± 0.01	0.79 ± 0.14	0.05 ± 0.02	0.77 ± 0.08
OH-JA-Ile	0.00 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.02 ± 0.00
COOH-JA-Ile	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
total AAs[Φ]	**259068.13 ± 94684.12**	**229582.41 ± 13613.39**	**227341.77 ± 16993.71**	**235212.26 ± 15343.19**	**260969.21 ± 14463.32**	**210236.28 ± 17155.63**
Alanine	70005.54 ± 77077.03	64122.10 ± 3857.69	55023.21 ± 5576.51	61943.25 ± 5526.44	71159.79 ± 4142.80	59015.69 ± 5536.44
Serine	11636.53 ± 2171.23	8577.56 ± 593.20	8692.04 ± 795.83	9582.74 ± 530.74	9791.38 ± 691.05	9291.09 ± 1063.64
Proline	1055.50 ± 106.78	1038.59 ± 44.11	818.59 ± 43.53	857.49 ± 35.01	919.87 ± 42.48	866.82 ± 47.61
Valine	1944.73 ± 167.74	1913.29 ± 79.95	1572.96 ± 56.17	1616.07 ± 70.26	1670.48 ± 68.39	1572.31 ± 69.19
Threonine	3790.79 ± 362.20	3319.92 ± 139.38	3062.42 ± 147.74	3071.99 ± 105.94	3340.47 ± 153.84	3031.11 ± 205.09

	Control	Control + herbivory	*C. populi* frass	*C. populi* frass + herbivory	*L. dispar* frass	*L. dispar* frass + herbivory
Isoleucine	1539.12 ± 124.40	1576.07 ± 74.73	1208.40 ± 54.12	1217.00 ± 58.58	1239.97 ± 57.40	1246.29 ± 54.87
Leucine	1406.89 ± 116.35	1497.43 ± 81.73	1153.64 ± 51.80	1259.93 ± 62.44	1273.59 ± 63.44	1265.01 ± 62.58
Glutamic acid	49600.31 ± 3972.93	51058.83 ± 2645.11	52159.57 ± 4357.88	60455.17 ± 2928.78	63192.61 ± 2470.65	51532.89 ± 4222.05
Phenylalanine	511.44 ± 64.83	528.89 ± 28.50	398.03 ± 23.14	435.19 ± 22.06	454.63 ± 29.62	462.05 ± 36.79
Tyrosine	552.67 ± 62.18	602.49 ± 32.38	459.27 ± 29.37	503.65 ± 27.25	507.86 ± 41.73	530.25 ± 39.90
Asparagine	6773.97 ± 591.89	6522.55 ± 342.70	5528.29 ± 371.99	5717.36 ± 342.01	6318.68 ± 291.68	5512.80 ± 387.55
Glutamine	110250.64 ± 9866.56	88824.69 ± 5693.91	97265.35 ± 5485.63	88552.42 ± 5633.68	101099.88 ± 6410.24	75909.97 ± 5429.92
total sugars$^{\Psi}$	**30.36 ± 1.70**	**25.46 ± 1.34**	**28.87 ± 1.66**	**27.89 ± 1.01**	**30.50 ± 1.17**	**25.35 ± 1.39**
Glucose	2.32 ± 0.16	1.88 ± 0.17	2.17 ± 0.13	1.79 ± 0.11	2.15 ± 0.14	1.72 ± 0.17
Fructose	5.18 ± 0.30	4.71 ± 0.36	3.77 ± 0.19	3.35 ± 0.21	4.06 ± 0.25	3.57 ± 0.26
Sucrose	21.79 ± 1.39	18.00 ± 0.92	22.03 ± 1.44	21.89 ± 0.95	23.31 ± 0.95	19.27 ± 1.13
Trisaccharides	0.86 ± 0.08	0.71 ± 0.07	0.75 ± 0.09	0.72 ± 0.10	0.81 ± 0.07	0.66 ± 0.05
Tetrasaccharides	0.21 ± 0.02	0.16 ± 0.01	0.16 ± 0.02	0.14 ± 0.02	0.18 ± 0.02	0.13 ± 0.00
Flavan-3-ols‡						
Catechin	3.02 ± 0.06	3.25 ± 0.07	3.31 ± 0.08	3.64 ± 0.10	3.32 ± 0.07	3.22 ± 0.11
Flavonoids‡						
Rutin	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Salicinoids‡						
Salicin	0.47 ± 0.05	0.43 ± 0.04	1.96 ± 0.09	2.17 ± 0.07	1.64 ± 0.07	1.46 ± 0.08
Sum higher-order salicinoids	13.27 ± 0.60	12.77 ± 0.65	13.09 ± 1.35	10.51 ± 1.07	10.56 ± 1.26	11.73 ± 0.95
Salicortin	4.59 ± 0.22	4.43 ± 0.24	4.52 ± 0.46	3.55 ± 0.36	3.60 ± 0.42	3.98 ± 0.34
Homalosid D	8.18 ± 0.36	7.90 ± 0.40	8.08 ± 0.82	6.60 ± 0.68	6.58 ± 0.77	7.32 ± 0.57

	Control	**Control + herbivory**	***C. populi* frass**	***C. populi* frass + herbivory**	***L. dispar* frass**	***L. dispar* frass + herbivory**
Tremulacin	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
6'-o-benzoylsalicortin	0.50 ± 0.04	0.44 ± 0.03	0.49 ± 0.08	0.36 ± 0.05	0.38 ± 0.08	0.43 ± 0.04
Phenolic acids						
Coumaric acid$^{\Theta}$	1.24 ± 0.07	1.47 ± 0.13	1.29 ± 0.07	1.44 ± 0.07	1.45 ± 0.09	1.27 ± 0.09
Caffeic acid$^{\Theta}$	5.18 ± 0.43	6.08 ± 0.57	6.94 ± 0.89	7.47 ± 0.66	6.63 ± 0.53	6.37 ± 0.45
Gallic acid$^{\Theta}$	0.12 ± 0.01	0.15 ± 0.02	0.11 ± 0.01	0.11 ± 0.00	0.11 ± 0.01	0.11 ± 0.01
trans-5-caffeoyl- quinic acid$^{\int}$	11851397 ± 739408	11497103 ± 586136	12844017 ± 869497	13008758 ± 1212375	10975793 ± 823693	12648639 ± 708016
cis-5-caffeoylquinic acid$^{\int}$	10461399 ± 930221	10684223 ± 1048266	11927049 ± 2427705	11744453 ± 827093	10134609 ± 964496	11375379 ± 1148842
cis-4-caffeoyl-quinic acid$^{\int}$	35500 ± 3271	38274 ± 4642	48786 ± 10218	50307 ± 8177	38760 ± 4169	40948 ± 8883
trans-4-caffeoyl-quinic acid$^{\int}$	143630 ± 10462	147711 ± 11260	180567 ± 11427	186918 ± 14321	145719 ± 10110	153069 ± 5440
Sum 3-QA$^{\int}$	2102 ± 101	5561129 ± 411760	5632519 ± 351537	7234538 ± 680466	5698341 ± 624127	5110741 ± 564385
trans-3-caffeoyl-quinic acid	1448 ± 87	4597910 ± 335186	4632145 ± 294195	5930521 ± 498377	4651626 ± 544942	4198550 ± 517952
cis-3-caffeoyl-quinic acid	653 ± 25	963220 ± 98614	1000374 ± 168511	1304018 ± 299969	1046716 ± 166021	912191 ± 111773

$^{¥}$ Fresh weights are given in g.

§ The leaf area loss is presented in %.

$^{‖}$ The unit of the residual leaf area is cm^2.

$^{\Delta}$ The height increase is given in cm.

$^{\Theta}$ Phytohormones and the phenolic acids caffeic acid, coumaric acid and gallic acid are stated in µg g^{-1}.

$^{\Phi}$ Amino acids are given in nmol g^{-1}.

$^{\Psi}$ Sugars are presented in µg mg^{-1}.

‡ Catechin, rutin and the salicinoids are stated in mg g^{-1}.

$^{\int}$ The unit of caffeoyl-quinic acids is peak area mg^{-1} dry weight.

Table A9. Results of GLS models of the main factors frass, herbivory and block for the residual leaf area, leaf and root hydroxyl- and carboxylated jasmonates (OH-JA, OH-JA-Ile, COOH-JA-Ile), total free amino acid content, coumaric and caffeic acid and total free sugars for the frass induction experiment (n= 9-12 for leaves and n= 12 for roots).

	Frass		Herbivory		Block	
	F	*P*	*F*	*P*	*F*	*P*
Residual leaf area	3.12	0.053	**6.95**	**0.02**	**35.56**	**<0.001**
Leaf hydroxyl- and carboxylated jasmonates[≠]	**3.34**	**0.05**	**414.31**	**<0.001**	0.50	0.61
Root hydroxyl- and carboxylated jasmonates	0.16	0.85	**160.23**	**<0.001**	2.59	0.09
Leaf free amino acids	**53.07**	**<0.001**	**159.77**	**<0.001**	**175.30**	**<0.001**
Root free amino acids	**262.37**	**<0.001**	**47.60**	**<0.001**	**2257.02**	**<0.001**
Leaf coumaric acid[¶]	1.28	0.29	**39.07**	**<0.001**	**10.05**	**<0.001**
Root coumaric acid	0.36	0.70	0.92	0.34	**6.60**	**<0.01**
Leaf caffeic acid[3]	**4.87**	**0.02**	0.19	0.66	**8.46**	**<0.001**
Root caffeic acid	**4.98**	**0.02**	**6.25**	**0.02**	**6.52**	**<0.01**
Leaf free sugars[†]	**156.32**	**<0.001**	**58.98**	**<0.001**	**56.24**	**<0.001**
Root free sugars	0.15	0.86	**20.46**	**<0.001**	**38.82**	**<0.001**

[≠] The two-way interactions between frass and herbivory (F= 4.91, P= 0.02) and herbivory-block (F= 16.31, P<0.001) were significant.

[¶] The two-way interaction of herbivory and block was tested to be significant (F= 3.44, P= 0.04).

[3] The two-way interaction between herbivory and block was significant (F= 6.18, P< 0.01).

[†] The two-way interaction of frass and block had a significant influence on free leaf sugars (F= 7.12, P< 0.001).

12. References

Ali JG and Agrawal AA (2012) Specialist versus generalist insect herbivores and plant defense. *Trends in plant science* 17(5): 293–302.

Babst BA, Harding SA and Tsai C-J (2010) Biosynthesis of phenolic glycosides from phenylpropanoid and benzenoid precursors in populus. *Journal of chemical ecology* 36(3): 286–297.

Barbehenn RV, Niewiadomski J and Kochmanski J (2013) Importance of protein quality versus quantity in alternative host plants for a leaf-feeding insect. *Oecologia* 173(1): 1–12.

Basu S, Varsani S and Louis J (2018) Altering Plant Defenses: Herbivore-Associated Molecular Patterns and Effector Arsenal of Chewing Herbivores. *Molecular plant-microbe interactions MPMI* 31(1): 13–21.

Boeckler GA, Gershenzon J and Unsicker SB (2011) Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses. *Phytochemistry* 72(13): 1497–1509.

Boeckler GA, Gershenzon J and Unsicker SB (2013) Gypsy moth caterpillar feeding has only a marginal impact on phenolic compounds in old-growth black poplar. *Journal of chemical ecology* 39(10): 1301–1312.

Boeckler GA, Paetz C, Feibicke P, et al. (2016) Metabolism of poplar salicinoids by the generalist herbivore Lymantria dispar (Lepidoptera). *Insect biochemistry and molecular biology* 78: 39–49.

Burse A, Frick S, Discher S, et al. (2009) Always being well prepared for defense: the production of deterrents by juvenile Chrysomelina beetles (Chrysomelidae). *Phytochemistry* 70(15-16): 1899–1909.

Caarls L, Pieterse CMJ and van Wees SCM (2015) How salicylic acid takes transcriptional control over jasmonic acid signaling. *Frontiers in plant science* 6: 1–11.

Carvalho AP and Malcata FX (2003) Kinetic Modeling of the Autotrophic Growth of Pavlova lutheri: Study of the Combined Influence of Light and Temperature. *Biotechnol. Prog.* 19(4): 1128–1135.

Chedgy RJ, Köllner TG and Constabel CP (2015) Functional characterization of two acyltransferases from Populus trichocarpa capable of synthesizing benzyl benzoate and salicyl benzoate, potential intermediates in salicinoid phenolic glycoside biosynthesis. *Phytochemistry* 113: 149–159.

Cheong J-J and Choi YD (2007) Signaling pathways for the Biosynthesis and action of Jasmonates. *Journal of Plant Biology* 50(2): 122–131.

Christenson LM, Lovett GM, Mitchell MJ, et al. (2002) The fate of nitrogen in gypsy moth frass deposited to an oak forest floor. *Oecologia* 131(3): 444–452.

Clifford MN, Johnston KL, Knight S, et al. (2003) Hierarchical scheme for LC-MS^n identification of chlorogenic acids. *Journal of agricultural and food chemistry* 51(10): 2900–2911.

Clifford MN, Kirkpatrick J, Kuhnert N, et al. (2008) LC–MS^n analysis of the cis isomers of chlorogenic acids. *Food Chemistry* 106(379-385).

Crawley MJ (2007) *The R book*. Chichester England, Hoboken N.J.: Wiley.

Debeljak M, Ficko A and Brus R (2015) The use of habitat and dispersal models in protecting European black poplar (Populus nigra L.) from genetic introgression in Slovenia. *Biological Conservation* 184: 310–319.

Demkura PV and Ballaré CL (2012) UVR8 mediates UV-B-induced Arabidopsis defense responses against Botrytis cinerea by controlling sinapate accumulation. *Molecular plant* 5(3): 642–652.

Derksen H, Rampitsch C and Daayf F (2013) Signaling cross-talk in plant disease resistance. *Plant science an international journal of experimental plant biology* 207: 79–87.

Dicke M, van Loon JJA and Soler R (2009) Chemical complexity of volatiles from plants induced by multiple attack. *Nature chemical biology* 5(5): 317–324.

Dixon RA, Achnine L, Kota P, et al. (2002) The phenylpropanoid pathway and plant defence—a genomics perspective. *Molecular Plant Pathology* 3(5): 371–390.

Dixon RA and Paiva NL (1995) Stress-lnduced Phenylpropanoid Metabolism. *The Plant Cell* 7: 1085–1097.

Eberl F, Hammerbacher A, Gershenzon J, et al. (2018) Leaf rust infection reduces herbivore-induced volatile emission in black poplar and attracts a generalist herbivore. *The New phytologist* 220(3): 760–772.

Erb M, Meldau S and Howe GA (2012) Role of phytohormones in insect-specific plant reactions. *Trends in plant science* 17(5): 250–259.

Eyles A, Bonello P, Ganley R, et al. (2010) Induced resistance to pests and pathogens in trees. *The New phytologist* 185(4): 893–908.

Fraser CM and Chapple C (2011) The phenylpropanoid pathway in Arabidopsis. *The arabidopsis book* 9: 1-19.

Frost CJ and Hunter MD (2007) Recycling of nitrogen in herbivore feces: plant recovery, herbivore assimilation, soil retention, and leaching losses. *Oecologia* 151(1): 42–53.

Frost CJ and Hunter MD (2008) Insect herbivores and their frass affect Quercus rubra leaf quality and initial stages of subsequent litter decomposition. *Oikos* 117(1): 13–22.

Frost CJ, Mescher MC, Carlson JE, et al. (2008) Plant defense priming against herbivores: getting ready for a different battle. *Plant physiology* 146(3): 818–824.

GOMI T, KIMURA T and HIROSE S (2008) Seasonal changes in the reproductive and life-history traits of Chrysomela populi L. (Coleoptera: Chrysomelidae). *Entomological Science* 11(1): 31–38.

Hilker M and Fatouros NE (2016) Resisting the onset of herbivore attack: plants perceive and respond to insect eggs. *Current opinion in plant biology* 32: 9–16.

Hillstrom M, Meehan TD, Kelly K, et al. (2010) Soil carbon and nitrogen mineralization following deposition of insect frass and greenfall from forests under elevated CO2 and O3. *Plant and Soil* 336(1-2): 75–85.

Holopainen JK (2011) Can forest trees compensate for stress-generated growth losses by induced production of volatile compounds? *Tree physiology* 31(12): 1356–1377.

Irmisch S, Jiang Y, Chen F, et al. (2014) Terpene synthases and their contribution to herbivore-induced volatile emission in western balsam poplar (Populus trichocarpa). *BMC plant biology* 14: 270.

Jankovic M and Petrovskii S (2013) Gypsy moth invasion in North America: A simulation study of the spatial pattern and the rate of spread. *Ecological Complexity* 14: 132–144.

Kagata H and Ohgushi T (2012) Non-additive effects of leaf litter and insect frass mixture on decomposition processes. *Ecological Research* 27(1): 69–75.

Kagata H and Ohgushi T (2012) Positive and negative impacts of insect frass quality on soil nitrogen availability and plant growth. *Population Ecology* 54(1): 75–82.

Kramell R, Schmidt J, Schneider G, et al. (1988) Synthesis of N-(jasmonoyl)amino acid conjugate. *Tetrahedron* 44(18): 5791–5807.

Lämke JS and Unsicker SB (2018) Phytochemical variation in treetops: causes and consequences for tree-insect herbivore interactions. *Oecologia* 187(2): 377–388.

Lawrence SD, Dervinis C, Novak N, et al. (2006) Wound and insect herbivory responsive genes in poplar. *Biotechnology letters* 28(18): 1493–1501.

Lazarevi´c J, Peri´c-Mataruga V, Stojkovi´c B, et al. (2002) Adaptation of the gypsy moth to an unsuitable host plant. *Entomologia Experimentalis et Applicata* 102: 75–86.

Li X, Schuler MA and Berenbaum MR (2002) Jasmonate and salicylate induce expression of herbivore cytochrome P450 genes. *Nature* 419(6908): 712–715.

Lovett GM and Ruesink AE (1995) Carbon and nitrogen mineralization from decomposing gypsy moth frass. *Oecologia* 104: 133–138.

Madritch MD, Donaldson JR and Lindroth RL (2007) Canopy herbivory can mediate the influence of plant genotype on soil processes through frass deposition. *Soil Biology and Biochemistry* 39(5): 1192–1201.

Maffei ME (2010) Sites of synthesis, biochemistry and functional role of plant volatiles. *South African Journal of Botany* 76(4): 612–631.

Major IT and Constabel CP (2007) Shoot–root defense signaling and activation of root defense by leaf damage in poplarThis article is one of a selection of papers published in the Special Issue on Poplar Research in Canada. *Canadian Journal of Botany* 85(12): 1171–1181.

Massad TJ, Trumbore SE, Ganbat G, et al. (2014) An optimal defense strategy for phenolic glycoside production in Populus trichocarpa--isotope labeling demonstrates secondary metabolite production in growing leaves. *The New phytologist* 203(2): 607–619.

McCormick AC, Reinecke A, Gershenzon J, et al. (2016) Feeding Experience Affects the Behavioral Response of Polyphagous Gypsy Moth Caterpillars to Herbivore-induced Poplar Volatiles. *Journal of chemical ecology* 42(5): 382–393.

McCormick AC, Boeckler GA, Köllner TG, et al. (2014) The timing of herbivore-induced volatile emission in black poplar (Populus nigra) and the influence of herbivore age and identity affect the value of individual volatiles as cues for herbivore enemies. *BMC plant biology* 14: 304.

McCormick AC, Irmisch S, Reinecke A, et al. (2014) Herbivore-induced volatile emission in black poplar: regulation and role in attracting herbivore enemies. *Plant, cell & environment* 37(8): 1909–1923.

Müller A, Kaling M, Faubert P, et al. (2015) Isoprene emission by poplar is not important for the feeding behaviour of poplar leaf beetles. *BMC plant biology* 15: 165.

Pauls G, Becker T, Rahfeld P, et al. (2016) Two Defensive Lines in Juvenile Leaf Beetles; Esters of 3-nitropropionic Acid in the Hemolymph and Aposematic Warning. *Journal of chemical ecology* 42(3): 240–248.

Philippe RN and Bohlmann J (2007) Poplar defense against insect herbivoresThis review is one of a selection of papers published in the Special Issue on Poplar Research in Canada. *Canadian Journal of Botany* 85(12): 1111–1126.

Pieterse CMJ and Dicke M (2007) Plant interactions with microbes and insects: from molecular mechanisms to ecology. *Trends in plant science* 12(12): 564–569.

R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Ray S, Alves PCMS, Ahmad I, et al. (2016) Turnabout Is Fair Play: Herbivory-Induced Plant Chitinases Excreted in Fall Armyworm Frass Suppress Herbivore Defenses in Maize. *Plant physiology* 171(1): 694–706.

Ray S, Basu S, Rivera-Vega LJ, et al. (2016) Lessons from the Far End: Caterpillar FRASS-Induced Defenses in Maize, Rice, Cabbage, and Tomato. *Journal of chemical ecology* 42(11): 1130–1141.

Ray S, Gaffor I, Acevedo FE, et al. (2015) Maize Plants Recognize Herbivore-Associated Cues from Caterpillar Frass. *Journal of chemical ecology* 41(9): 781–792.

Ruuhola T, Ossipov V, Lempa K, et al. (2003) Amino acids during development of mountain birch leaves. *CHEMOECOLOGY* 13(2): 95–101.

Scala A, Allmann S, Mirabella R, et al. (2013) Green leaf volatiles: a plant's multifunctional weapon against herbivores and pathogens. *International journal of molecular sciences* 14(9): 17781–17811.

Scanlon JT and Willis DE (1985) Calculation of Flame Ionization Detector Relative Response Factors Using the Effective Carbon Number Concept. *Journal of Chromatographic Science* 23: 333–340.

Shuxin L, Lei Y, Baoli D, et al. (2017) Sex-specific responses of Populus deltoides to defoliation. *Acta Societatis Botanicorum Poloniae* 86(4): 3566.

Tuskan GA, DiFazio S, Jansson S, et al. (2006) The Genome of Black Cottonwood, Populus trichocarpa. *Science* 313: 1596–1604.

Unsicker SB, Kunert G and Gershenzon J (2009) Protective perfumes: the role of vegetative volatiles in plant defense against herbivores. *Current opinion in plant biology* 12(4): 479–485.

Vanhanen H, Veteli TO, Päivinen S, et al. (2007) Climate change and range shifts in two insect defoliators: gypsy moth and nun moth – a model study. *Silva Fennica* 41(4): 621–638.

Vassão DG, Wielsch N, Gomes AMdMM, et al. (2018) Plant Defensive β-Glucosidases Resist Digestion and Sustain Activity in the Gut of a Lepidopteran Herbivore. *Frontiers in plant science* 9.

Venkatesan R (2015) Biosynthesis and Regulation of Herbivore-Induced Plant Volatile Emission. *Journal of the Indian Institute of Science* 95(1): 25–34.

Vogt T (2010) Phenylpropanoid biosynthesis. *Molecular plant* 3(1): 2–20.

War AR, Paulraj MG, Ahmad T, et al. (2012) Mechanisms of plant defense against insect herbivores. *Plant signaling & behavior* 7(10): 1306–1320.

Zuur AF, Ieno EN, Walker N, et al. (2009) *Mixed effects models and extensions in ecology with R*. New York, NY: Springer New York.

Eigenständigkeitserklärung

Ich erkläre, dass ich die vorliegende Arbeit selbständig und nur unter Verwendung der angegebenen Hilfsmittel und Quellen angefertigt habe. Die eingereichte Arbeit wurde bisher keiner anderen Prüfungsbehörde vorgelegt und ist weder in deutscher noch einer anderen Sprache als Veröffentlichung erschienen.

Jena, den 20. November 2019

E. Mindt