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Abteilung Biochemie

Metabolische Flusskontrolle des MEP-Stoffwechselweges

The effect of *Myzus persicae* infestation on the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway in *Arabidopsis thaliana*

Masterarbeit

Zur Erlangung des Grades eines

Master of Sciences Biochemistry

vorgelegt von

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JENA, November 2015

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1 Zusammenfassung

Trotz ihrer geringen Größe können Blattläuse einen starken Einfluss auf die Landwirtschaft haben. Daher ist es besonders wichtig die Interaktionen zwischen Pflanze und Blattlaus näher zu untersuchen und zu verstehen, wie die Resistenz der Pflanzen erhöht werden kann. Es ist bekannt, dass *Myzus persicae* eine Salizylsäure (SA) abhängige Stressantwort in *Arabidopsis thaliana* auslöst. Jedoch wird immer noch darüber diskutiert, ob SA lediglich induziert wird, um eine Verteidigung mittels Jasmonsäure (JA) zu verhindern. Xiao *et al.* fanden 2012 heraus, dass 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphat (MEcDP) in der Pflanze als retrogrades Signal genutzt werden kann, um die SA Konzentration zu erhöhen. Der verantwortliche 2-*C*-methyl-D-erythritol 4-phosphat (MEP) Biosyntheseweg wurde erst vor kurzem entdeckt und ist von großem wissenschaftlichem Interesse. Die Rolle des MEP Weges in Reaktionen auf biotischem Stress ist noch nicht vollständig geklärt. Daher beschäftigt sich diese Arbeit mit dem Einfluss von *M. persicae* auf den MEP Biosyntheseweg in *A. thaliana* und behält hierbei auch beteiligte pflanzliche Komponenten im Auge.

Für die Untersuchungen, die im Rahmen dieser Arbeit durchgeführt wurden, wurden auf jeder Pflanze 40 Blattläuse ausgesetzt und die Pflanzen anschließend nach verschiedenen Zeiträumen geerntet. Im Gegensatz zu den bisherigen Forschungsergebnissen konnte kein Einfluss der Blattläuse auf die Photosynthese und einige der untersuchten Pigmente beobachtet werden. Nachfolgende Untersuchungen des MEP Biosyntheseweges ergaben, dass einige der im Chloroplast gelegenen Metabolite beeinflusst werden. Besonders MEcDP Konzentrationen stiegen innerhalb der ersten drei Stunden nach Blattlausbefall an und DXP konnte nach sechs Stunden in erhöhten Mengen nachgewiesen werden. Dies kann anhand der Aktivität der DXP Synthase (DXS) erklärt werden. Überraschender Weise lag weder das zytosolische MEcDP, noch die zytosolischen Degradationsprodukte 2-*C*-methyl-D-erythritrol (ME) und ME-glc, in erhöhten Konzentration vor. Weitere Mediatoren der Verteidigungsantwort von *A. thaliana* auf *M. persicae*, wie zum Beispiel SA, JA und reaktive Sauerstoffspezies, zeigten keine Reaktion auf den Blattlausbefall. Diese Ergebnisse weisen auf eine Anpassung von *M. persicae* an *A. thaliana* hin, welche vermutlich durch die Kultivierung der Blattlaus auf der gleichen Wirtspflanze induziert wurde. Die Anpassung beinhaltet jedoch nicht die erwartete Unterdrückung von JA durch SA.

2 Abstract

Aphids, albeit small, can have an immense detrimental impact on agriculture if their numbers increase too much. Therefore, it is important to study the aphid-plant interactions and to understand which mechanisms of the plants could be utilized to increase plant resistance. *Myzus persicae* is known to induce an increase in salicylic acid (SA) in *Arabidopsis thaliana*. The debate about a possible decoy mechanism to inhibit a jasmonic acid (JA) induced defense response is still ongoing. Xiao *et al.* (2012) have found that retrograde signaling through 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate (MEcDP) increases the SA content. The responsible 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway is a comparatively 'new' metabolic pathway which holds the interest of many scientists. The involvement of the MEP pathway in responses to biotic stress has not been completely elucidated, yet. Consequently, this thesis investigates the effect of *M. persicae* feeding on the MEP pathway in *A. thaliana* but also looks at other plant responses connected to the MEP pathway.

Upon investigating the importance of the MEP pathway in *M. persicae–A. thaliana* interactions new insights have been gained in the presented study. 40 aphids have been caged on 40 days old rosette plants for different periods of time. Contradicting the previous findings in literature no changes in photosynthesis and most of the involved pigments have been observed. However, subsequent analysis of the plastidial MEP pathway intermediates revealed that this pathway is affected by aphid infestation. Especially MEcDP levels were increased in the first three hours, whereas DXP levels increased after six hours of infestation. DXP synthase (DXS) activity appears to be the main reason for MEcDP accumulation. However, no cytosolic MEcDP was found. In contrast, mainly the cytosolic 2-*C*-methyl-D-erythritol (ME) seems to be influenced by the feeding aphid. Different players involved in the defense responses of *A. thaliana* to *M. persicae* such as SA, JA and reactive oxygen species have also been investigated and were found to be indifferent to aphid feeding. These results prove an adaptation of *M. persicae* to *A. thaliana* due to the rearing executed on the same host plant. However, the expected decoy mechanism has not been observed.

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3 Introduction

Everything we see during our daily lives has some form of pigmentation. During spring time especially plants stand out with their differently colored flowers (Beale, 1999; Tanaka *et al.*, 2008). Some of the pigments involved in this coloration are terpenoids, such as β-carotene, which consist of isoprene units. In addition to coloration terpenoids are also involved in defense responses (Thompson and Goggin, 2006). Xanthophylls (Jahns and Holzwarth, 2012) and β-carotene (Ramel *et al.*, 2012) for example are involved in scavenging reactive oxygen species. Diterpene glycosides on the other hand are involved in defense against insect herbivores (Kerchev *et al.*, 2012).

All terpenoids have in common that their subunits are produced via two possible routes, namely the Mevalonate pathway and the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway. For a long time it has been thought that the Mevalonate pathway was the only pathway responsible for isoprene production (Duvoid *et al.*, 1997). However, during one of many experiments to better understand the primary isoprenoid metabolism it became obvious that evolution had invented more than one way to produce isopentenyl diphosphate (IDP) (Rohmer, 1999). First closer hints about this pathway had been found in bacteria while doing a feeding assay with stable isotope labeling (Flesh and Rohmer, 1988). Here the authors found a surprising compartmentalization of the isoprenoid precursors. This alternative pathway was initially titled mevalonate-independent pathway or non-mevalonate pathway but was later renamed to MEP pathway after its first unique metabolite (Duvoid *et al.*, 1997; Proteau, 2004; Lichtenthaler, 2010).

3.1 2-*C*-Methyl-D-Erythritol 4-Phosphate Pathway

3.1.1 An Overview of the MEP pathway

The MEP pathway is located in the chloroplast and responsible for the biosynthesis of precursors for isoprenoid production (Rohmer *et al.*, 1996; Duvoid *et al.*, 1997; Lichtenthaler, 1999).

D-glyceraldehyde 3-phosphate (G3P), derived from freshly assimilated carbon dioxide (CO_2), and pyruvate are the precursors of the MEP pathway (Rohmer *et al.*, 1996; Sharkey and Monson, 2014). The exact origin of pyruvate is still unknown, because chloroplasts lack glycolytic enzymes which could synthesize pyruvate from G3P. In addition the import of pyruvate from the cytosol into the chloroplast has not yet been elucidated (Banerjee and Sharkey, 2014).

The mentioned substrates are used by the first enzyme DXP synthase (DXS) to form 1-deoxy-D-xylulose 5-phosphate (DXP; Fig. 3-1) (Vranova *et al.*, 2013). During this condensation reaction CO_2 is produced. Subsequently the DXP reductoisomerase (DXR) reduces DXP to MEP with NADPH as reducing agent (Lichtenthaler, 2010). Further steps lead to the synthesis of 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate (MEcDP) via the usage of cytidine triphosphate (CTP) involving three enzymes (Rohdich *et al.*, 2000; Wright and Phillips, 2014). Subsequently, the circular structure is linearized by the (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate (HMBDP) synthase (HDS) (Banerjee and Sharkey, 2014). HMBDP represents the last unique compound of the MEP pathway. Therefrom the isomeric compounds isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP) are produced. The enzyme catalyzing this step to IDP/DMADP is called HMBDP reductase (HDR), whereas the IDP isomerase (IDI) regulates the ratio of IDP to DMADP (Banerjee and Sharkey, 2014; Wright *et al.*, 2014).

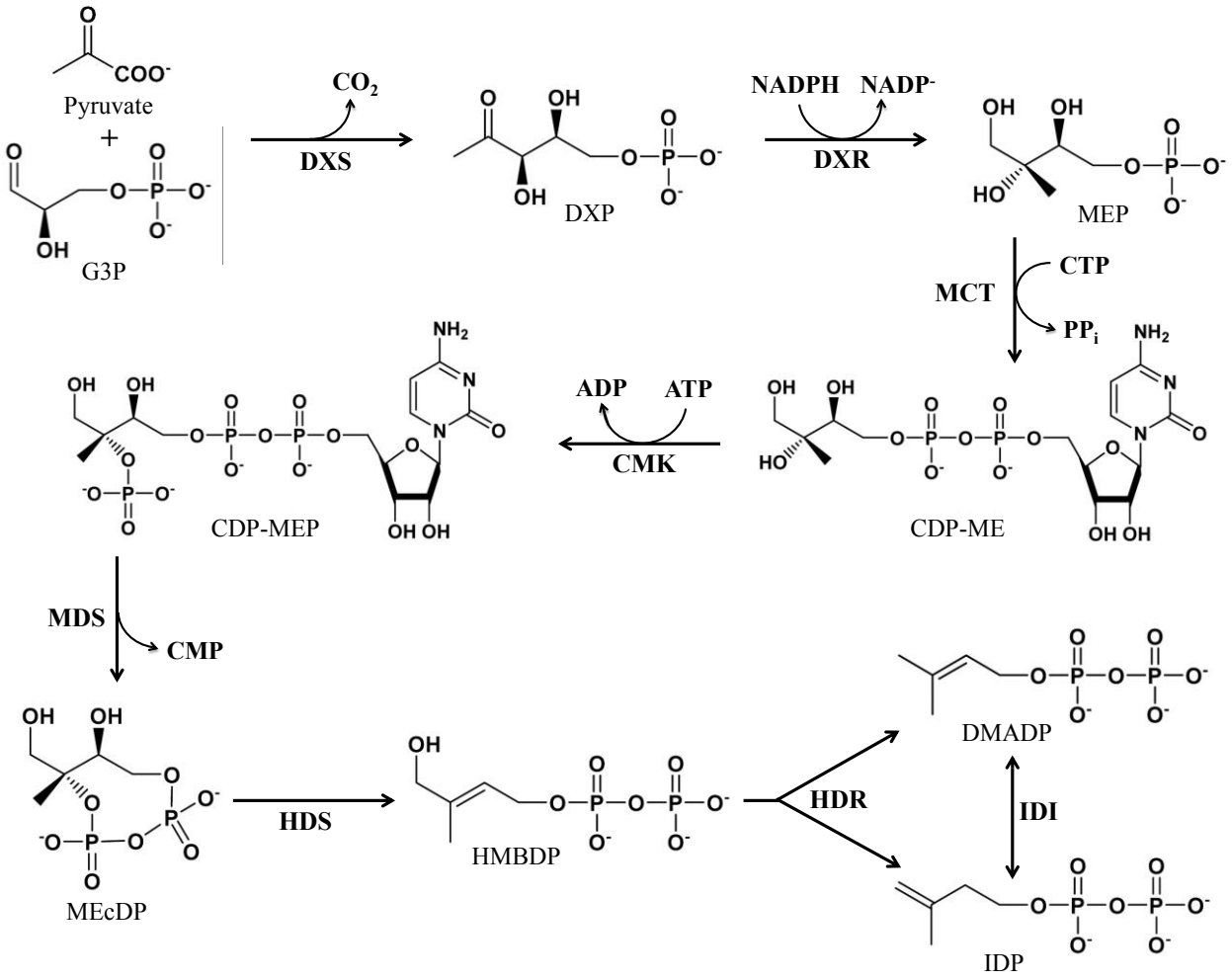

Fig. 3-1: Overview of the MEP pathway in plants. Metabolites are abbreviated as follows: G3P – D-glyceraldehyde 3-phosphate; DXP – 1-deoxy-D-xylulose 5-phosphate; MEP – 2-*C*-methyl-D-erythritol 4-phosphate; CDP-ME – 4-(cytidine diphosphate)-2-*C*-methyl-D-erythritol; CDP-MEP – 2-phospho-4-(CDP)-2-*C*-methyl-D-erythritol; MEcDP – 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate; HMBDP – (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate; DMADP – dimethylallyl diphosphate; IDP – isopentenyl diphosphate. Enzymes are abbreviated as follows: DXS – DXP synthase; DXR – DXP reductase; MCT – MEP cytidylyltransferase; CMK – CDP-MEP kinase; MDS – MEcDP synthase; HDS – HMBDP synthase; HDR – HMBDP reductase; IDI – IDP isomerase.

3.1.2 Regulation of the MEP pathway

Similar to other primary metabolic pathways the MEP pathway is regulated through various mechanisms (Cordoba *et al.*, 2009; Banerjee and Sharkey, 2014). These mechanisms are sensitive to environmental conditions such as light because the MEP pathway obtains substrates from the photosynthesis. Therefore, the photosynthetic rate influences the flux through the pathway and, consequently, the pathway regulation on the enzymatic level. Other signals in the plant cell may also be involved in enzymatic regulation.

Environmental Regulation and Photosynthesis

Photosynthesis influences the flux of the MEP pathway and is connected to this via three major points: the light-dependent reactions of photosynthesis, the light-independent (dark) reactions of photosynthesis and IDP/DMADP.

The light-dependent reactions supply the required energy necessary for the dark reactions and the functioning of the MEP pathway in the form of ATP and NADPH (Proteau, 2004; Chenu and Scholes, 2015; Kaiser *et al.*, 2015). A cooperation of light harvesting complexes (LHC) and photosystems (PS) I and II photochemically harnesses the energy contained in the sunlight (Shikanai, 2014). Subsequently, the LHCs transfer electrons in a photo initiated process towards PSII (Chenu and Scholes, 2015; Kaiser *et al.*, 2015) and constitutively to PSI (Munekage *et al.*, 2004; Shikanai, 2014). During this process a proton gradient is established through oxidation of water which is then used to power the ATP synthase, thus, producing energy in form of ATP (van Amerongen and Croce, 2013; Shikanai, 2014). LHCs are composed of isoprenoid units containing chlorophyll A and B, the main pigments involved in light harvesting (Caffarri *et al.*, 2014; Chenu and Scholes, 2015).

The Calvin-Benson-Bassham cycle or Calvin cycle describes the dark reactions of photosynthesis which use the energy gained from the light reactions to assimilate carbon containing intermediates by fixating CO_2 (Raines, 2002; Biel and Fomina, 2015). The carbon fixation is catalyzed by the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RubisCO). Produced intermediates are used to synthesize e.g. starch and sucrose (Raines, 2002). The intermediate G3P is used among others as precursor for isoprenoid production via the MEP pathway (Delwiche and Sharkey, 1993; Rohmer *et al.*, 1996; Lichtenthaler, 1999).

Last but not least, in *A. thaliana* IDP and DMADP, produced by the MEP pathway, are mainly used as precursors for the biosynthesis of chlorophyll and β-carotene, the pigments required for photosynthesis (Beale, 1999; Fellermeier *et al.*, 1999; Estevez *et al.*, 2001; Bode *et al.*, 2009). Besides helping with harvesting the light energy the produced pigments are also involved in absorbing excess energy or resulting reactive oxygen species (ROS) via the xanthophyll cycle or β-carotene (Müller *et al.*, 2001; Ramel *et al.*, 2012; Shikanai, 2014). Furthermore, it has been reported that the MEP pathway enzymes' transcript levels follow a

diurnal rhythm (Loivamäki *et al.*, 2007). Therefore, light influences the MEP pathway in a direct (via diurnal rhythm) and indirect (via substrates and chemical energy provided by photosynthesis) manner.

Enzymatic Regulation and Involvement in Stress Responses

The flux through the MEP pathway is controlled by DXS, the first enzyme of this pathway (Estevez *et al.*, 2001; Wright *et al.*, 2014). In poplar, an isoprene emitting plant, it has been found that DXS can be regulated by a feedback loop through the amount of the accumulated end product IDP/DMADP (Ghirardo *et al.*, 2014). However, under normal physiological conditions this regulation does not seem to play a role in *Arabidopsis thaliana* Col-0 a non-isoprene-emitting plant (Wright *et al.*, 2014). Furthermore, the transcript abundance of DXS is increased by the phytohormones methyl jasmonate and abscisic acid (ABA; Yang *et al.* (2012)).

The intermediate MEcDP has been found to be influenced by two enzymes of the MEP pathway. An increase in DXS levels and activity has been found to increase the total pool of MEcDP (Wright *et al.*, 2014). The second enzyme is HDS and was identified as a controlling enzyme during a mutant screen by Xiao *et al.* (2012). They found that if the function of HDS is impaired, MEcDP accumulates in great quantities. Due to this constellation the total amount of MEcDP may increase whereas the other MEP pathway metabolites decrease in their concentration.

An increase in reactive oxygen species (ROS) caused by abiotic or biotic stress disrupts the HDS [4Fe-4S]-cluster's integrity and leads to an accumulation of MEcDP, the substrate of HDS (Seemann and Rohmer, 2007; Rivasseau *et al.*, 2009; Mongelard *et al.*, 2011; Banerjee and Sharkey, 2014). MEcDP has the ability to scavenge reactive oxygen species (ROS) and may also act as an antioxidant but is mainly exported out of the chloroplast (Ostrovsky *et al.*, 2003; Xiao *et al.*, 2012). Consequently, MEcDP accumulation is also under close control of oxidative stress.

Mainly in response to abiotic stress the accumulation of MEcDP leads to an export out of the chloroplast into the cytosol, as mentioned previously. The cytosolic MEcDP is dephosphorylated to 2-*C*-methyl-D-erythritol (ME) which is subsequently glycosylated

(Gonzalez-Cabanelas *et al.*, 2015). Currently it is debated whether cytosolic MEcDP or ME is actually responsible in transmitting a retrograde signal (Xiao *et al.*, 2012; Gonzalez-Cabanelas *et al.*, 2015). Nonetheless, as a consequence of the retrograde signaling the level of the phytohormone salicylic acid (SA) increases and further defense responses are induced (Xiao *et al.*, 2012).

3.1.3 Pigments – Products of the MEP Pathway

As already mentioned pigments are derived from the end products IDP and DMADP of the MEP pathway. The most important ones are chlorophyll A and B which contain a tetrapyrrole ring and an isoprenoid unit (Beale, 1999; Eckhardt *et al.*, 2004). Metabolites completely comprised of isoprene units are called terpenoids.

The carotenoids are a family of pigments which is very closely connected to the MEP pathway (Tanaka *et al.*, 2008; Cazzonelli, 2011). Their biosynthesis starts with IDP and DMADP, the last compounds of the MEP pathway (Tanaka *et al.*, 2008). Through several steps the tetraterpene lycopene (C40) is produced, which is the precursor for α- and β-carotene synthesis (see Fig. 3-2). In contrast to lycopene these carotenoids contain a C6-ring at each end. While α-carotene is the precursor of lutein, the pigments involved in the xanthophyll cycle are synthesized from β-carotene. These xanthophylls are violaxanthin and neoxanthin which are also used as precursors for the biosynthesis of abscisic acid (Tanaka *et al.*, 2008).

During stress responses xanthophylls quench the induced reactive oxygen species and are recycled via the xanthophyll cycle (Müller *et al.*, 2001; Jahns and Holzwarth, 2012), whereas β-carotene is degraded during the quenching process (Ramel *et al.*, 2012). Two of the major degradation products are β-cyclocitral (β-CC) and β-ionone (β-I) both of which can also act as stress signals (Ramel *et al.*, 2012; Lv *et al.*, 2015). Especially β-CC upregulates salicylic acid signaling (Lv *et al.*, 2015). ROS are also increased upon aphid feeding; therefore, β-CC can be used in both abiotic and biotic stress responses (Moloi and van der Westhuizen, 2006; Thompson and Goggin, 2006).

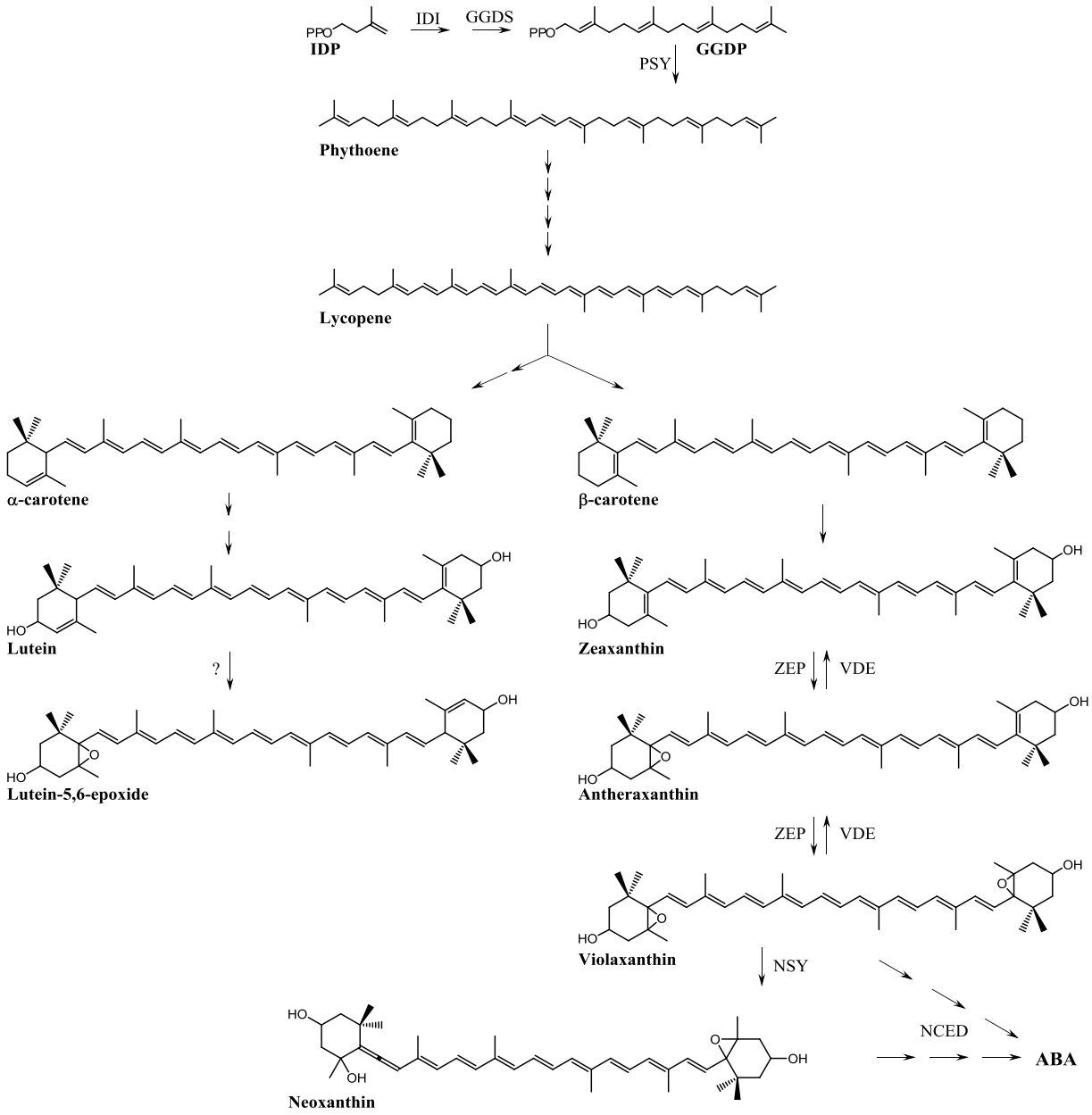

Fig. 3-2: Overview of selected carotenoid biosynthesis in *A. thaliana*. Only all-*trans*-configurations and selected enzymes are shown. Metabolites are abbreviated as follows: IDP – isopentenyl diphosphate; GGDP – geranylgeranyl diphosphate; ABA – abscisic acid. Enzymes are abbreviated as follows: IDI – IDP isomerase; GGDS – GGDP synthase; PSY – phytoene synthase; ZEP – zeaxanthin epoxidase; VDE – violaxanthin de-epoxidase; NSY – neoxanthin synthase; NCED – 9-*cis*-epoxycarotenoid dioxygenase; ? – unknown enzyme.

3.2 Aphids

Aphids are sucking insects which feed from the phloem sap of their host plant. A too large population may induce such strong responses in the host that the growth is reduced or the plants die (Girousse *et al.*, 2005; Blackman and Eastop, 2007; Louis and Shah, 2013; Will *et al.*, 2013). Especially generalists may become a problem for farmers (Blackman and Eastop, 2007). *Myzus persicae*, also known as the green peach aphid, is a generalist that can infest over 500 different plant species from 40 plant families (Blackman and Eastop, 2007). Some of those plants are e.g. peach, potatoes and all members of the Brassicaceae (Collier and Finch, 2007).

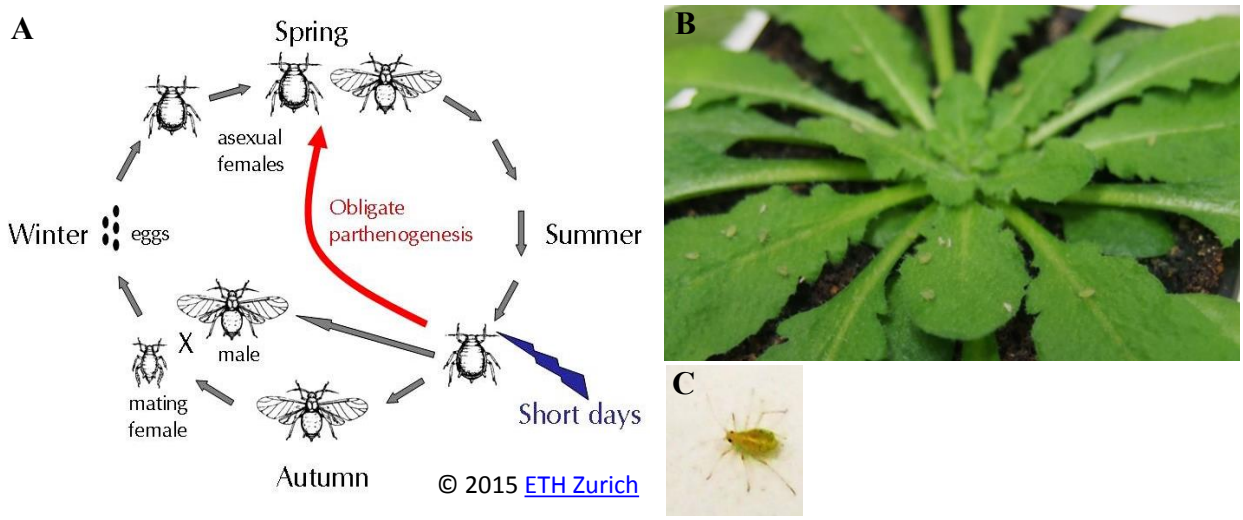

Fig. 3-3: *Myzus persicae*. A: Overview over the life cycle of *M. persicae*. During spring and summer female asexual forms are generated which may develop wings under less optimal conditions such as overcrowding of the plant. Shorter days and colder weather induce migration to their primary host plant (peach) and the formation of the sexual forms. The winter months are weathered by so-called "overwintering" eggs. Picture was taken from the web page of the department of environmental system sciences of the ETH Zurich (from 01.11.2015). **B**: Infested *A. thaliana* as used for rearing. **C**: *M. persicae* nymph as used during experiments.

Under suitable conditions aphids can reproduce by obligate parthenogenesis (Blackman and Eastop, 2007; Louis and Shah, 2013). The aphids reproduce asexually in so called telescopic generations. Each neonate is a genetical clone of its mother. Especially during summer time when the plants are thriving, barely any winged aphid can be seen. The asexual winged form is usually induced upon crowding of the infested plant, thus, the aphids can spread more easily. Shorter days and generally colder weather induce the production of sexual winged

female which are drawn to their primary host plant (for life cycle see Fig. 3-3). Upon mating of the sexual male and female forms *M. persicae* deposits its eggs on the green peach tree (Blackman and Eastop, 2007).

As opposed to chewing insects, aphids do not induce defense responses in plants through additional mechanical damage (Tjallingii and Hogen Esch, 1993; Zhu-Salzman *et al.*, 2005). On the way to the phloem they navigate with their stylets in between the cells of the plants (see Fig. 3-4). Occasionally they pierce a cell to test for the general location of their stylet (Elzinga and Jander, 2013; Will *et al.*, 2013). However, the plant might detect proteins (defense elicitors) secreted in the aphid's saliva during movement towards the phloem and during feeding (Zhu-Salzman *et al.*, 2005; Bos *et al.*, 2010). This induces a whole range of defense reactions (Louis and Shah, 2013), such as the first and most practical plant defense to stop nutrient loss by clogging the phloem. A general approach found in most plants is the clogging via callose formation (Will and van Bel, 2006). This is induced either through Ca^{2+}-signaling or via hydrogen peroxide (H_2O_2; Miller *et al.* (2009); Will *et al.* (2013)). Additionally, in legumes proteins called forisomes can provide a fast occlusion (Will and van Bel, 2006; Will *et al.*, 2013). Furthermore, oxylipin (Benning *et al.*, 2012), indole-glucosinolates (Zhu-Salzman *et al.*, 2005; Kim *et al.*, 2008) or antibiosis inducing factors (Pegadaraju *et al.*, 2007) can be utilized as defenses.

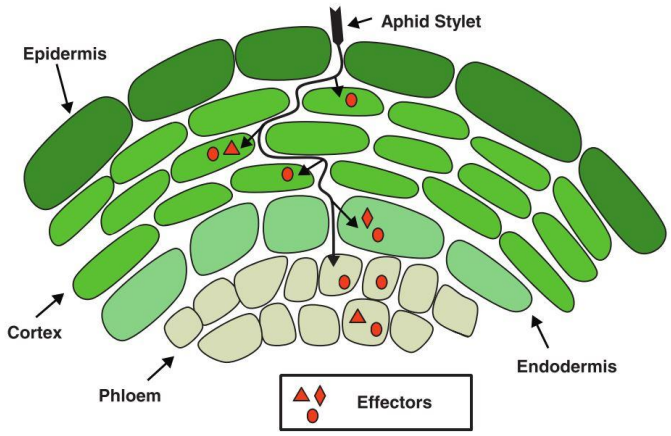

Fig. 3-4: Aphids navigating their stylet in between the plant cells. Depicted is a cross-section of an *A. thaliana* flower stalk. On the way to the phloem the aphid penetrates the cells with its stylet to test for cell composition and the exact location of the stylet in the plant. During this process saliva is secreted which contains effectors that help facilitate infestation but may also be detected by the plant and lead to a defense response. Picture taken from Elzinga and Jander (2013).

Phytohormones are generally induced as signaling compounds in response to stress. Commonly jasmonic acid (JA) accumulation is associated with herbivory and SA induction in response to biotrophic pathogens. However, there is a lot of research into the exact role of SA in plant-aphid interactions. Whereas the infestation of *A. thaliana* by *M. persicae* is known to increase the level of SA, its direct influence on the aphids is currently under debate (Morkunas *et al.*, 2011; Louis and Shah, 2013). Moran and Thompson (2001) reported that an increase in SA tends to result in a decrease of aphid fecundity. However, different studies have shown that SA may act as an antagonist to JA, thus, preventing the plant from effectively inducing defense reactions. Therefore, an aphid induced increase in SA concentration may function as a decoy (Mewis *et al.*, 2005; Pegadaraju *et al.*, 2005). JA signaling in response to aphid infestation leads to an increase of defense compounds such as indole-glucosinolates and camalexin (Zhou *et al.*, 1999; De Vos and Jander, 2009). Furthermore, Pegadaraju *et al.* (2005) observed that *M. persicae* infestation leads to chlorophyll degradation and subsequently leaf senescence in *A. thaliana*. Interestingly De Vos *et al.* (2005) have found that neither pigmentation nor SA levels are affected by *M. persicae* feeding. This contradiction highlights the discrepancies found in aphid research. Aphids can also suppress the defense reaction of a plant. Thus, they can feed from their host plants in an undisturbed manner. The deciding factors for this are the defense elicitors in the secreted saliva (De Vos and Jander, 2009).

3.3 Hypothesis

The impact of aphid infestation on plants and their defense system has been widely studied (Louis and Shah, 2013). Also different fecundity assays have been executed to investigate e.g. the influence of drought stress, CO_2, phytohormone overexpression etc. on the vitality of aphids (Mewis *et al.*, 2012). Despite numerous studies on plant-aphid interactions, no one looked at the effect of aphids on the metabolism of isoprenoid precursor biosynthesis. Experiments with *Spodoptera littoralis* revealed that the MEP metabolites are decreased whereas MEcDP accumulates and is exported out of the chloroplast in a defensive reaction (unpublished data). Therefore my Master's thesis investigates "The influence of *Myzus persicae* infestation on the MEP pathway in *Arabidopsis thaliana*" in the context of previously found plant responses.

The MEP pathway is closely connected to both pigment biosynthesis and photosynthesis. *M. persicae* (from here on also referred to as aphid) has been reported to influence the pigment level of the infested plants due to induced leaf senescence. Therefore, I expected that the photosynthesis and the MEP pathway will be influenced by aphid feeding. Based on these reports I hypothesize that *Myzus persicae* feeding will result in:

- A reduction in chlorophyll levels. Consequently, the photosynthetic rate is expected to be decreased, as well.
- The MEP pathway metabolites are decreased upon infestation by *M. persicae*. But, MEcDP is increased and exported out of the chloroplast as a retrograde signal.

Xiao *et al.* found, that MEcDP export leads to an increase in SA (Xiao *et al.*, 2012). This increase of SA levels has also been found during several plant-aphid interactions. Thus, it is expected that:

- The SA concentration is increased, whereas the concentration in JA is decreased.

If the accumulated data is in agreement to the named hypotheses new insights into the MEP pathway as an additional defense inducing system will be gained.

4 Materials and Methods

4.1 Materials

4.1.1 Buffers

Chemicals used for the buffers were supplied by Sigma Aldrich (Steinheim, D) and Roth GmbH and Co. KG (Karlsruhe, D) in analytical grade quality, if not noted otherwise. All buffers listed below (see Tab. 4-1) were prepared with deionized and filtered water acquired from a Milli-Q integral water purification system (pore size: 0.22 µm, Merck Millipore, Darmstadt, D). Solvents used for LC-MS/MS or HPLC-UV/Vis were supplied by VWR PROLABO BDH chemicals (Leuven, B) in analytical grade quality.

Tab. 4-1: List of buffers and their composition.

Name	Composition
DXS assay buffer	50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 10 % glycerol; to be added fresh: 2.5 mM DTT, 1 mM TPP, 10 mM sodium pyruvate, 10 mM glyceraldehyde-3-phosphate, phosphatase inhibitor (2 mM imidazole, 1 mM NaF, 1.15 mM molybdate), 1 % plant protease inhibitor (Sigma-Aldrich)
Protein (DXS) extraction buffer	50 mM Tris-HCl (pH 8.0), 10 % glycerol, 0.5 % Tween 20, 1 % w/v PVPP insoluble, phosphatase inhibitor (2 mM imidazole, 1 mM NaF, 1.15 mM molybdate); to be added fresh: 10 mM DTT, 1 mM ascorbic acid, 100 µM TPP, 1 % plant protease inhibitor cocktail (Sigma-Aldrich)
MEP metabolite analysis solvent A	20 mM NH_4HCO_3 (from 200 mM NH_4HCO_3 pH 10.5; adjusted with NH_4OH)
MEP metabolite analysis solvent B	20 mM NH_4HCO_3 (from 200 mM NH_4HCO_3 pH 10.5; adjusted with NH_4OH) in 80 % ACN
MEP metabolite extraction buffer	5 mM NH_4Ac in 50 % ACN (pH 9.0; adjusted with NH_4OH)
Phytohormone analysis solvent A	0.05 % formic acid in deionized water
Phytohormone analysis solvent B	100 % acetonitrile

Tab. 4-1: List of buffers and their composition; continued from previous page.

Name	Composition
Phytohormone extraction buffer	100 % MeOH spiked with 40 ng μL^{-1} D_2JA, D_4SA, D_6ABA; 8 ng μL^{-1} $^{13}C_6$JA-Ile
Pigment analysis solvent A	1 mM $NaHCO_3$
Pigment analysis solvent B	100 % acetone
RNA-loading dye	60 % Glycerol, 10 mM Tris-HCl (pH 7.6), 60 mM EDTA (pH 8.0), 0.03% bromophenol blue, 0.03 % xylene cyanol
ROS bleaching solution	60 % EtOH, 20 % acetic acid, 20 % glycerol
ROS staining solution (protect from light)	Add 50 mg DAB to 45 mL water and adjust with HCl to pH 3.0; then add 25 µL Tween 20 and 2.5 mL 200 mM Na_2HPO_4; (final concentration: 1 mg mL^{-1} DAB in 0.05 % v/v Tween 20 and 10 mM Na_2HPO_4)
1 x Tris-borate-EDTA (TBE) buffer	90 mM Tris-HCl (BioRad), 90 mM boric acid, 2 mM Na_2EDTA (from 0.5 mM pH 8.0)

4.1.2 Standards

To quantify the investigated analytes each analysis was executed with its own set of standards. The composition and final concentration of each standard can be gathered from Tab. 4-2 shown below.

Tab. 4-2: Standards used for analysis and their composition; for external standards (std.) only the first concentration of the dilution series is shown.

Analysis of	Composition and final concentration
MEP metabolite DXP (internal std.)	0.5 ng μL^{-1} [3,4,5-$^{13}C_3$] DXP (a kind gift from F. Rohdich, A. Bacher and W. Eisenreich from the Technical University Munich)
MEP metabolites (external std.)	0.3 ng μL^{-1} DXP (Sigma Aldrich), 0.89 ng μL^{-1} MEP, 1.08 ng μL^{-1} MEcDP, 0.94 ng μL^{-1} HMBDP (Echelon Biosciences, Salt Lake City, USA), 1 ng μL^{-1} DMADP (Sigma Aldrich); in MEP metabolite extraction buffer
MEP metabolites (internal std. for labeled analysis)	0.5 ng μL^{-1} DXP, 0.02 ng μL^{-1} MEP, 0.62 ng μL^{-1} MEcDP, 0.02 ng μL^{-1} HMBDP, 0.2 ng μL^{-1} DMADP
Phytohormones (internal std.)	40 ng mL^{-1} D_6JA, D_4SA (Sigma Aldrich), D_6ABA (Santa Cruz Biotechnology, Santa Cruz, USA); 8 ng mL^{-1} $^{13}C_6$JA-Ile (synthesized in house as described by Kramell *et al.* (1988)); in 100 % MeOH

Tab. 4-2: Standards used for analysis and their composition; continued from previous page.

Analysis of	Composition and final concentration
Pigments (external std.)	0.1 mg mL^{-1} chlorophyll a, chlorophyll b, β-carotene (Sigma-Aldrich), lutein (Roth GmbH and Co. KG); in 80 % acetone
Total ROS (external std.)	5 mg mL^{-1} H_2O_2 in 10 mM Tris-HCl pH 7.2
β-ionone (β-I) **and β-cyclocitral** (βCC) (internal std.)	0.02 ng mL^{-1} β-I (Alfa Aeser, Ward Hill, USA), 10 ng μL^{-1} β-CC (Sigma Aldrich) in 100 % MeOH

4.1.3 Machinery and Equipment

Tab. 4-3: Utilized machinery and equipment.

Machinery	Name/ Specifications	Company (Headquarters)
96 well plate qPCR	Hard-Shell® PCR plates 96 well, thin-wall	BioRad (Hercules, USA)
96 well plate ROS assay	F bottom crystal clear, Microeon D 200	Greiner bio-one (Frickenhausen, D)
Balance	PG 1003-S	Mettler Toledo (Columbus, USA)
Balance	BP3-OCEV1	Sartorius (Göttingen, D)
Cellophane bag	18.8 x 39 cm, air permeable	Armin Zeller, Nachf. Schütz & Co. (Langenthal, CH)
Centrifuge	5424; 5424 R; 5810	Eppendorf (Hamburg, D)
Chemiluminescence Measurement	Tecan, infinite 200	Tecan Austria GmbH 2005 (Grödig, A)
Climate Chamber	Growth chamber	York Refrigeration (York, USA)
Climate Chamber	AR-36L with 101 controller	Percival Scientific (Perry, USA)
Column XBridge amide	150 x 2; 1 mm, 2.5 µm	Waters (Milford, USA)
Column Zorbax Eclipse XDB-C18	50 x 4.6 mm, 1.8 µm	Agilent Technologies (Santa Clara, USA)
Column Supelcosil™ LC-18	7.5 cm x 4.6 mm, 3 µm	Supelco® Analytical Sigma-Aldrich (Steinheim, D)
Fine Balance	XP 26	Mettler Toledo(Columbus, USA)
Freeze Dryer	Alpha 1-4 LD plus	Christ (Osterode am Harz, D)
Gel documentation system	Gene Genius Bio Imaging System	Syngene (Cambridge, UK)
Heating block	ThermoMixerC	Eppendorf (Hamburg, D)
HPLC	1260 & 1290 Infinity	Agilent Technologies (Santa Clara, USA)

Tab. 4-3: Utilized machinery and equipment; continued from previous page.

Machinery	Name/ Specifications	Company (Headquarters)
Light sources	OsramL 36 W/77 Fluora	Osram GmbH (Munich, D)
Mass spectrometer	API5000 LC/MS/MS System	AB Sciex (Darmstadt, D)
Milli-Q® (0.22 µm filter)	Synthesis A10	Merck Millipore (Darmstadt, D)
Mixer	Vortex Genie 2™	Scientific Industries (Bohemia, USA)
NanoDrop	2000C Spectrophotometer	PEQlab Biotechnologie GmbH (Erlangen, D)
PCR system	GeneAmp® PCR System 2700	Applied Biosystems, ThermoFischer Scientific (Waltham, USA)
Portable Photosynthesis Measurement System	LI-6400XT	Li-Cor Biosciences (Lincoln, USA)
qPCR machine	CFX Connect™ Real-Time System	BioRad (Hercules, USA)
Shaker	KS 130 basic	IKA (Staufen, D)
Stuart rotator	SB3	Bibby Scientific Limited (Staffordshire, UK)
UV-Vis (HPLC)	Series 1100; Agilent	Hewlett Pachard/ Agilent Technologies (Santa Clara, USA)
Vacuum pump	Rotary Vane Vacuum pump RZ6	Vacuubrand GmbH + Co. KG (Wertheim, D)
Vial for HPLC analysis	Microvials PP; 0.3 mL with short thread	VWR International (Radnor, USA)

4.1.4 Software

For acquisition and analysis of the obtained data the following software programs have been used: Adobe Photoshop CS5 (64bit), Agilent ChemStation Rev. A.09.01[1206], Analyst Software 1.5, Bio-Rad CFX Manager 3.1, DataTrans, EndNote X7.2, GeneSnap, i-control 1.5, Microsoft Office 2010, SigmaPlot 11.0 and RStudio.

4.2 Methods

4.2.1 Plant Cultivation

All experiments were carried out with *Arabidopsis thaliana* Columbia wild type (Col-0) plants. For germination the seeds were sown directly into soil and kept in darkness at 4 °C for 72 h. The plants were grown and kept at 21 °C in short day conditions (10:14 hours light:dark) with a light intensity of about 80 to 100 $\mu mol\ m^{-2}\ s^{-1}$. Soil pots used for germination and growth of *A. thaliana* were sized 7 x 7 x 8 cm. For aphid rearing plants were grown in round pots (10 cm diameter).

4.2.2 Rearing of *Myzus persicae*

The generalist *Myzus persicae* was obtained from the in house culture of Dr. G. Kunert from the Biochemistry department at the Max-Planck-Institute for chemical ecology in Jena (Kunert *et al.*, 2010). The aphids were reared at a temperature of 21 °C in long day conditions (16:8 hours light:dark) on wild type (WT) *A. thaliana* of the Col-0 genotype. The plants were watered as little as possible to increase the stress (Khan *et al.*, 2010) and to reduce the chances of a fungal infestation. For experiments adult aphids were isolated on a fresh WT plant and removed after 48 h. Five days after removing the adults, the synchronized nymphs (about 3rd instar) were used to infest the experimental plants. To prevent the aphids from escaping the plants were covered with transparent, air-permeable cellophane bags (18.8 x 39 cm).

4.2.3 General Experimental Set-up

Six weeks old rosette *A. thaliana* plants were infested with 40 aphids (about 3rd instar) and covered by cellophane bags which were fastened with an elastic band. Non-infested plants were bagged similarly as infested plants and used as control. The plants were then exposed to a light intensity of 100 $\mu mol\ m^{-2}\ s^{-1}$, in short day conditions, for a given time period. The harvest of plants was always executed between 10 a.m. and 4 p.m. Before harvesting, all

aphids were removed and the control plants mock-examined. However, labelled plants were harvested directly after stopping the $^{13}CO_2$ airflow. The leaves intended for local staining of reactive oxygen species (ROS) accumulation were also harvested without previous removal of the aphids. Aerial parts of the rosette plants were immediately submerged and frozen in liquid nitrogen (supplied by Linde Group, Pullach, D). Subsequently, the samples were homogenized under liquid nitrogen. The powdered tissue was weighed in batches as required into plastic containers precooled with liquid nitrogen and stored at -80 °C. Previous to MEP pathway metabolite extraction the tissue was dried by a vacuum freeze dryer at -76 °C and 10^{-4} mbar.

4.2.4 Photosynthesis Measurements

Photosynthesis was measured with an LI-6400XT Portable Photosynthesis System as described by Wright *et al.* (2014). The photosynthetic activity was measured between 10 a.m. and 4 p.m. Fig. 4-1 shows the complete set-up used during measurements. A custom-built *A. thaliana* cuvette (Loivamäki *et al.*, 2007) was used for measuring the photosynthetic rate of the complete plant. The air was humidified by a bubbler and dew point controller. For this, two wash bottles partially filled with water were connected in series to the air inlet of the Li-Cor System. A water bath cooled the second bottle and passing air down to 10 °C. The final water content was adjusted with the DRIERITE® desiccant (ACROS organics, ThermoFischer Scientific). To stabilize the humidity within the cuvette and minimize CO_2 emission through soil respiration, the plant pot and soil were wrapped with plastic foil. The soda lime absorber (Sigma-Aldrich) filtered all CO_2 introduced through ambient air. Subsequently, a CO_2 cartridge (LISS Kft., Répcelak, H) and a flow controller introduced a defined amount of CO_2 to the plant cuvette. The total CO_2 concentration was measured within the infra-red gas analyzer (IRGA) via infra-red spectroscopy. An external light source supplied light with an intensity of 140 µmol m^{-2} s^{-1}. With a fan the air was constantly mixed in the cuvette and the temperature kept at room temperature (21 °C) through a Peltier element. The settings used can be viewed in Tab. 4-4. Each plant was kept in the cuvette and the gas exchange parameters were measured until the photosynthetic rate was stable. For the final rate analysis 30 consecutive data points of the stable phase, logged every 5 s, were averaged.

Tab. 4-4: Settings used during photosynthetic rate measurements.

Light intensity	140 µmol m^{-2} s^{-1}
Air flow	500 µmol s^{-1}
Supplied CO_2 concentration	400 µmol mol^{-1}
Supplied humidity	17700 – 17900 mmol mol^{-1}
Cuvette temperature	21 °C

To determine the leaf area of the plant, a photograph of each measured plant and a reference square was taken before measurements. For the normalization of the photosynthetic rate to the according leaf area of the plant, the area was imaged and measured with Adobe Photoshop CS5.

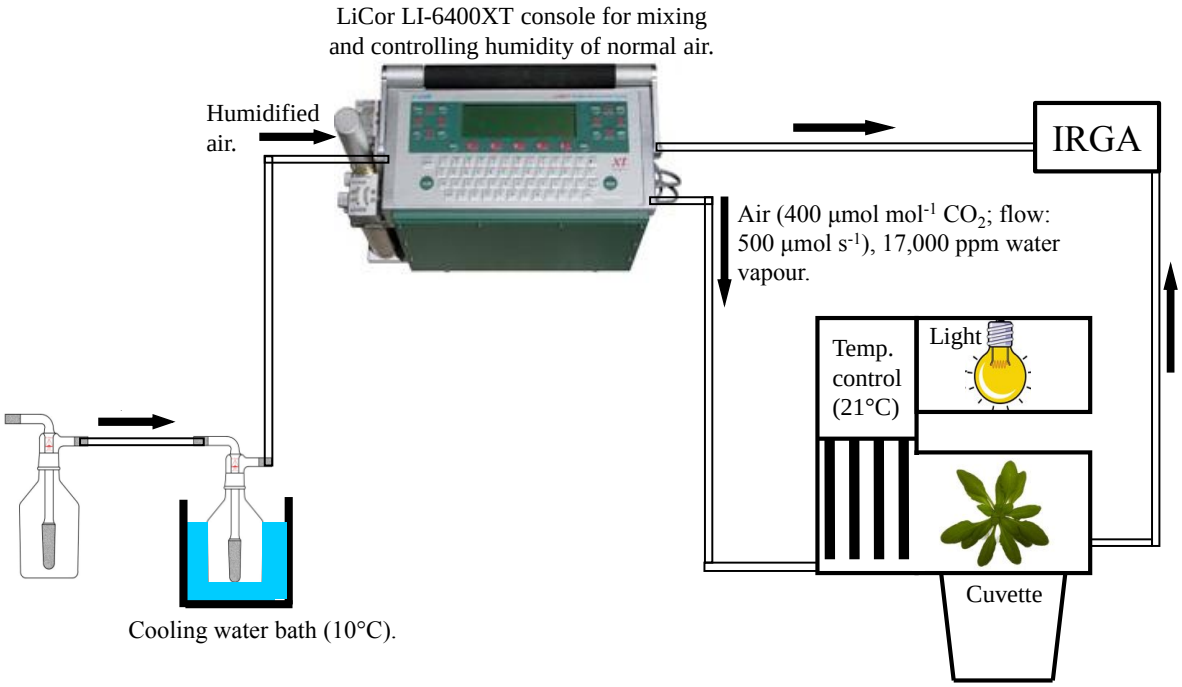

Fig. 4-1: Simplified model of the set-up for photosynthesis measurements. A cooling water bath was used to control the humidity. In the console the ambient air composition was controlled using desiccants to remove excess water vapor and CO_2 after which CO_2 levels were adjusted by injecting pure CO_2 using a flow controller. The airstream (flow of 500 µmol s^{-1}) was split into a reference and a sample to determine the amount of CO_2 assimilated and water respired in the plant cuvette. The light intensity (140 µmol m^{-2} s^{-1}) and temperature (21 °C) were tightly controlled in the cuvette and the water vapor and CO_2 levels were measured with the infra-red gas analyzer (IRGA) connected to the outlet of the cuvette.

4.2.5 $^{13}CO_2$ Labeling

The $^{13}CO_2$ labeling was executed under similar conditions as photosynthetic rate measurements. As opposed to the commonly used air flow of 500 µmol s^{-1} the flow was changed to 550 µmol s^{-1}. An external flow meter was used to adjust the flow of the isotope containing gas to 0.8 liter per minute which equates to a flow of 550 µmol s^{-1} and contains 400 µmol mol^{-1} of $^{13}CO_2$. After an adjustment period with CO_2 from a cartridge with natural isotope abundance for 20 min the inlet was switched to the synthetic air containing $^{13}CO_2$. The plants were labelled for 30 min, harvested and immediately flash frozen in liquid nitrogen.

4.2.6 Pigment Analysis

Extraction

The following protocol was executed at 4 °C in semidarkness to protect the samples from direct light. The pigments were extracted from 100 mg powdered fresh tissue with 1.5 mL acetone. After six hours of incubation in complete darkness at 400 rpm and 4 °C the samples were centrifuged for five minutes at 2300 x g. 800 µL of the supernatant were transferred into a new tube and mixed with 200 µL Milli-Q water. A second short spin followed at 2300 x g and subsequently the solution was transferred into a brown HPLC vial.

Analysis

The samples were analyzed with an HPLC Agilent 1100 Series with a diode-array-detector (DAD) using a Supelcosil LC-18 column (7.5 cm x 4.6 mm, 3 µm). The sample (50 µL) was injected and separated on the column through a concentration gradient in the mobile phase (see Tab. 4-5) using a flow rate of 1.5 mL min^{-1}. Afterwards the chromatograms at 445 nm (carotenoids) and 650 nm (chlorophylls) were analyzed. A serially diluted external standard (see Tab. 4-2) was used to quantify the measured peak areas of the obtained chromatogram and 80 % acetone served as negative control. The peak area was extracted from the generated files with the DataTrans program and imported to an excel file. After evaluating the retention times with the applicable standards, the pigments were found to elute as shown in Tab. 4-6.

Tab. 4-5: Mobile phase composition during HPLC-UV/Vis; solvent A contains 1 mM $NaHCO_3$ and solvent B 100 % acetone.

Time point [min]	Solvent A	Solvent B
0	35 %	65 %
4	35 %	65 %
12	10 %	90 %
20	0 %	100 %
22	0 %	100 %
22.5	35 %	65 %
25	35 %	65 %

Tab. 4-6: Retention times of pigments on a Supelcosil LC-18 column; retention times were evaluated previously with the applicable standards.

Pigment	Retention time [min]
Neoxanthin	5.6
Violaxanthin	5.9
Lutein	9.3
Chlorophyll B	12.5
Chlorophyll A	13.1
β-Carotene	15.3

4.2.7 MEP Pathway Metabolite Analysis

Extraction

The extraction of the MEP pathway intermediates and products was done as previously described by Wright *et al.* (2014). The metabolites were extracted from 5 mg of freeze-dried, powdered tissue with 250 µL of an acetonitrile/ammonium acetate mixture (ACN: 10 mM NH_4Ac 1:1 v:v) by vortexing for 5 min. After centrifuging for 8 min at maximum speed (~20000 x g) the supernatant (200 µL) was transferred to a new tube. The pellet was resuspended in 250 µL of the ACN:NH_4Ac mixture and vortexed for 5 min. After a second centrifugation step (5 min, 20000 x g) the new supernatant (200 µL) was combined with the first and the pellet discarded. The pooled supernatant was fully evaporated under a constant stream of nitrogen gas at 40 °C The dried residues were dissolved in 100 µL of 10 mM NH_4Ac (pH 9.0) by vortexing for 5 min. Afterwards 100 µL of chloroform ($CHCl_3$) were added and the suspension vortexed once again for 5 min. Centrifugation for 5 min at

20000 x g separated the two phases. The aqueous phase (50 µL) was added to 50 µL ACN. After mixing by short vortexing the sample was centrifuged again. The supernatant (80 µL) was transferred to a vial with inlet and subsequently analyzed by LC-MS/MS.

Methyl-erythritol-glycosides (ME-glc) are products of MEcDP which can be found in the vacuole of the plant (Gonzalez-Cabanelas *et al.*, 2015). To extract these compounds 500 µL 60 % methanol (MeOH) were added to 5 mg powdered and freeze dried tissue and the samples were subsequently vortexed for 5 min. After centrifugation (20000 x g for 8 min) 400 µL of the supernatant were transferred to a new tube and centrifuged again for 5 min at 20000 x g. Subsequently, 100 µL of the new supernatant were analyzed.

Analysis

An XBridge amide column (2.5 µm, 150 x 2.1 mm) was used for separation of the analytes prior to MS/MS analysis. The guard column (2.5 µm, 20 x 2.1 mm) contained the same sorbent. For separation of the injected sample (1 µL) the gradient shown in Tab. 4-7 was applied with a constant flow rate of 0.5 mL min^{-1}. The settings applied before monitoring the precursor ion – product ion reactions via scheduled multiple reaction monitoring (MRM) are displayed in Tab. 4-8. It also shows the expected retention times and mass-charge-values (*m/z* values) for all MEP metabolites. Samples being analyzed for ME-glc were run on the same XDB-C18 column as phytohormones. However water and acetonitrile were used as mobile phase with a flow of 1.1 mL/min as shown in Tab. 4-7. The peak areas of the observed spectra were integrated with the Analyst Software.

Tab. 4-7: Mobile phase composition applied during LC-MS/MS used for MEP pathway metabolite analysis. **XBridge amide column**: solvent A contains 20 mM NH_4HCO_3 and solvent B 20 mM NH_4HCO_3 in 80 % acetone. **XDB-C18 column** (for ME-glc): solvent A contains water, solvent B 100 % acetonitrile.

XBridge amide column			**XDB-C18 column**		
Time point [min]	Solvent A	Solvent B	**Time point**	Solvent A	Solvent B
0	0 %	100 %	**0**	100	0
5	16 %	84 %	**2.5**	100	0
10	16 %	84 %	**5**	0	100
11	40%	60%	**7.5**	0	100
15	40 %	60 %	**7.6**	100	0
15.10	0 %	100 %	**10**	100	0
25	0 %	100 %			

Tab. 4-8: Retention times (RT) **of MEP pathway metabolites during LC-MS/MS, *m/z* values and utilized voltages for MEP pathway metabolite analysis.** For labelled MEP metabolites applies: each additional ^{13}C increases Q1 by 1 Da.

Compound	Q1 mass [Da]	Q3 mass [Da]	RT [min]	DP [V]	EP [V]	CE [V]	CXP [V]
DXP	212.95	96.9	6.7	-60	-10	-16	-15
MEP	214.97	78.9	7.5	-70	-10	-36	-11
IDP	244.92	78.9	6.3	-45	-10	-24	-11
HMBDP	260.93	78.9	7.8	-70	-10	-28	-11
MEcDP	276.96	78.9	5.6	-50	-10	-38	-11
ME	135.1	102.9	1.8	-60	-10	-14	-15
ME-glc	297.3	59	0.9 & 1.45	-120	-10	-46	-9

4.2.8 Phytohormone Analysis

Extraction

1 mL of the phytohormone extraction buffer spiked with internal standard (Tab. 4-2) was added to 100 mg powdered fresh plant tissue kept on ice. The samples were mixed for 5 min by vortexing and subsequently centrifuged at 4 °C for 20 min at 20000 x g. The supernatant was then transferred to a new tube and centrifuged again for 10 min. The supernatant was transferred to an HPLC vial and analyzed by LC-MS/MS.

Analysis

A Zorbax Eclipse XDB-C18 column (50 x 4.6 mm, 1.8 µm) was used to separate the phytohormones through liquid chromatography prior to analyzing the fragments on the MS/MS system via scheduled MRM (see Tab. 4-10). The gradient utilized to separate the different hormones contained in the injected 5 µL is shown in Tab. 4-9. 100 % MeOH was used as a negative control. The general set-up of the MS/MS and the expected retention times as well as the *m/z* values are listed in Tab. 4-10. The API5000 was run in negative ionization mode and the Analyst software was used for analyzing the obtained data.

Tab. 4-9: Mobile phase composition during LC-MS/MS used for phytohormone analysis, solvent A contains 0.05 % formic acid (FA), solvent B contains 100 % acetonitrile.

Time point [min]	Solvent A	Solvent B
0	95 %	5 %
0.5	95 %	5 %
9.5	42 %	58 %
9.52	0 %	100 %
11	0 %	100 %
11.1	95 %	5 %
14	95 %	5 %

Tab. 4-10: Retention times (RT) **and *m/z* values of phytohormones during LC-MS/MS; as well as utilized ionization voltage.**

Compound	Q1 mass [Da]	Q3 mass [Da]	RT [min]	DP [V]	EP [V]	CE [V]	CXP [V]
D_4-SA	140.93	97	5.9	-35	-8	-22	0
SA	136.93	93	5.9	-35	-8	-22	0
D_6-JA	215	59	7.4	-35	-9	-24	0
JA	209.07	59	7.4	-35	-9	-24	0
D_6-ABA	269	159.2	6.4	-35	-12	-22	-2
ABA	263	153.2	6.4	-35	-12	-22	-2
JA-$^{13}C_6$-Ile	328.19	136.1	8.6	-50	-4.5	-30	-4
JA-Ile	322.19	130.1	8.6	-50	-4.5	-30	-4
OPDA	290.9	165.1	10.7	-45	-12	-24	-2

4.2.9 Reactive Oxygen Species Analysis

Total ROS Estimation

For a complete extraction of ROS samples had to be kept on ice at all times. In a 2 mL tube 1 mL of 10 mM Tris-HCl (pH 7.2) was added to 100 mg ground fresh tissue. After inverting the samples once they were centrifuged at 12000 x g for 20 min at 4 °C. The supernatant was then transferred into a fresh tube and centrifuged for 10 min to remove all tissue. The supernatant (10 µL) was diluted with 400 µL 10 mM Tris-HCl (pH 7.2). For measurement the samples were distributed into a 96 well plate. The diluted supernatant was split into two batches of 200 µL each. Into one well per sample 2 µL of 1 mM 2,7-dichlorofluorescin

diacetate (DCFDA; Sigma-Aldrich) were added to a final concentration of 10 µM. The samples were then incubated for 10 min at room temperature in complete darkness. For measurement the Tecan system was used with settings as shown in Tab. 4-11.

Tab. 4-11: Instrument settings for Chemiluminescence measurements executed with the Tecan infinite 200 system.

Duration	10 s
Gain	Optimal
Number of flashes	5
Excitation	485 nm
Emission	530 nm

Local ROS Estimation

The accumulation of hydrogen peroxide (H_2O_2) was detected locally according to a protocol adapted from Daudi and O'Brien (2012). After infesting the plants for a given time period as described in 4.2.3 three of the most infested source leaves and three same aged leaves of the control plants were cut at the base and immediately submerged in 25 mL of freshly prepared ROS staining solution. As a negative control, one leaf per treatment was submerged in 10 mM Na_2HPO_4. For proper infiltration of the leaves, vacuum was applied gently for about 5 min. Afterwards the leaves were incubated in darkness under constant shaking for 6 h. Subsequently, the staining solution was replaced by the bleaching solution and the samples were boiled for 15 min at 95 °C in a water bath to remove all chlorophylls from the leaves. The tubes were opened under the fume hood and the old bleaching solution replaced with fresh solution. After 30 min of resting pictures were taken of the stained leaves and the leaves were then stored at 4 °C.

4.2.10 β-cyclocitral and β-ionone Analysis

β-cyclocitral (β-CC) and β-ionone (β-I) were extracted analogue to the phytohormones. Methanol (1 mL) was added to ~100 mg fresh tissue and this suspension was mixed by vortexing for 5 min. After centrifuging the samples for 20 min at 4 °C and at 20000 x g, the supernatant was removed and spun again for 10 min. Afterwards 200 µL of the new supernatant were transferred into an HPLC vial with inlet. Analog to the phytohormones the

prepared samples were separated on an XDB-C18 column with the same buffers (ACN and 0.05 % FA) before MS/MS analysis. The exact buffer gradient used with a flow of 0.5 mL min^{-1} can be seen in Tab. 4-12. At the expected retention time of 5.8 to 5.9 min the following *m/z* values were used for detecting β-CC: 153.191 → 109.0, precursor ion → production ion. β-I was expected to eluate after ca. 6.8 min and precursor ion → product ion *m/z* values of 193.276 → 175.0 have been used. Due to a contamination by, presumably, a plasticizer at an *m/z* transition of 193 → 109 for β-I this scan combination was excluded. The Analyst software has been utilized again to analyze the obtained chromatograms.

Tab. 4-12: Mobile phase composition during LC-MS/MS used for β-CC and β-I analysis. Buffer A contains 0.05 % formic acid (FA); buffer B contains 100 % acetonitrile.

Time point [min]	Buffer A	Buffer B
0	95 %	5 %
2	50 %	50 %
3	50 %	50 %
9	0 %	100 %
11	0 %	100 %
11.10	95 %	5 %
14	95 %	5 %

4.2.11 Transcript Abundance Analysis

RNA Extraction

All subsequent work was executed under RNase free conditions with filter pipette tips. A maximum of 100 mg fresh tissue per sample were weighed for RNA extraction. For the extraction the innuPREP Plant RNA Kit from Analytik Jena (D) was used. As described in the manufacturer's protocol, first the genomic DNA was removed and the RNA bound to the column matrix. Salts and other contaminants were removed during several washing steps. For the elution of RNA 30 µL RNase free water were added on top of the column and spun for 1 min at 6000 x g. This step was repeated once for optimal elution. The eluted RNA was immediately stored at 4 °C or at -80 °C for long term storage.

The integrity of the isolated RNA was visualized by agarose gel electrophoresis. RNA (2 µL) was mixed with 4 µL RNA loading dye and loaded on the 1 % agarose gel containing 200 ng mL^{-1} ethidium bromide (Roth) as staining agent. An electrical potential of 100 V was

applied for 20 min after the gel was submerged in 1 x TBE. The RNA was visualized under UV light and an image recorded with the Gene Genius Bio Imaging System and the concentration determined with the NanoDrop 2000C spectrophotometer. In the same step the purity of the RNA was monitored through the ratios of the absorption at different wavelength. A minimal ratio of 1.8 was accepted for protein contamination (260 nm / 280 nm) and chaotrophic salt and other contaminants (260 nm / 230 nm).

First-Strand cDNA Synthesis

The components for the cDNA synthesis were provided by Invitrogen (ThermoFischer Scientific). In a total volume of 20 µL 0.5 µg of the isolated RNA were transcribed into cDNA. For this the primer mix, consisting of oligo$(dT)_{20}$, dNTP Mix, 0.1% diethylpyrocarbonate (DEPC) treated water and RNA (see Tab. 4-13), was heated up to 65 °C for 5 min. Immediately afterwards the samples were chilled on ice for at least 1 min. The reaction mix, consisting of 5 x First Strand RNA Buffer, DTT, RNase OUT and SuperScript™ III reverse transcriptase, was added to the chilled samples. The reverse transcriptase PCR occurred for 60 min at 50 °C followed by an inactivation step for 15 min at 70 °C. All samples were diluted with 80 µL water to a final volume of 100 µL and stored at -20 °C until further use.

Tab. 4-13: Composition of reaction mix for first-strand cDNA synthesis.

	Component	Volume
Primer Mix	50 µM oligo$(dT)_{20}$	1 µL
	10 mM dNTP Mix (10 mM each)	1 µL
	DEPC water	11 – x µL
	0.5 µg RNA	x µL
Reaction Mix	5 x First Strand RNA Buffer	4 µL
	0.1 mM DTT	1 µL
	40 units μL^{-1} RNase OUT	1 µL
	200 units μL^{-1} SuperScript™ RT	1 µL
	Total	20 µL

RT-qPCR

The SsoAdvanced™ universal SYBR® Green supermix from BioRad has been used for the real-time quantitative PCR (RT-qPCR). Utilized primers can be viewed in Tab. 10-1. All components (see Tab. 4-14) except the cDNA were dispensed into the 96 well qPCR plate.

Subsequently the cDNA and nuclease-free water were added to a final reaction volume of 20 µL. Each plate contained as control at least one well without template per primer pair and a two times dilution series obtained from three non-infested replicates of the same time point. The Bio-Rad® real-time PCR system has been used in the SYBR® only mode. The thermal cycling protocol can be viewed in

Tab. 4-15. For all three tested transcripts the adenine phosphoribosyl transferase 1 (APT) transcript has been used as housekeeping gene since it is known for being expressed relatively constant (Moffat *et al.*, 1994).

Tab. 4-14: Set-up for real-time qPCR reaction.

Component	Volume	Final concentration
2 x SsoAdvanced™ universal SYBR® Green supermix	10 µL	1 x
Forward and reverse primers	1 µL each	250 nM
Template cDNA	4 µL	1 ng μL^{-1}
Nuclease free water	4 µL	–
Total	20 µL	–

Tab. 4-15: Thermal cycling protocol for real-time qPCR.

Step		Temperature	Time	Cycles
Activation		95 °C	5 min	1
Amplification	**Denaturation**	95 °C	15 sec	40
	Annealing/ extension & plate read	60 °C	30 sec	

4.2.12 DXS Enzyme Assay

The extraction of the DXP synthase (DXS) in a crude plant protein extract and the subsequent enzyme activity assays were performed as previously described by Wright and Phillips (2014).

Extraction

All mentioned steps were executed at 4 °C. Enzymes were extracted from 5 mg of lyophilized and powdered plant material with 1 mL DXS extraction buffer (see Tab. 4-1). The suspension was mixed with a Stuart Rotator for 15 min at 20 rpm. To achieve proper separation of

supernatant and plant material, the samples were centrifuged for 20 min at 20000 x g and 4 °C. Subsequently, the supernatant was transferred into a fresh tube.

Enzyme Assay

The crude enzyme extract (30 µL) was added to 70 µL of the DXS assay buffer (see Tab. 4-1) in a 2 mL tube. The assay suspension was incubated for 2 h at 25 °C. To stop the enzyme reaction 100 µL of chloroform were added and the samples mixed by vortexing vigorously. Phase separation was achieved by centrifugation at room temperature (21 °C) for 5 min at 20000 x g. 45 µL of the upper aqueous phase were transferred into an HPLC vial with inlet. 5 µL of the [3,4,5-$^{13}C_3$] DXP internal standard and 50 µL 100 % MeOH were added.

Analysis

Enzymatically produced DXP was analyzed similar to the other MEP pathway metabolites on the XBridge amide column. Gradient and flow rate corresponded to the general analysis shown in Tab. 4-7. However, used buffers had a pH of 10.0 as opposed to 10.5. An internal standard of [3,4,5-$^{13}C_3$] DXP was additionally used for quantification. The observed *m/z* scan combinations for DXP and $^{13}C_3$ DXP were 212.95/138.9 and 215.95/140.9, respectively. Other settings corresponded to the data shown in Tab. 4-8.

4.2.13 Statistical Analysis

For statistical analysis most data sets were tested for significance with SigmaPlot 11.0. Here Two Way ANOVA ($p \leq 0.05$) informed about statistical significant differences between each treatment. However, the data set of chlorophyll A had to be transformed with the natural logarithm, whereas SA and JA were square root transformed. For better handling data sets of ABA, OPDA, β-I, ME, MEcDP and IPP were analyzed in RStudio. ABA, β-I and MEcDP had to be transformed with the natural logarithm. IDP was square root transformed. Due to the strong but not significant increases (according to Two Way ANOVA) in the early time points of the MEP pathway metabolites they were additionally tested with One Way ANOVA independent of the factor time. Data is represented as mean ± standard error (SE).

5 Results of *M. persicae* Infestation

5.1 Effect on Photosynthesis

Pegadaraju *et al.* (2005) showed that *Myzus persicae* infestation induces chlorosis and leaf senescence in *Arabidopsis thaliana* Col-0. Thus, it was expected that the photosynthesis would be impaired after infestation, as well. *Spodoptera littoralis* has been found to cause a decrease in photosynthesis within one hour of infestation (unpublished data). Therefore, especially shorter time points have been investigated (see Fig. 5-1). The longest time point was investigated to prevent any later responses from being missed. The measurements were executed with a whole plant cuvette connected to an LI-6400XT Portable Photosynthesis System. In this partially open system ambient conditions were maintained.

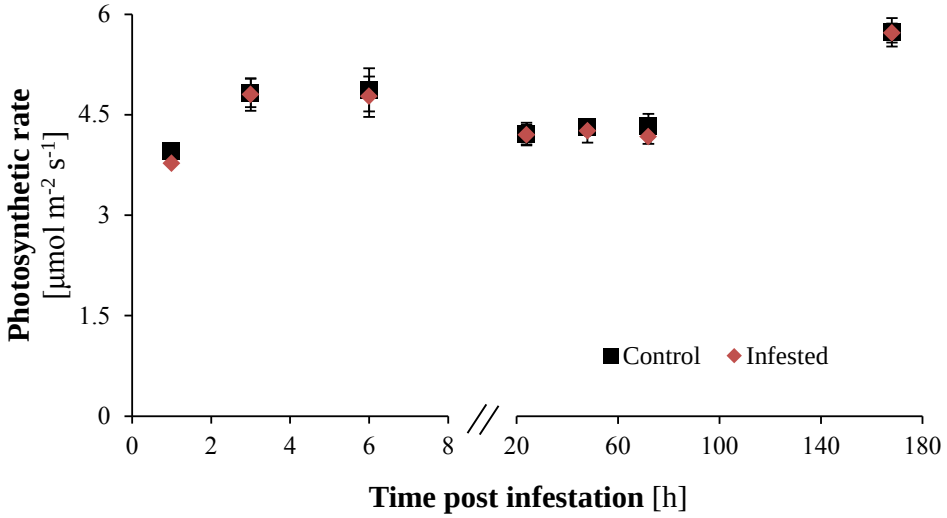

Fig. 5-1: *Myzus persicae* infestation does not alter the photosynthetic rate of *Arabidopsis thaliana*. 40 days old rosette plants were infested with 40 aphids each. Measurements were executed under the following conditions: light 140 µmol m^{-2} s^{-1} PPFD, CO_2: 400 mmol mol^{-1}, humidity: 170000 mmol mol^{-1}, air flow: 500 µmol s^{-1}. Measurements were taken 1, 3, 6, 24, 48, 72 and 168 h post infestation. Values are means of three replicates per treatment; error bars indicate the standard error (SE). The data was analyzed with Two Way ANOVA.

During the measurement of the photosynthetic rate it became obvious that there was no change after different periods of *M. persicae* infestation (Fig. 5-1). On the other hand, a difference in the photosynthetic rate has been observed between different sets of plants. This difference was bigger than the changes between infested and control plants. Therefore, it can be concluded that *M. persicae* does not influence the photosynthetic rate of *A. thaliana* in this experiment.

5.2 Effect on Pigmentation

A constant level of photosynthetic activity suggests that the photosynthetically relevant pigments are not affected by aphid infestation which stands in contrast to the expected decrease in pigmentation. Therefore, a direct investigation of the pigment levels during infestation was the next logical step.

The main pigments involved in photosynthesis are chlorophyll A and B (Caffarri *et al.*, 2014). No significant changes in their concentration have been observed upon aphid infestation of 40 d old *A. thaliana* plants (see Fig. 5-2 **A, B**). Thus, *M. persicae* infestation did not influence the chlorophyll levels.

Another pigment relevant to photosynthesis is β-carotene which also protects the plant from oxidative stress. The treatment did not significantly influence the β-carotene levels (see Fig. 5-2 **C**). However, the β-carotene level tends to increase after three hours of infestation with *M. persicae* (23 %). β-carotene is a pigment which is also used by the plant as an intermediate for the biosynthesis of e.g. violaxanthin and neoxanthin (Tanaka *et al.*, 2008). Despite the slight increase in their precursor after three hours of infestation neither violaxanthin nor neoxanthin showed increased concentration at this time point (see Fig. 5-2 **E, F**). However, a general influence of aphid attack has been observed for both ($p = 0.032$ and $p = 0.0269$ accordingly). Similar to chlorophylls *Myzus persicae* has no influence on these carotenoids.

Not only β-carotene is produced from lycopene but also α-carotene (Moehs *et al.*, 2001; Tanaka *et al.*, 2008). The downstream product lutein has been investigated to analyze this side of produced pigments. In contrast to other analyzed pigments an impact of aphid attack on the plant has been observed as presented in Fig. 5-2 **D**. Increases in lutein levels of the infested plants occurred after 6 h ($p = 0.018$) and 48 h ($p = 0.032$) of infestation. However, after 7 d of aphid cultivation the lutein level decreased in the infested plants ($p = 0.049$). This makes lutein the only significantly affected pigment.

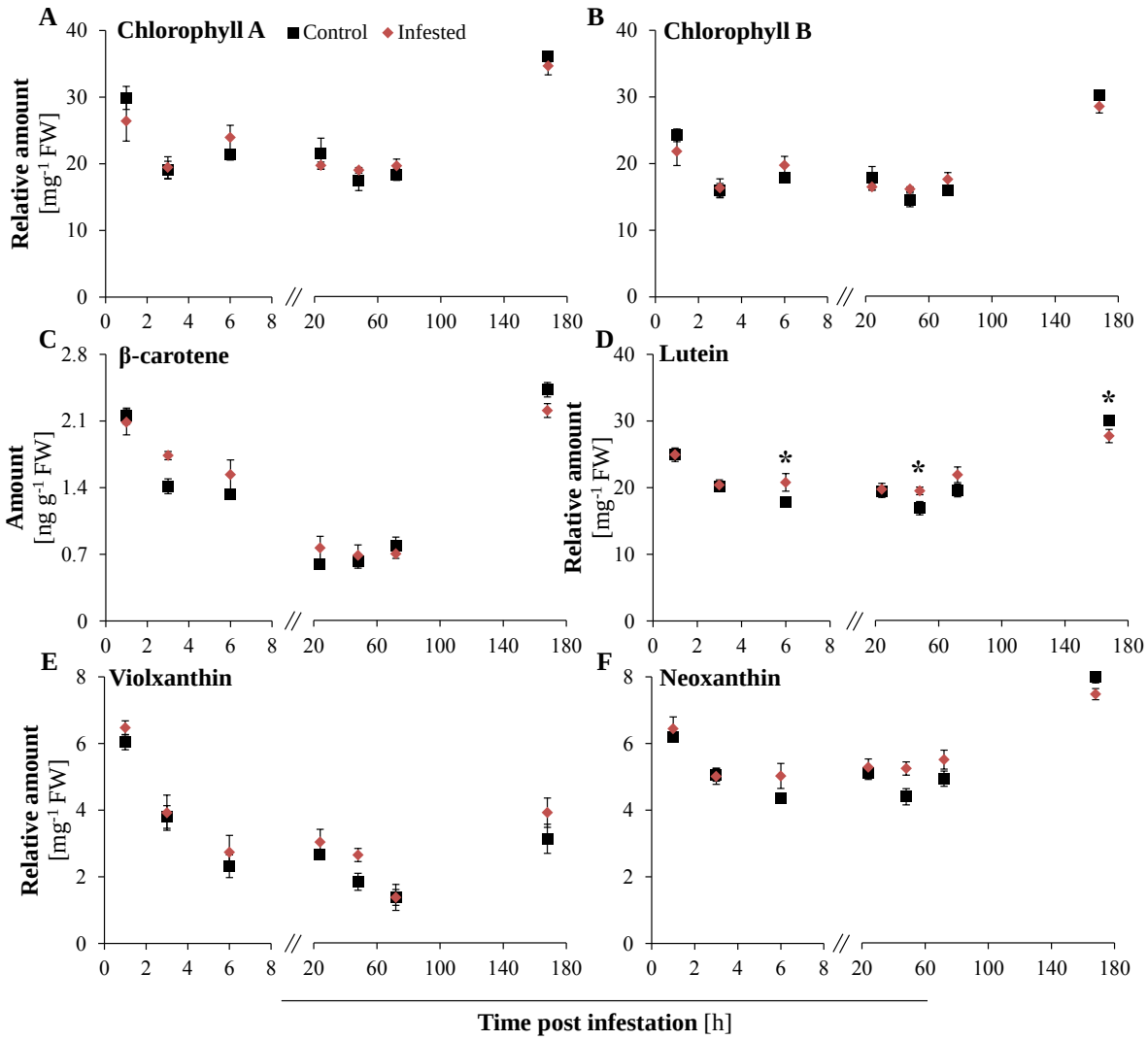

Fig. 5-2: Relative pigment levels after different periods of infestation. 40 days old *A. thaliana* rosette plants were infested with 40 aphids each. **A** and **B**: neither chlorophyll A nor chlorophyll B levels were affected by *Myzus persicae*. **C**: β-carotene shows a slight increase after 3 h but was not significantly affected. **D**: lutein levels changed significantly after 6 h ($p = 0.018$), 48 h ($p = 0.032$) and 168 h ($p = 0.049$) of infestation. **E** and **F**: violaxanthin and neoxanthin levels were not changed significantly. Values are means of four replicates per treatment; error bars indicate the standard error (SE). Asterisks represent significant differences at $p \leq 0.05$. The data was analyzed with Two Way ANOVA. FW – fresh weight.

5.3 Effect on MEP Pathway Metabolites

5.3.1 Metabolite Levels

Contrary to what was expected, neither the photosynthesis nor the pigment levels were affected by aphid infestation. The MEP pathway and, therefore, the production of DXP and other intermediates is closely connected to photosynthesis (Banerjee and Sharkey, 2014; Wright *et al.*, 2014). Since the photosynthetic rate stayed stable upon infestation by aphids (Fig. 5-1), the DXP concentration is expected to be unaffected. Yet, the analysis of the first three time points with One Way ANOVA revealed a significant increase by 30 % (0.032) 6 h post infestation (Fig. 5-3 **A**). Both of these results contradict the hypothesis about an infestation induced decrease of MEP pathway metabolites introduced at the beginning.

Changes in DXP concentration may lead to changes in the level of MEcDP. Moreover, MEcDP has been reported to be a regulator for the expression of nuclear stress-response genes through export out of the chloroplast and was therefore under special scrutiny (Xiao *et al.*, 2012; Gonzalez-Cabanelas *et al.*, 2015). In response to stress the change in its total concentration is independent of the rest of the MEP pathway metabolite accumulation (Xiao *et al.*, 2012; Wright *et al.*, 2014). Indeed, an increase of 42 % was observed three hours prior to the increase in DXP (Fig. 5-3 **B**). Unexpectedly neither this increase after 3 h nor the increase after 6 h (57 %) of *M. persicae* infestation was significant according to Two Way ANOVA. Therefore, these time points were tested with One Way ANOVA. This presented a significant effect of *M. persicae* on the metabolites after 1 h ($p = 0.035$) and after 3 h ($p = 0.035$) of infestation.

Metabolites found in the earlier steps of a metabolic pathway usually offer information about the concentration of later metabolites. Since DXP concentration did not change significantly upon attack no such changes were expected for IDP either. By contrast, increased levels of lutein (Fig. 5-2 **D**) suggested an increase in the precursor IDP. The lack of significant changes in IDP and DMADP levels after aphid infestation (Fig. 5-3 **C**) falsified the expectations of an increase. These results also contradict the starting hypothesis about a decrease in MEP pathway metabolites but support the data acquired by the measurement of the photosynthetic rate.

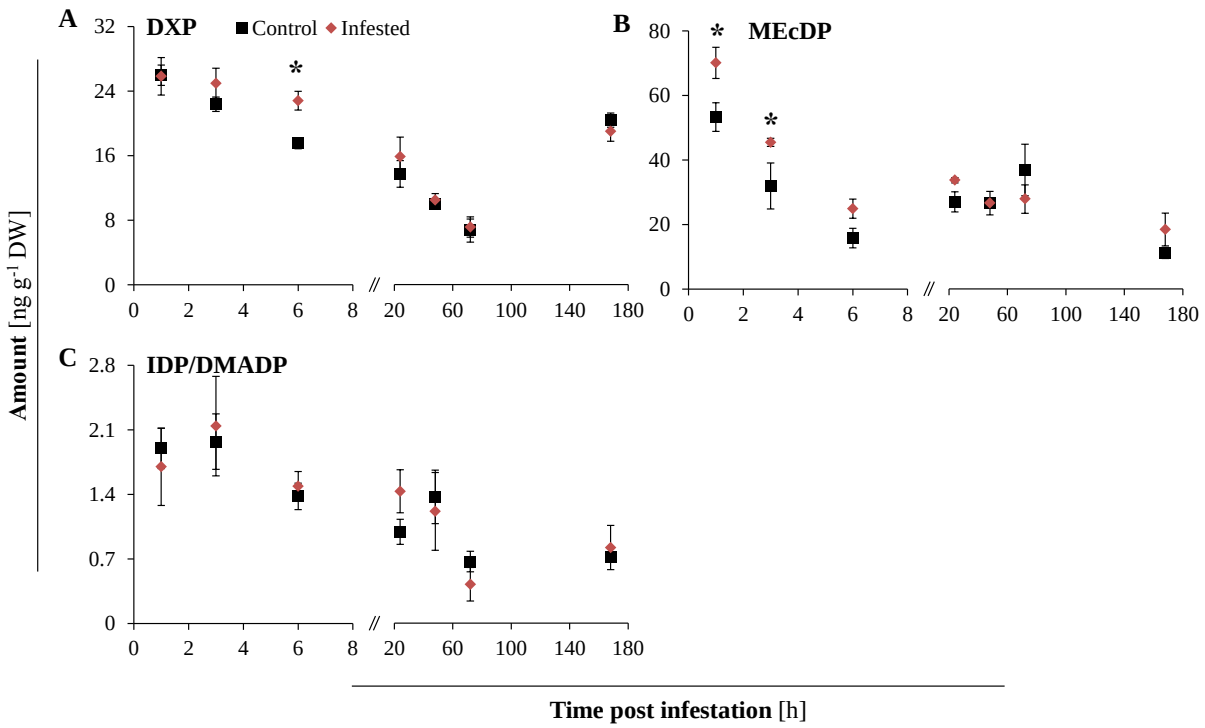

Fig. 5-3: MEP pathway metabolites were partially affected by *M. persicae* infestation. 40 days old *A. thaliana* rosette plants were infested with 40 aphids each. **A**: 1-deoxy-D-xylulose 5-phosphate (DXP) concentration increased after 6 h of infestation ($p = 0.032$). **B**: 2-*C*-methyl-D-erytrhitol 4-phosphate (MEcDP) concentration increased after 1 h ($p = 0.035$) and 3 h ($p = 0.035$) of aphid infestation. **C**: isopentenyl diphosphate/dimethylallyl diphosphate (IDP/DMADP) concentration did not change after aphid infestation. Values are means of four replicates per treatment; error bars indicate the standard error (SE). Asterisks represent significant differences at $p \leq 0.05$. The data was analyzed with Two Way ANOVA. However, due to strong increases, early time points of DXP and MEcDP were tested with One Way ANOVA independent of the factor time. DW – dry weight.

5.3.2 Involvement of Key-Enzyme DXS

The increases in DXP and MEcDP levels observed in the first six hours of aphid infestation left the need for a stronger argument concerning the significance of these observations. Therefore, the key enzyme of the MEP pathway has been investigated. This enzyme is the 1-deoxy-D-xylulose 5-phosphate synthase (DXS; Wright *et al.* (2014)). Long term regulation of enzyme abundance and, therefore, their products usually occurs via the regulation of the transcript level of a given enzyme. However, enzyme activity and the flux through the pathway can also be regulated directly in a contemporary manner.

The transcript abundance of the gene encoding DXS (*DXS*) was analyzed via RT-qPCR (Fig. 5-4 **A**) and revealed that there are no differences between treatments. It has been recognized that the transcript level of DXS does not necessarily represent the actual activity of this enzyme (Wright and Phillips, 2014; Wright *et al.*, 2014). Thus, an enzyme assay has been executed to test for a possible influence of *M. persicae* infestation on DXS activity in *A. thaliana*. The first two investigated sample sets (1 and 3 h of infestation) did not show any significant change in DXS activity (Fig. 5-4 **C**). However, after 6 h of aphid infestation the DXP synthase activity increased by 30 % ($p = 0.056$). This increase coincides with the increase of DXP after 6 h ($p = 0.032$) and points towards a weak influence of *M. persicae*.

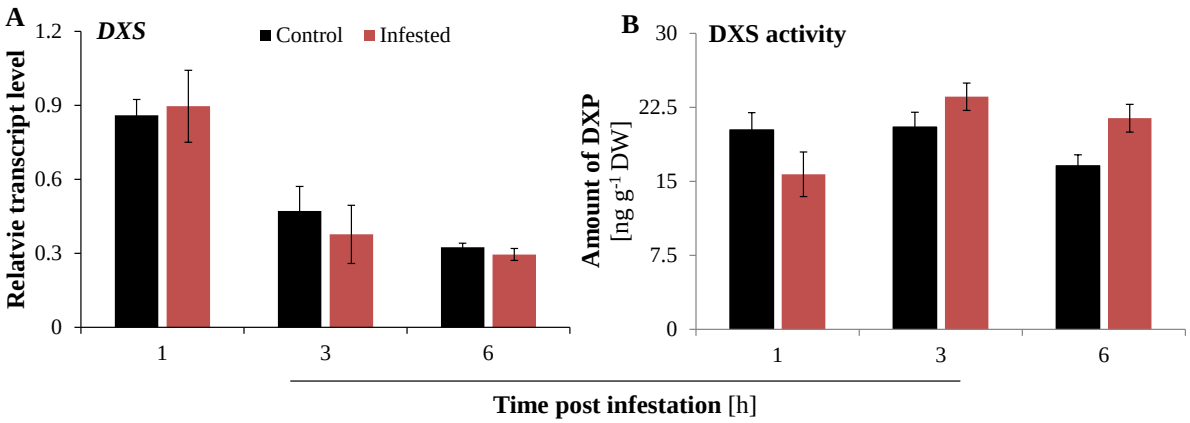

Fig. 5-4: The activity of the 1-deoxy-D-xylulose 5-phosphate synthase (DXS) but not the transcript abundance were affected by *M. persicae* infestation. 40 days old *A. thaliana* rosette plants were infested with 40 aphids each. **A**: *DXS* transcript levels are not affected by *M. persicae* infestation. **B**: DXS activity was analyzed through an enzyme assay. *M. persicae* infestation did not significantly influence the produced amount of DXP. Values are means of three replicates per treatment; error bars indicate the standard error (SE). The data was analyzed with Two Way ANOVA. DW - dry weight.

5.4 Phytohormone Responses

The increase in MEcDP concentration hinted towards an export of this compound. In response to abiotic stress this retrograde signal has been found to lead to an increased accumulation of the phytohormone salicylic acid (SA; Xiao *et al.* (2012)). Studies in recent years revealed that infestation of *A. thaliana* by *M. persicae* leads to an increase of SA accompanied by a decrease in the concentration of jasmonic acid (JA; Xiao *et al.* (2012); Sun *et al.* (2013)). Therefore, it was of interest to examine the influence of MEcDP on SA in this pathosystem.

Surprisingly, SA did not increase after different periods of infestation as expected (Fig. 5-5 **A**). On the contrary, the SA level tends to decrease by 40 % within one hour of *M. persicae* infestation. Therefore, the increase in MEcDP (~ 50 %) after infestation is not translated to an increase of SA. This result leads to the conclusion, that there is most probably no export of MEcDP and no production of ROS in the attacked tissue.

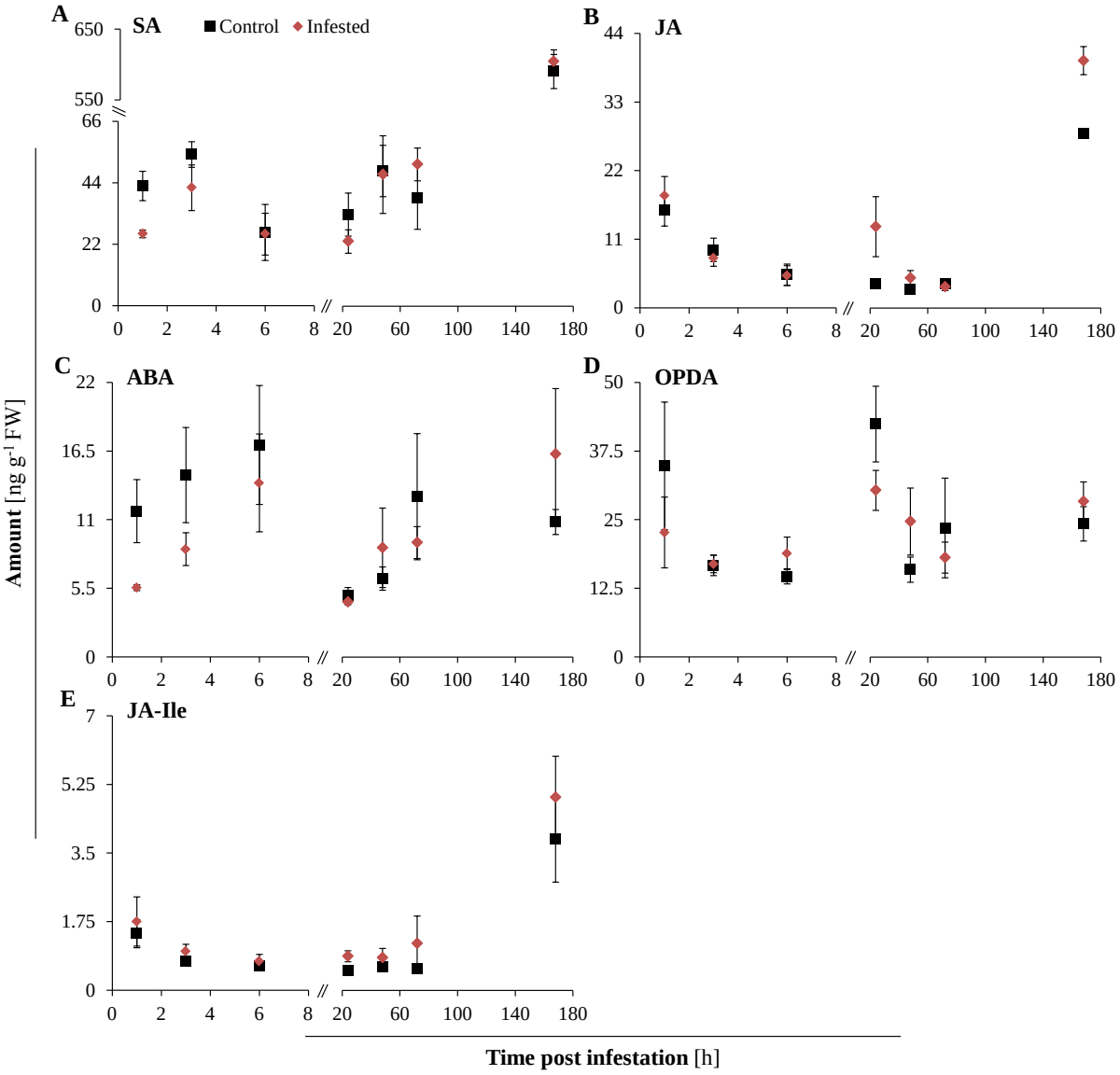

Fig. 5-5: Phytohormone levels were not affected significantly by *M. persicae* infestation. 40 days old *A. thaliana* rosette plants were infested with 40 aphids each. **A**: amount of salicylic acid (SA). **B**: amount of jasmonic acid (JA). **C**: amount of abscisic acid (ABA). **D**: amount of JA precursor 12-oxo-phytodienoic-acid (OPDA). **E**: The active form of JA, JA-isoleucine (JA-Ile), was not affected by aphid infestation. Values are means of four replicates per treatment; error bars indicate the standard error (SE). The data was analyzed with Two Way ANOVA. FW – fresh weight.

Furthermore, the concentration levels of the often antagonistically to SA acting JA are not increased either (Fig. 5-5 **B**). To check the possible influence of *M. persicae* on other components in the JA signaling system the precursor OPDA (12-oxo-phytodienoic acid) and the active compound JA-isoleucine (JA-Ile) were investigated as well (Fig. 5-5 **D, E**). Neither OPDA nor JA-Ile showed any significant changes in their concentration.

Abscisic acid (ABA) is a phytohormone which is known to act in response to draught stress and as a growth regulator (Hirai *et al.*, 2000; Nambara and Marion-Poll, 2005). *M. persicae* was observed to have a general influence on the precursors violaxanthin and neoxanthin, therefore the influence on ABA was of interest as well. However, the accumulation of ABA did not change after aphid infestation (Fig. 5-5 **C**). Therefore, it can be concluded that no water stress was induced by the aphids. In total no effect of *M. persicae* infestation on *A. thaliana* was observed at the phytohormone level.

5.5 Reason for Discrepancy in MEcDP and SA Levels

The results of the phytohormone response contradict the starting hypothesis about a defensive response. This leads to questioning the defense mechanism of *A. thaliana* to *M. persicae*: Is there indeed no response to the infestation or are the monitored responses simply blocked at an earlier stage? Despite the increase in MEcDP levels it is possible that it was not exported out of the chloroplast and, therefore, is not inducing the accumulation of SA through retrograde signaling. Another element involved in the accumulation of SA is the isochorismate synthase (ICS) (Yang *et al.*, 2015; Zheng *et al.*, 2015). Analyzing ICS transcript abundance to check for other influences will help to solve this puzzle.

5.5.1 MEcDP Export

Attack by chewing insects (unpublished data) or mechanical damage (Xiao *et al.*, 2012) has been found to increase the total amount of MEcDP. Subsequently, part of this MEcDP is exported into the cytosol and functions as stress response signal (Xiao *et al.*, 2012; Gonzalez-Cabanelas *et al.*, 2015). This led to the question whether *M. persicae* also induced an export.

Intra-Cell Distribution of MEcDP

The increase of MEcDP has been observed in the earlier stages, consequentially, a possible export was expected during the first three hours of infestation. To determine the distribution of MEcDP in the cell all MEP pathway intermediates were labelled with $^{13}CO_2$ fumigation. The second pool of MEcDP not present in the chloroplast and, hence, not directly involved in the MEP pathway will not be labelled during short fumigation periods. The result will be a relative decrease in ^{13}C incorporation detected in the total MEcDP pool, due to the dilution of label through the second unlabeled pool. The distribution of MEcDP did not differ between infested and non-infested control plants (Fig. 5-6 **A**, **B**). However, upon export of MEcDP dephosphorylation and subsequent glycosylation takes place, which were also analyzed for possible differences (Gonzalez-Cabanelas *et al.*, 2015).

Cytosolic MEcDP Degradation Products

Methyl-erythritol (ME) and ME-glucosides (ME-glc) were analyzed by LC-MS/MS as a second indicator towards the MEcDP export (Gonzalez-Cabanelas *et al.*, 2015). Neither ME nor one of the ME-glc isomers showed any change in their total concentration during the earlier time points of infestation (Fig. 5-6 **C**, **D**). This is in accordance with the results of the labeling experiment. However, 72 h post infestation the amount of ME was significantly decreased in the *M. persicae* infested plants ($p < 0.001$).

Myzus persicae does not seem to induce retrograde signaling via MEcDP in *Arabidopsis thaliana* during the earlier time points of infestation. This explains why SA did not increase in accordance to the MEcDP concentration. As a matter of fact, the SA levels seem to decrease after 1 h of infestation.

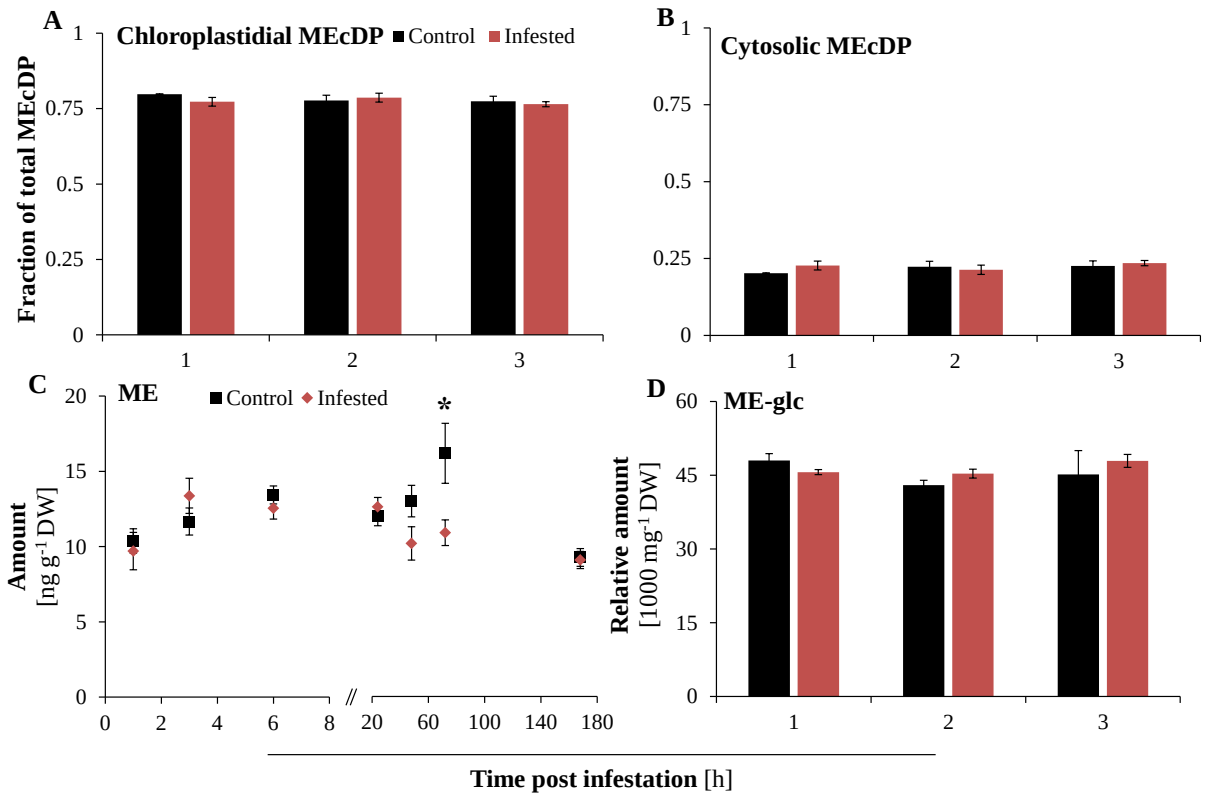

Fig. 5-6: MEcDP localization and cytosolic degradation products after *Myzus persicae* infestation. 40 days old *A. thaliana* rosette plants were infested with 40 aphids each. **A** and **B**: no increase in cytosolic MEcDP has been observed after aphid infestation. **C**: the amount of cytosolic dephosphorylated 2-*C*-methyl-D-erythritrol (ME) was significantly decreased after 72 h of aphid infestation ($p < 0.001$). **D**: the amount of the glucosylated form of (ME-glc) was not influenced by aphid feeding. Values of **A, B and D** are means of three replicates per treatment sampled during labeling of short term infested plants, values of **C** are means of four replicates per treatment; error bars indicate the standard error (SE). The data was analyzed with Two Way ANOVA. Asterisks represent significant changes. DW – dry weight.

5.5.2 Involvement of Isochorismate Synthase

To get a full picture on the interactions between MEcDP and SA levels the ICS transcript abundance has been investigated, as well. In accordance to the SA concentration the transcript levels of ICS were decreased significantly at two time points: after 1 h ($p = 0.017$) and 24 h ($p = 0.001$) of infestation (see Fig. 5-7). Yet, the drop in SA concentration was not as significant. This data indicates that the level of SA is not regulated by MEcDP export and that ICS transcript levels alone do not explain the detected SA amounts.

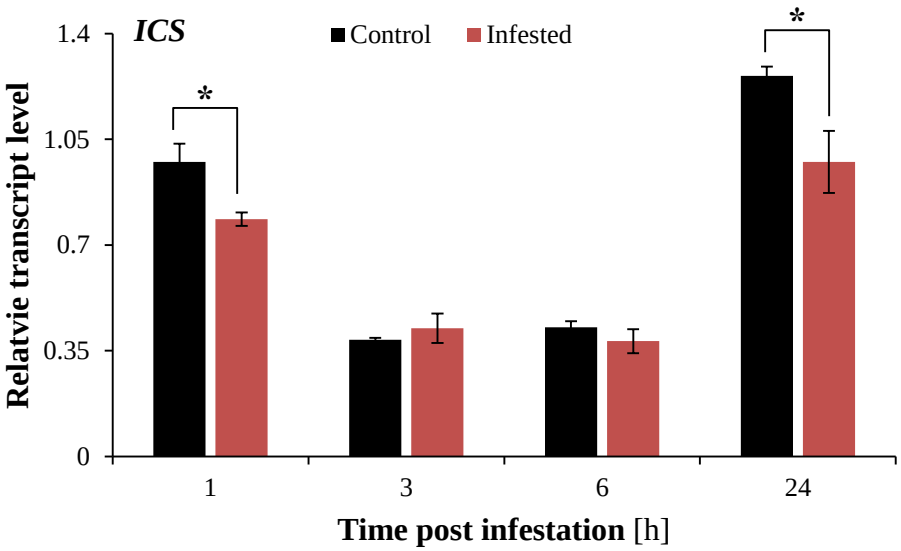

Fig. 5-7: ICS transcript levels were decreased after *M. persicae* infestation; 40 days old *A. thaliana* rosette plants were infested with 40 aphids each. A decrease in ICS (isochorismate synthase) transcript levels has been observed after 1 h ($p = 0.017$) and 24 h ($p = 0.001$) of infestation. Values are means of three replicates per treatment; error bars indicate the standard error (SE). The data was analyzed with Two Way ANOVA. Asterisks represent significant changes.

5.6 Involvement of an Oxidative Burst

Upon insect feeding, the plant usually produces reactive oxygen species (ROS) as a defense response (Thompson and Goggin, 2006; De Vos and Jander, 2009; Ren *et al.*, 2014). Those ROS can be scavenged by β-carotene, thus, producing β-cyclocitral (β-CC), which in turn can act as a signaling molecule (Ramel *et al.*, 2012; Lv *et al.*, 2015). This induces an increase in SA which mobilizes further defense responses. Subsequently SA can induce a decrease in the accumulation of ROS (Lv *et al.*, 2015). It has been discovered that ROS are involved in the increase of MEcDP concentration by inhibiting the 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate synthase (HDS) and the subsequent catalysis of MEcDP to HMBDP (Banerjee and Sharkey, 2014). Since SA did not increase, even though MEcDP showed an increase after aphid infestation, it was unknown if ROS played a role during infestation. Therefore, these molecules were investigated to better understand the absence of a SA mediated defense response in *A. thaliana* Col-0.

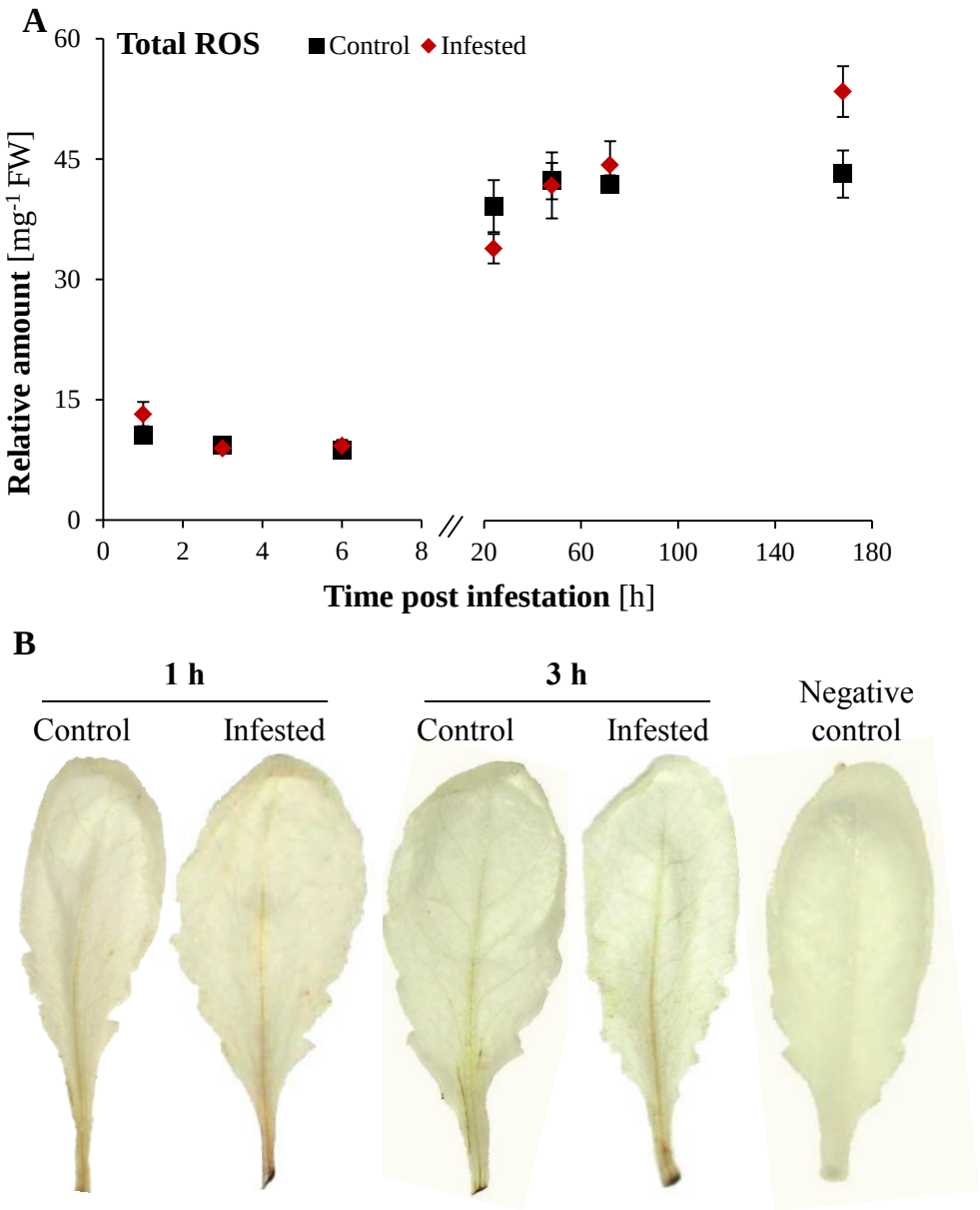

Fig. 5-8: ROS accumulation after different periods of infestation. 40 days old *A. thaliana* rosette plants were infested with 40 aphids each. **A**: values are means of four replicates per treatment; error bars indicate the standard error (SE). According to Two Way ANOVA aphid infestation did not significantly influence ROS accumulation. FW – fresh weight. **B**: 3,3'-diaminobenzidine (DAB) staining did not reveal any local accumulation of H_2O_2 after 1 and 3 h in infested leaves or at feeding sites. The negative control was treated as the other leaves but DAB was omitted from the staining solution.

5.6.1 Accumulation of Reactive Oxygen Species

Despite the slight increase of total ROS 7 days post infestation, the total amount of ROS did not change significantly after *M. persicae* infestation (Fig. 5-8 A). Additionally, the infested leaves were investigated locally for hydrogen peroxide (H_2O_2). Accumulation of H_2O_2 during an oxidative burst can be visualized by distinctive staining with 3,3'-diaminobenzidine (DAB). The stained leaves did not show any local accumulation of H_2O_2 (see Fig. 5-8 B), supporting the total ROS results. However, an early increase would have been expected in the presence of a defensive response (Kusnierczyk *et al.*, 2008). On the other hand it is possible that the ROS accumulation may not be detectable due to fast scavenging.

5.6.2 Accumulation of Oxidation Products β-cyclocitral and β-ionone

If the absence of detectable ROS is due to fast scavenging it can be expected that the ROS mediated degradation product of β-carotene, β-CC, will increase (Lv *et al.*, 2015). To validate this β-CC and β-ionone (β-I) were measured (Fig. 5-9). In agreement with the unchanged ROS levels detected in the investigated plants, neither β-CC nor β-I levels were significantly increased or decreased by aphid infestation. Therefore, the decrease in ICS transcript abundance cannot be explained by changes in ROS levels.

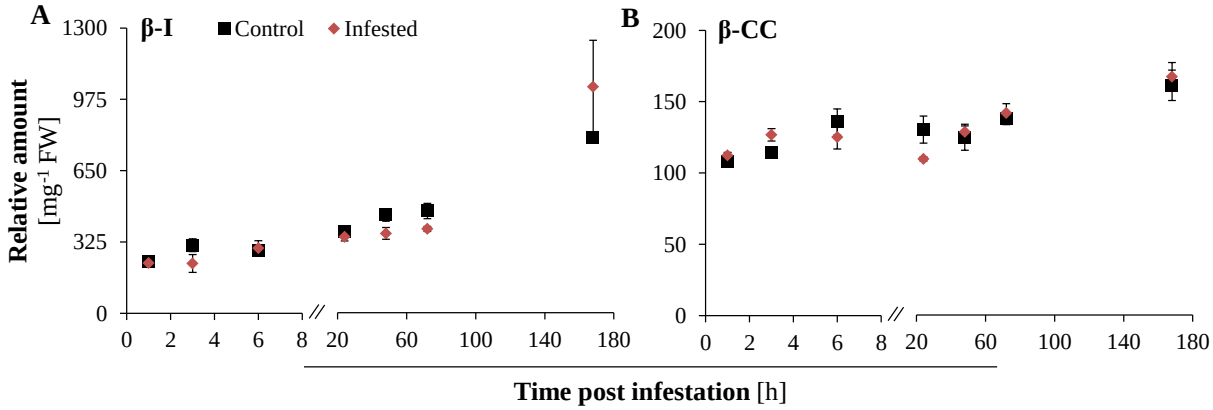

Fig. 5-9: β-CC and β-I were not affected by *M. persicae* infestation. 40 days old *A. thaliana* rosette plants were infested with 40 aphids each. **A**: relative amount of accumulated β-ionone (β-I). **B**: amount of accumulated β-cyclocitral (β-CC). Values are means of four replicates per treatment; error bars indicate the standard error (SE). The data was analyzed with Two Way ANOVA. FW – fresh weight.

5.6.3 Influence on HDS

ROS disrupts the function of HDS causing an increase in MEcDP and consequently an increase in SA (Xiao *et al.*, 2012). However, ROS accumulation has not visibly increased after aphid infestation, which is most likely the reason that no export of MEcDP took place. To check for the effect of other possible influences the *HDS* transcript level was investigated. As presented in Fig. 5-10, there is no significant change in *HDS* mRNA concentration at any of the investigated time points, thus, aphid infestation has no influence. However, the enzymes activity was not investigated and is still an unknown in plant-aphid interaction.

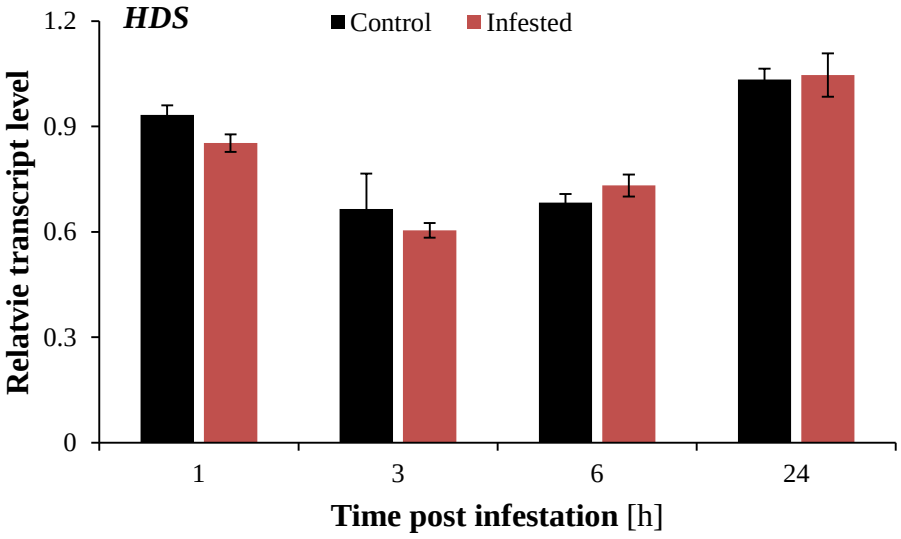

Fig. 5-10: 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate synthase (HDS) mRNA levels are not affected by *M. persicae* infestation. 40 d old *A. thaliana* rosette plants were infested with 40 aphids each. Values are means of three replicates per treatment. The data was analyzed with Two Way ANOVA. Error bars indicate the standard error (SE).

6 Discussion

6.1 Influence on Photosynthesis and Pigmentation

In the introduction a hypothesis was put forth based on the data of Pegadaraju *et al.* (2005) and Macedo and colleagues (2003). These groups had found that *Myzus persicae* induces chlorosis and subsequent leaf senescence in *Arabidopsis thaliana* (Pegadaraju *et al.*, 2005) and that the soybean aphid impairs photosynthesis in soybean (Macedo *et al.*, 2003). The consecutive hypothesis put forth proposed that *M. persicae* infestation of *A. thaliana* would lead to chlorosis and would, therefore, impair the photosynthesis, leading to a decrease in the MEP pathway metabolites. Yet, own experiments with *A. thaliana* and *M. persicae* showed no influence of infestation on photosynthesis. Successive analysis revealed the pigmentation to not be affected as much as expected. While especially the chlorophylls were expected to be degraded upon aphid attack, lutein levels were instead found to increase in infested plants.

The lack of the anticipated influence may be explained by the experimental set-up or different aphid clones. Pegadaraju *et al.* (2005) infested four weeks old *A. thaliana* Col-0 plants with 15 *M. persicae* each and observed leaf senescence after seven days. Additionally, during the rearing of *M. persicae* on *A. thaliana* no increased leaf senescence was observed even after four weeks of incubation on the same plant. Each host plant may react differently to infestation by the same aphid (e.g. *Aphis craccivora* on *Vigna unguiculata* or *V. sesquipedalis*; Shannag *et al.* (1998)). This usually results in an adaptation of the aphid to the host plant for higher vitality and fecundity if they live on the same host for several generations. An extreme example for distinct biotypes is the family of pea aphids which possesses different host races known to cope differently on the same plant, while each has its optimal host (Peccoud *et al.*, 2015). Pegadaraju and colleagues used different plants of the family of Brassicaceae (*Brassica juncea* and *Raphanus sativus*) for rearing the aphids. This avoided a possible adaption to *A. thaliana* previous to their experiments. Contrary to Pegadaraju *et al.* we cultivated *M. persicae* on *A. thaliana* which might have resulted in host adaptation and consequently decreased or aborted plant defense through leaf senescence. Furthermore, De Vos *et al.* also received different results from Pegadaraju and others after rearing their *M. persicae* on *Brassica chinensis* (De Vos *et al.*, 2005). These studies support

the hypothesis that the discrepancies in reported observations were caused by different *M. persicae* biotypes/clones that may have a mutation in effectors involved in inducing certain plant responses. One solution to reveal different biotypes would be a genomic comparison between the clones used by the different groups.

Independent of the insect's biotype the plant can detect the attack through different effectors secreted by the aphid during the feeding process. The effector Mp10 was found by Bos *et al.* (2010) to be responsible for leaf senescence by inducing a secondary sink upon recognition by the plant (Bos *et al.*, 2010). Induction of the secondary sink is accomplished by the plant through redirecting carbon into starch (Louis and Shah, 2013). Loss or mutation of Mp10 might explain the lack of leaf senescence and involved changes in leaf pigment composition, which would consequently explain the unaltered levels in chlorophyll.

Chlorophylls are the main pigments involved in photosynthesis and as such they need strong protection against oxidation, therefore, pigments involved in this protection were also investigated. No changes were observed in most of these pigments, despite the expected oxidative burst induced through aphid feeding. Interestingly, the level of lutein did increase during the experiment. Just as the other xanthophylls it is involved in photoprotection (Jahns and Holzwarth, 2012). More precisely lutein-5,6-epoxide (Lx) is involved in non-photochemical quenching (García-Plazaola *et al.*, 2007). During this process it is transformed into lutein, thus, the increase of lutein observed after six and 48 hours of aphid infestation may be explained through a small increase of excited triplet chlorophyll (^{3}Chl*; Jahns and Holzwarth (2012)). Although the synthesis of Lx from lutein is not yet known (García-Plazaola *et al.*, 2007; Tanaka *et al.*, 2008), it might explain the decrease of lutein after seven days of *M. persicae* infestation. Due to a lack of ^{3}Chl* and the continuous production of Lx from lutein Lx levels increase. Given this line of thought infested plants appear to be more susceptible towards light induced damage during the early stages of infestation and are, therefore, in greater need of photoprotective processes. In support of this other pigments involved in photoprotection showed a similar pattern of increase and decrease as lutein, thus, a repetition of the experiment with more replicates is of importance to better understand the possible involvement of the pigments.

6.2 Influence on the MEP Pathway

This thesis was started with the hypothesis that photosynthesis and pigment levels would be impaired by aphid infestation. Consequently, the amounts of MEP pathway metabolites were expected to be decreased, whereas MEcDP would be increased and used for defense signaling. The unaffected photosynthesis and even increased pigment levels were in contrast to this hypothesis. Seeing that the MEP pathway derived IDP and its isomer DMADP are important precursors for the production of isoprenoids and for pigment synthesis (Lichtenthaler, 2010), they were now expected to also behave in contrast to the starting hypotheses. In agreement to this IDP/DMADP not significantly influenced by aphid infestation. The increase in DXP and MEcDP after aphid infestation was in compliance with the investigated pigment levels.

At certain metabolic conditions of the plant the synthesis of chlorophylls, carotenoids and ABA may be controlled by the DXP synthase (Lindgren *et al.*, 2003; Ducreux *et al.*, 2005; Taylor *et al.*, 2005; Wright *et al.*, 2014), therefore, this enzyme was investigated. The measured DXS transcript abundance did not change in infested plants, yet, the activity of the enzyme was slightly increased after six hours of infestation. This coincides with the increase in DXP and shows a connection to the pigment levels, thereby proofing a weak influence of *M. persicae*. However, *Myzus persicae* is known to be the vector to no less than 50 different types of viruses (Blackman and Eastop, 2007). Most recently the HC-pro protein of the potato virus Y (PVY) has been reported to interact with DXS in *Nicotiana benthamiana* (Li *et al.*, 2015). This interaction leads to an increase of DXS activity five days post infection. For this thesis, no tests were done to exclude a viral infection vectored by *M. persicae*. Therefore, the observed results may have been influenced by viral infection. A possible way to exclude a viral transmission would be a PCR test for known viral promotors and see whether the aphids are contaminated or not (Li *et al.*, 2015).

MEcDP levels changed in a similar pattern as DXP whereas IDP/DMADP levels stayed stable. An export of MEcDP or an inhibition of the HMBDP synthase by e.g. H_2O_2 would have explained this phenomenon (Wright *et al.*, 2014); however, no influence of *M. persicae* on the export or the transcript abundance could be detected. The increased MEcDP levels,

being the largest metabolite pool in the MEP pathway, most probably increased due to a slight increase in the flux of the MEP pathway (Wright *et al.*, 2014).

The difficulty in observing significant changes in concentration of the MEP pathway metabolites may be explained by alterations in the sink/source-leaf patterns. Commonly the distribution of the MEP pathway metabolite concentrations varies in accordance with the pattern of sink, source and transition leaf (see Fig. 10-1). The flux of the MEP pathway is believed to be higher in sink leaves than mature source leaves. Aphids are known to induce a change in the sink/source metabolism of the plant leaves by inducing a sink at their feeding site (Girousse *et al.*, 2005; Bos *et al.*, 2010). Therefore, a change in the sink/source-leaf pattern may have disguised a significant change in the local composition of the metabolites. Although feeding damage can reduce the MEP pathway flux, changing the leaf from a source to a sink leaf can result in increased flux, thereby disguising the opposite effects. To circumvent this problem it would be necessary to confine the aphids to one type of leaf, however, this is difficult without inducing early defensive responses due to wounding with the use of clip cages. Improving this method would help to elucidate the local responses of the plant to aphid feeding, yet, the confinement would only give information about a local effect.

Besides adaptation, the number of aphids used and the infestation period might also influence leaf senescence and plant defense. For instance the effect of cotton aphids on the photosynthetic rate was shown to be dependent on the number of aphids and the infestation period (Shannag *et al.*, 1998). During experiments the number of *M. persicae* doubled in seven days of infestation. This increased aphid load may influence the responses of the plant in later time points. Due to its small size it would be impractical to remove *M. persicae* offspring from the plants regularly without influencing the plants' defense response. However, before harvesting the plant the aphids have been removed in most experiments. This procedure could explain the high variability in phytohormone and MEP pathway metabolite concentrations which are very sensitive to the environmental conditions such as light intensity and wounding. Metabolite extractions from 40 *M. persicae* revealed no measurable compound concentrations, consequently, aphids should not be removed in future experiments to minimize experimental variation.

6.3 Defensive Reactions

Initially it was hypothesized that SA concentration would be increased upon aphid infestation. Seeing that the first two starting hypothesis about an impaired photosynthesis and subsequent decrease in MEP pathway metabolites were mostly disproven the defensive reaction were also expected to behave unexpected. In addition, the aphid induced defense responses described in literature are very controversial. De Vos *et al.* (2005) found no changes in any of the investigated marker genes for defensive signaling and also did not observe any changes in the phytohormone levels. This is very much in contrast to the results presented by Pegadaraju and coworkers (2005) and indicates possible mutations in the aphid clones or effects due to differences in experimental set-up.

Usually experiments with aphids are executed under long day conditions to prevent the aphids from transforming into their sexual forms. For this thesis the experiments were done in short day conditions to be able to compare it to the effects of simulated *Spodoptera littoralis* attack. This might explain some differences to the published literature. Additionally, studies described in the literature usually spanned longer time periods (Hawkins *et al.*, 1987). Independent of the difference in day length, the results of this thesis are in agreement with the results in total plant measurements of De Vos *et al.* (2005). However, they did find local responses to *Myzus persicae* infestation through an increase in local SA responsive genes (De Vos *et al.*, 2005). Future experiments can address this short coming by measuring effects at or near the sites of feeding.

A common problem in using aphids is the variability in the time of feeding initiation. This may lead to a delay in observed reaction periods after infestation and, subsequently, increase the variances especially in the earlier time points. However, the measured concentration levels of metabolites do not vary too much, which indicates that this influence is marginal.

As opposed to the reported increases in SA in response to *Myzus persicae* infestation (Moran and Thompson, 2001; Sun *et al.*, 2013) no significant changes were observed in phytohormone levels in this thesis. *M. persicae* has been reported to utilize SA as a decoy to the plant's JA-signaling to prevent a defense response (Ellis *et al.*, 2002; De Vos *et al.*, 2007; Sun *et al.*, 2013). SA and JA signaling function antagonistic to each other, so that a stimulated

SA response will down regulate the JA mediated defense response. In contrast to published results, the SA level seemed to decrease one hour post infestation, suggesting that the reported decoy hypothesis is not active in the investigated system. However, the previously mentioned change in the source/sink-leaf composition of *A. thaliana* should also lead to a change in SA concentration. A higher concentration of SA would be expected because the sink leaves were found to have higher SA levels in addition to increased MEP pathway metabolite levels (see Fig. 10-1). To further investigate the lack of a SA response the transcript abundance of the isochorismate synthase (ICS) was analyzed. In contrast to the measured SA levels the ICS transcript abundance significantly decreased after one and 24 hours. The results after one hour of infestation agree with the decrease in SA concentration. However, the correlation at 24 hours is not as clear, due to high SA variability. Nonetheless, these results support the idea that *M. persicae* is not able to induce the decoy response to facilitate its own fecundity. Additionally, next to the downregulation of ICS transcript abundance the increase of SA is blocked at an earlier stage in signaling. Another way to investigate the SA response would be the pathogenesis related (*PR-1*) marker gene which is known to respond to the abundance of SA (De Vos *et al.*, 2005).

SA signaling mediated by MEcDP has been proven by Gonzalez-Cabanelas *et al.* (2015) to be of biological significance upon infestation of *Arabidopsis thaliana* (Col-0) with *Brevicoryne brassicae*. However, this does not prove the involvement of MEcDP in the actual defensive signaling induced by aphids. The plant mutant upon which their work was based produced MEcDP levels more than two orders of magnitude higher than the levels occurring in wild type plants. Additionally, the continuous over-production of MEcDP in these plants resulted in constant activation of the MEcDP mediated defense response which does not necessarily occur after aphid infestation. As the presented results suggest, no such involvement was found for *M. persicae* infestation. It would be of interest to compare the different aphid species directly to determine a possible species specific effect. This specificity may also be mediated by mobilizing different forms of SA. SA is active in the free form, but also occurs in the plant as inactive glycosylated forms. Xiao and others (2012) reported an increase in free SA upon export of MEcDP. In contrast to these findings Gonzalez-Cabanelas *et al.* (2015) reported an increase in total but not free SA. In this thesis only the free SA content has been investigated

which should be remedied in future studies. Nonetheless, *M. persicae* may elicit a different signaling pathway for inhibiting a plant defense response.

In the cytosol MEcDP is dephosphorylated to ME (Gonzalez-Cabanelas *et al.*, 2015). This downstream product was found to be decreased 72 hours post infestation relative to control plants. The concentration of ME seems to be stable in infested plants whereas there is an increase in non-infested plants two and three days post infestation. Especially at earlier time points ME is increased in its total concentration for both treatments, which is most likely caused in response to the bags keeping the aphids confined to the plants. The suppressed increase of ME in infested plants hints towards an influence of *Myzus persicae*. In regards to the decoy hypothesis the decrease in ME accumulation would only make sense if MEcDP is the signal for an increase in SA levels: Inhibiting the dephosphorylation would result in more MEcDP being available for signaling and therefore inhibiting the JA pathway. This is supported by the findings of Gonzalez-Cabanelas *et al.* (2015) who reported a correlation between increasing ME concentration and a decrease in the hydroperoxide lyase (HPL) transcript abundance. Subsequently, a decreased amount of the free SA was found. Yet, in the conducted experiments no increase in MEcDP levels was observed. This might be explained by the dependence of the effects of ME and MEcDP on the developmental stage of the plant. Furthermore, the effects may also be antagonistic or synergistic depending on the metabolic and developmental stage (Gonzalez-Cabanelas *et al.*, 2015) making it vital to closely monitor the metabolic age of the plant. Another explanation for the decrease in ME in infested plants may be an increased glucosylation to ME-glc in the later stages of aphid infestation which were not measured due to time constrains. Further investigations in this direction shall help elucidating unanswered question.

In accordance to the slight decrease in SA, JA increased slightly after 24 hours of aphid infestation. In contrast neither the precursor OPDA nor the active downstream product JA-Ile showed any defensive related changes in their concentration. A loss of the defensive SA-elicitor should lead to an increased response of the plant with JA signaling and, thus, decrease of aphid fecundity (Mewis *et al.*, 2005; Louis and Shah, 2013). This has not been observed and may be explained by an ongoing interaction between plant and aphid prompting a status quo where no signal prevails. Therefore, preventing the plant from inducing an effective

defensive response but also keeping *M. persicae* from inducing an increase in SA and directly manipulating the plants defense response.

As already mentioned stress signaling is not only executed by phytohormones but also involves ROS (Kerchev *et al.*, 2012, 2012; Ren *et al.*, 2014). Yet, the expected oxidative burst was not observed during the earlier investigated time points. In support of this Kuśnierczyk *et al.* (2008) found no local accumulation of H_2O_2 in *Arabidopsis thaliana* (Ler-biotype) induced by *Brevicoryne brassicae* (Kusnierczyk *et al.*, 2008). This lack of observable H_2O_2 accumulation is thought to be caused by a fast and efficient detoxification, yet, producing enough ROS at the time of infestation to induce a defense response (Kusnierczyk *et al.*, 2008). After seven days of aphid infestation the ROS accumulation was increased, but neither the MEcDP nor the SA levels were affected. In contrast, the JA levels were increased. Therefore, ROS influenced neither the HMBDP synthase nor the MEcDP levels. The efficient detoxification of ROS involves β-carotene as a scavenger and, thus, induces the production of β-I and β-CC (Pogson *et al.*, 2005; Ramel *et al.*, 2012). However, most likely another mode of detoxification was utilized by *Arabidopsis thaliana*, since no increases in these scavenging products were observed despite an increase in β-carotene. For example the reported increase in lutein accumulation may be due to this previously explained alternative mode of detoxification. For further investigation the transcript abundance of genes involved in detoxification of radicals could be measured via RT-qPCR (Kusnierczyk *et al.*, 2008).

The third investigated phytohormone abscisic acid showed a different pattern in its accumulation than its investigated pigment precursors. Park and colleagues (2006) reported an involvement in defense whereas Voelckel *et al.* (2004) did not find an induction of factors involved in ABA biosynthesis (Morkunas *et al.*, 2011), making the involvement of ABA in a defense response somewhat controversial. However, the changes in ABA accumulation may be caused by the cellophane bag used to confine the aphids on the plant. This is true for most presented data and is reflected in the graphs by a decrease of metabolites in the first six hours independent of the treatment. Yet, a comparison between bagged and unbagged plants after 24 hours did not show a significant difference in MEP pathway metabolite concentration (Fig. 10-2); nevertheless the comparison did not include earlier time points. This ‘bag effect’ may be circumvented in future experiments by bagging the plants 24 hours before aphid

infestation. Either way, in this thesis ABA is clearly not involved in a defense response of *A. thaliana* against *M. persicae*.

Another problem found upon evaluating the recorded data was the lack of significances. There are a lot of increases which can only be reported as tendencies, probably due to the small number of replicates. However, the changes in concentration do make a lot of sense when put into context. Thus, a repetition of the experiments with at least ten replicates per treatment would be of great help in validating the reported data. The practical difficulty in rearing the necessary number of aphid offspring regretfully prevented a higher number of replicates in this study.

The small number of neonates on *A. thaliana* observed during rearing could be caused by an adaptation to glucosinolates. These compounds are produced by members of the Brassicaceae upon induction of a defensive response (Mewis *et al.*, 2005; Kim *et al.*, 2008). During an arms race between *M. persicae* and *A. thaliana* it is possible that the aphid had to accept a decrease in fecundity in exchange for higher vitality. Due to this adaptation of the aphid the plant would have to deal with a less effective defensive response, which might further explain the weak responses recorded in this thesis. Another challenge for the plant is the comparatively subtle attack of the aphid when compared to chewing herbivores. Effectors located in the aphid saliva which can be recognized by the plant or utilized to further aphid infestation are therefore of interest in future experiments (De Vos and Jander, 2009).

7 Conclusion

As far as the data with four replicates per treatment suggests, the 2-*C*-methyl-D-erythritol 4-phosphate pathway is influenced by aphid feeding. Increases in DXS activity, DXP and MEcDP levels were observed whereas IDP/DMADP concentration did not change. This discrepancy could not be explained by an export of MEcDP into the cytosol or by the investigated degradation products. Interestingly, these MEcDP degradation products revealed that the cytosolic 2-*C*-methyl-D-erythritrol (ME) seems to be decreased by aphid feeding. Increased levels of ME were previously correlated with decreased levels of free SA. A suppression of this by the aphid may be part of the decoy signaling of SA to prevent an increase in JA due to the antagonistic behavior of JA and SA. Analyzing MEcDP and ME in pathosystems with reported increases in SA may give new insight into the decoy hypothesis. However, in the monitored pathosystem the actual decoy increase of SA may have been lost in the arms race between *A. thaliana* and *M. persicae*. In connection to this it seems prudent to analyze the transcript abundance of genes activated by stress related ROS formation to detect ROS that may have been missed by the presented approaches. Furthermore, the increases observed in intermediates of the MEP pathway are independent of photosynthesis and correlate only partially with the investigated pigment levels. This gives rise to questions regarding the detailed regulation of the MEP pathway besides the previously mentioned enzymes and photosynthesis. The differences between our results and the literature can in future be addressed by performing the experiments with similar aphid genotypes. Applying the suggested improvements will give better and even more exact data concerning this pathosystem.

Most reactions were observed during the shorter infestation periods (1 to 24 hours) but it is possible that an influence of *Myzus persicae* on the MEP pathway after seven days has been missed. All in all it can be said that hypotheses introduced in the introduction were disproven. However, *M. persicae* has an, albeit small, effect on the MEP pathway in *Arabidopsis thaliana.*

8 Outlook

Mild winters such as the previous will make it easier for aphids to survive in their asexual form and, subsequently, easier to multiply their numbers. Therefore, it is important to contain aphid infestation in future years. Understanding the reason behind the observed differences in the same pathosystem may help to understand the exact mechanisms underlying these differences. The following experiments concerning plant-aphid interaction will help further this understanding and in the long term this can lead to better control of aphid pests in agriculture.

To verify the reported findings it is of importance to repeat the experiment with more replicates. Furthermore, a comparative test about the influence of long and short day conditions on the performance of *Myzus persicae* on *Arabidopsis thaliana* should be executed in the same lab. This will help to exclude the different day lengths as reasons for the deviating plant responses. Influenced by the day length the aphid returns to its primary host plant during fall and switches to its sexual form. Day length effects on the aphid performance and plant defense responses will, thus, give new insights into the cues for host change of aphids during seasonal changes. At the same time the investigation of longer periods of infestation may reveal novel effects on the MEP pathway. Additionally, investigating local effects and more enzymes for activity and transcript abundance might uncover unknown plant-aphid interactions. Another way of amplifying the influence of aphids on plants would be an ‘artificial saliva’ infiltration experiment. The responses usually confined to a local environment would be more global and, thus, more easy to identify.

Nothing is known about the interaction of the MEP pathway with the C_2 compound ethylene, another phytohormone known to be involved in defensive signaling (Morkunas *et al.*, 2011). Ethylene has been reported in *A. thaliana* to both induce and reduce resistance to *M. persicae* infestation (Louis and Shah, 2013). Analyzing the effect of ethylene on the MEP pathway may reveal novel insights.

9 References

Banerjee A, Sharkey TD (2014) Methylerythritol 4-phosphate (MEP) pathway metabolic regulation. Nat Prod Rep **31:** 1043-1055

Beale SI (1999) Enzymes of chlorophyll biosynthesis. Photosynth Res **60:** 43-73

Benning UF, Tamot B, Guelette BS, Hoffmann-Benning S (2012) New aspects of Phloem-mediated long-distance lipid signaling in plants. Front Plant Sci **3:** 53

Biel K, Fomina I (2015) Benson-Bassham-Calvin cycle contribution to the organic life on our planet. Photosynthetica **53:** 161-167

Blackman RL, Eastop VF (2007) Taxonomic Issues. *In* van Emden HF, Harrington R, eds, Aphids as Crop Pests, Vol 1. CAB International, UK, pp 1-29

Bode S, Quentmeier CC, Liao PN, Hafi N, Barros T, Wilk L, Bittner F, Walla PJ (2009) On the regulation of photosynthesis by excitonic interactions between carotenoids and chlorophylls. Proc Natl Acad Sci U S A **106:** 12311-12316

Bos JI, Prince D, Pitino M, Maffei ME, Win J, Hogenhout SA (2010) A functional genomics approach identifies candidate effectors from the aphid species Myzus persicae (green peach aphid). PLoS Genet **6:** e1001216

Caffarri S, Tibiletti T, Jennings RC, Santabarbara S (2014) A Comparison Between Plant Photosystem I and Photosystem II Architecture and Functioning. Curr Protein Pept Sci **15:** 296-331

Cazzonelli CI (2011) Goldacre Review: Carotenoids in nature: insights from plants and beyond. Functional Plant Biology **38:** 833-847

Chenu A, Scholes GD (2015) Coherence in energy transfer and photosynthesis. Annu Rev Phys Chem **66:** 69-96

Collier RH, Finch S (2007) IPM Case Studies: Brassicas. *In* van Emden HF, Harrington R, eds, Aphids as Crop Pests, Vol 1. CAB International, UK, pp 549-559

Cordoba E, Salmi M, Leon P (2009) Unravelling the regulatory mechanisms that modulate the MEP pathway in higher plants. J Exp Bot **60:** 2933-2943

Daudi A, O'Brien JA (2012) Detection of Hydrogen Peroxide by DAB Staining in Arabidopsis Leaves. bio-protocol **2:** e263

De Vos M, Jander G (2009) Myzus persicae (green peach aphid) salivary components induce defence responses in Arabidopsis thaliana. Plant Cell Environ **32:** 1548-1560

De Vos M, Kim JH, Jander G (2007) Biochemistry and molecular biology of Arabidopsis-aphid interactions. Bioessays **29:** 871-883

De Vos M, Mueller MJ, Van Oosten VR, Buchala AJ, Van Poecke RMP, Métraux J-P, Van Pelt JA, Van Loon LC, Pozo MJ, Dicke M, Pieterse CMJ (2005) Signal Signature and Transcriptome Changes of Arabidopsis During Pathogen and Insect Attack. Molecular Plant-Microbe Interactions **18:** 923-937

Delwiche CF, Sharkey TD (1993) Rapid appearance of 13C in biogenic isoprene when 13CO2 is fed to intact leaves. Plant, Cell Environ. **16:** 587-591

Ducreux LJ, Morris WL, Hedley PE, Shepherd T, Davies HV, Millam S, Taylor MA (2005) Metabolic engineering of high carotenoid potato tubers containing enhanced levels of beta-carotene and lutein. J Exp Bot **56:** 81-89

Duvoid T, Bravo J-M, Pale-Grosdemange C, Rohmer M (1997) Biosynthesis of 2-C-Methyl-D-erythritol, a Putative C5 Intermediate in the Mevalonate Independent Pathway for Isoprenoid Biosynthesis. Tetrahedron Letters **38:** 4769-4772

Eckhardt U, Grimm B, Hortensteiner S (2004) Recent advances in chlorophyll biosynthesis and breakdwon in higher plants. Plant Mol. Biol. **56:** 1-14

Ellis C, Karafyllidis I, Turner JG (2002) Constitutive Activation of Jasmonate Signaling in an Arabidopsis Mutant Correlates with enhanced Reistance to Erysiphe cichoracearum, Pseudomonas syringae, and Myzus persicae. Mol. Plant Microbe Interact. **15:** 1025-1030

Elzinga DA, Jander G (2013) The role of protein effectors in plant-aphid interactions. Curr Opin Plant Biol **16:** 451-456

Estevez JM, Cantero A, Reindl A, Reichler S, Leon P (2001) 1-Deoxy-D-xylulose-5-phosphate synthase, a limiting enzyme for plastidic isoprenoid biosynthesis in plants. J Biol Chem **276:** 22901-22909

Fellermeier M, Kis K, Sagner S, Maier U, Bacher A, Zenk MH (1999) Cell-free conversion of 1-deoxy-D-xylulose 5-phosphate and 2-C-methyl-D-erythritol 4-phosphate into 13-carotene in higher plants and its inhibition by fosmidomycin. Tetrahedron Letters**:** 2743-2746

Flesh G, Rohmer M (1988) Prokaryotic hopanoids: the biosynthesis of the bacteriohopane skeleton Formation of isoprenic units from two distinct acetate pools and a novel type of carbon/carbon linkage between a triterpene and D-ribose. Eur. J. Biochem.**:** 405

García-Plazaola JI, Matsubara S, Osmond CB (2007) The lutein epoxide cycle in higher plants: its relationships to other xanthophyll cycles and possible functions. Functional Plant Biology **34:** 759-773

Ghirardo A, Wright LP, Bi Z, Rosenkranz M, Pulido P, Rodriguez-Concepcion M, Niinemets U, Bruggemann N, Gershenzon J, Schnitzler JP (2014) Metabolic flux analysis of plastidic isoprenoid biosynthesis in poplar leaves emitting and nonemitting isoprene. Plant Physiol **165:** 37-51

Girousse C, Moulia B, Silk W, Bonnemain JL (2005) Aphid infestation causes different changes in carbon and nitrogen allocation in alfalfa stems as well as different inhibitions of longitudinal and radial expansion. Plant Physiol **137:** 1474-1484

Gonzalez-Cabanelas D, Wright LP, Paetz C, Onkokesung N, Gershenzon J, Rodriguez-Concepcion M, Phillips MA (2015) The diversion of 2-C-methyl-D-erythritol-2,4-cyclodiphosphate from the 2-C-methyl-D-erythritol 4-phosphate pathway to hemiterpene glycosides mediates stress responses in Arabidopsis thaliana. Plant Journal **82:** 122-137

Hawkins CDB, Aston MJ, Whitecros MI (1987) Short-term effects of aphid feeding on photosynthetic CO2 exchange and dark respiration in legume leaves. Physiol. Plant **71:** 379-383

Hirai N, Yoshia R, Todoroki Y, Ohigashi H (2000) Biosynthesis of Abscisic Acid by the Non-mevalonate Pathway in Plants, and by the Mevalonate Pathway in Fungi. Biosci. Biotechnol. Biochem. **64:** 1448-1458

Jahns P, Holzwarth AR (2012) The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. Biochim Biophys Acta **1817:** 182-193

Kaiser E, Morales A, Harbinson J, Kromdijk J, Heuvelink E, Marcelis LF (2015) Dynamic photosynthesis in different environmental conditions. J Exp Bot **66:** 2415-2426

Kerchev PI, Fenton B, Foyer CH, Hancock RD (2012) Infestation of potato (Solanum tuberosum L.) by the peach-potato aphid (Myzus persicae Sulzer) alters cellular redox status and is influenced by ascorbate. Plant Cell Environ **35:** 430-440

Kerchev PI, Fenton B, Foyer CH, Hancock RD (2012) Plant responses to insect herbivory: interactions between photosynthesis, reactive oxygen species and hormonal signalling pathways. Plant Cell Environ **35:** 441-453

Khan MAM, Ulrichs C, Mewis I (2010) Influence of water stress on the glucosinolate profile of Brassica oleracea var. italica and the performance of Brevicoryne brassicae and Myzus persicae. Entomologia Experimentalis et Applicata **137:** 229-236

Kim JH, Lee BW, Schroeder FC, Jander G (2008) Identification of indole glucosinolate breakdown products with antifeedant effects on Myzus persicae (green peach aphid). Plant J **54:** 1015-1026

Kramell R, Schmidt J, Schneider G, Sembdner G, Schreiber K (1988) Synthesis of N-(jasmonyl)amino acid conjugate. Tetrahedron **44:** 5791-5807

Kunert G, Reinhold C, Gershenzon J (2010) Constitutive emission of the aphid alarm pheromone, (E)-beta-farnesene, from plants does not serve as a direct defense against aphids. BMC Ecol **10**

Kusnierczyk A, Winge P, Jorstad TS, Troczynska J, Rossiter JT, Bones AM (2008) Towards global understanding of plant defence against aphids--timing and dynamics of early Arabidopsis defence responses to cabbage aphid (Brevicoryne brassicae) attack. Plant Cell Environ **31:** 1097-1115

Li H, Ma D, Jin Y, Tu Y, Liu L, Leng C, Dong J, Wang T (2015) Helper component-proteinase enhances the activity of 1-deoxy-D -xylulose-5-phosphate synthase and promotes the biosynthesis of plastidic isoprenoids in Potato virus Y-infected tobacco. Plant Cell Environ **38:** 2023-2034

Lichtenthaler HK (1999) The 1-Deoxy-D-Xylulose-5-Phosphate Pathway of Isoprenoid Biosynthesis in Plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **50:** 47-65

Lichtenthaler HK (2010) The Non-mevalonate DOXP/MEP (Deoxyxylulose 5-Phosphate/Methylerythritol 4-Phosphate) Pathway of Chloroplast Isoprenoid and Pigment Biosynthesis. *In* Rebeiz CA, Benning C, Bohnert HJ, Daniell H, Hoober JK, Lichtenthaler HK, Portis AR, Tripathy BC, eds, The Chloroplast: Basics and Applications, Vol 31. Springer, pp 95-118

Lindgren LO, Stalberg KG, Hoglund AS (2003) Seed-specific overexpression of an endogenous Arabidopsis phytoene synthase gene results in delayed germination and increased levels of carotenoids, chlorophyll, and abscisic acid. Plant Physiol **132:** 779-785

Loivamäki M, Louis S, Cinege G, Zimmer I, Fischbach RJ, Schnitzler JP (2007) Circadian rhythms of isoprene biosynthesis in grey poplar leaves. Plant Physiol **143:** 540-551

Louis J, Shah J (2013) Arabidopsis thaliana-Myzus persicae interaction: shaping the understanding of plant defense against phloem-feeding aphids. Front Plant Sci **4:** 213

Lv F, Zhou J, Zeng L, Xing D (2015) beta-cyclocitral upregulates salicylic acid signalling to enhance excess light acclimation in Arabidopsis. J Exp Bot **66:** 4719-4732

Macedo TB, Bastos CS, Higley LG, Ostlie KR, Madhavan S (2003) Photosynthetic Responses of Soybean to Soybean Aphid (Homoptera: Aphididae) Injury. Journal of Economic Entomology **96:** 188-193

Mewis I, Appel HM, Hom A, Raina R, Schultz JC (2005) Major signaling pathways modulate Arabidopsis glucosinolate accumulation and response to both phloem-feeding and chewing insects. Plant Physiol **138:** 1149-1162

Mewis I, Khan MA, Glawischnig E, Schreiner M, Ulrichs C (2012) Water stress and aphid feeding differentially influence metabolite composition in Arabidopsis thaliana (L.). PLoS One **7:** e48661

Miller M, Schlauch K, Tam R, Cortes D, Torres MA, Shulaev V, Dangl JL, Mittler R (2009) The Plant NADPH Oxidase RBOHD Mediates Rapid Systemic Signaling in Response to Diverse Stimuli. Sci. Signal. **2:** ra45

Moehs CP, Tian L, Osteryoung KW, DellaPenna D (2001) Analysis of carotenoid biosynthetic gene expression during marigold petal development. Plant Mol. Biol. **45:** 281-293

Moffat BA, McWhinnie EA, Agarwal SK, Schaff DA (1994) The adenine phosphoribosyltransferase-encoding gene of Arabidopsis thaliana. Gene **143:** 211-216

Moloi MJ, van der Westhuizen AJ (2006) The reactive oxygen species are involved in resistance responses of wheat to the Russian wheat aphid. J Plant Physiol **163:** 1118-1125

Mongelard G, Seemann M, Boisson AM, Rohmer M, Bligny R, Rivasseau C (2011) Measurement of carbon flux through the MEP pathway for isoprenoid synthesis by (31)P-NMR spectroscopy after specific inhibition of 2-C-methyl-d-erythritol 2,4-cyclodiphosphate reductase. Effect of light and temperature. Plant Cell Environ **34:** 1241-1247

Moran PJ, Thompson GA (2001) Molecular responses to aphid feeding in Arabidopsis in relation to plant defense pathways. Plant Physiol **125:** 1074-1085

Morkunas I, Mai VC, Gabryś B (2011) Phytohormonal signaling in plant responses to aphid feeding. Acta Physiologiae Plantarum **33:** 2057-2073

Müller P, Li X-P, Niyogi KK (2001) Non-Photochemical Quenching. A Response to Excess Light Energy. Plant Physiol **125:** 1558-1566

Munekage Y, Hashimoto M, Miyake C, Tomizawa K-I, Endo T, Tasaka M, Shikanai T (2004) Cyclic electron flow around photosystem I is essential for photosynthesis. Nature **429:** 579-582

Nambara E, Marion-Poll A (2005) Abscisic Acid Biosynthesis and Catabolism. Annu. Rev. Plant Biol. **56:** 165-185

Ostrovsky DN, Dyomina GR, Deryabina YI, Goncharenko AV, Eberl M, Shumaev KB, Shashkov AS (2003) Properties of 2-C-Methyl-D-Erythritol 2,4-Cyclopyrophosphate, an Intermediate in Nonmevalonate Isoprenoid Biosynthesis. Appl. Biochem. Microbiol. **39:** 497-502

Park SJ, Huang Y, Ayoubi P (2006) Identification of expression profiles of sorghum genes in response to greenbug phloem-feeding using cDNA subtraction and microarray analysis. Planta **223:** 932-947

Peccoud J, Mahéo F, de la Huerta M, Laurence C, Simon J-C, Leather SR (2015) Genetic characterisation of new host-specialised biotypes and novel associations with bacterial symbionts in the pea aphid complex. Insect Conservation and Diversity **8:** 484-492

Pegadaraju V, Knepper C, Reese J, Shah J (2005) Premature leaf senescence modulated by the Arabidopsis PHYTOALEXIN DEFICIENT4 gene is associated with defense against the phloem-feeding green peach aphid. Plant Physiol **139:** 1927-1934

Pegadaraju V, Louis J, Singh V, Reese JC, Bautor J, Feys BJ, Cook G, Parker JE, Shah J (2007) Phloem-based resistance to green peach aphid is controlled by Arabidopsis PHYTOALEXIN DEFICIENT4 without its signaling partner ENHANCED DISEASE SUSCEPTIBILITY1. Plant J **52:** 332-341

Pogson BJ, Rissler HM, Frank HA (2005) The Role of Carotenoids in Energy Quenching. *In* Wydrzynski TJ, Satoh K, eds, Advances in Photosynthesis and Respiration, Vol 22. Springer-Verlag, pp 515-537

Proteau PJ (2004) 1-Deoxy-D-xylulose 5-phosphate reductoisomerase: an overview. Bioorg Chem **32:** 483-493

Raines CA (2002) The Calvin cycle revisited. Photosynth Res **75:** 1-10

Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylides C, Havaux M (2012) Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. Proc Natl Acad Sci U S A **109:** 5535-5540

Ren G, Wang X, Chen D, Wang X, Liu X (2014) Effects of aphids Myzus persicae on the changes of Ca2+and H2O2 flux and enzyme activities in tobacco. Journal of Plant Interactions **9:** 883-888

Rivasseau C, Seemann M, Boisson AM, Streb P, Gout E, Douce R, Rohmer M, Bligny R (2009) Accumulation of 2-C-methyl-D-erythritol 2,4-cyclodiphosphate in illuminated plant leaves at supraoptimal temperatures reveals a bottleneck of the prokaryotic methylerythritol 4-phosphate pathway of isoprenoid biosynthesis. Plant Cell Environ **32:** 82-92

Rohdich F, Wungsintaweekul J, Luttgen H, Fischer M, Eisenreich W, Schuhr CA, Fellermeier M, Schramek N, Zenk MH, Bacher A (2000) Biosynthesis of terpenoids: 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase from tomato. Proc Natl Acad Sci U S A **97:** 8251-8256

Rohmer M (1999) The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. Nat. Prod. Rep. **16:** 565-574

Rohmer M, Seemann M, Horbach S, Bringer-Meyer S, Sahm H (1996) Glyceraldehyde 3-Phosphate and Pyruvate as Precursors of Isoprenic Units in an Alternative Non-mevalonate Pathway for Terpenoid Biosynthesis. J. Am. Chem. Soc. **118:** 2564-2566

Seemann M, Rohmer M (2007) Isoprenoid biosynthesis via the methylerythritol phosphate pathway: GcpE and LytB, two novel iron–sulphur proteins. Comptes Rendus Chimie **10:** 748-755

Shannag HK, Thorvilson H, El-Shatnawi MK (1998) Changes in photosynthetic and transpiration rates of cotton leaves infested with the cotton aphid, Aphis gossypii: Unrestricted infestation. Ann. appl. Biol. **132:** 13-18

Sharkey TD, Monson RK (2014) The future of isoprene emission from leaves, canopies and landscapes. Plant Cell Environ **37:** 1727-1740

Shikanai T (2014) Central role of cyclic electron transport around photosystem I in the regulation of photosynthesis. Curr Opin Biotechnol **26:** 25-30

Sun Y, Guo H, Zhu-Salzman K, Ge F (2013) Elevated CO2 increases the abundance of the peach aphid on Arabidopsis by reducing jasmonic acid defenses. Plant Sci **210:** 128-140

Tanaka Y, Sasaki N, Ohmiya A (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J **54:** 733-749

Taylor IB, Sonneveld T, Bugg TDH, Thompson AJ (2005) Regulation and Manipulation of the Biosynthesis of Abscisic Acid, Including the Supply of Xanthophyll Precursors. Journal of Plant Growth Regulation**:** 253-273

Thompson GA, Goggin FL (2006) Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. J Exp Bot **57:** 755-766

Tjallingii WF, Hogen Esch T (1993) Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. Physiol Entomol **18:** 317-328

van Amerongen H, Croce R (2013) Light harvesting in photosystem II. Photosynth Res **116:** 251-263

Voelckel C, Weisser WW, Baldwin IT (2004) An analysis of plant-aphid interactions by different microarray hybridization strategies. Mol Ecol **13:** 3187-3195

Vranova E, Coman D, Gruissem W (2013) Network analysis of the MVA and MEP pathways for isoprenoid synthesis. Annu Rev Plant Biol **64:** 665-700

Will T, Furch AC, Zimmermann MR (2013) How phloem-feeding insects face the challenge of phloem-located defenses. Front Plant Sci **4:** 336

Will T, van Bel AJ (2006) Physical and chemical interactions between aphids and plants. J Exp Bot **57:** 729-737

Wright LP, Phillips MA (2014) Measuring the activity of 1-deoxy-D-xylulose 5-phosphate synthase, the first enzyme in the MEP pathway, in plant extracts. Methods Mol Biol **1153:** 9-20

Wright LP, Rohwer JM, Ghirardo A, Hammerbacher A, Ortiz-Alcaide M, Raguschke B, Schnitzler JP, Gershenzon J, Phillips MA (2014) Deoxyxylulose 5-Phosphate Synthase Controls Flux through the Methylerythritol 4-Phosphate Pathway in Arabidopsis. Plant Physiol **165:** 1488-1504

Xiao Y, Savchenko T, Baidoo EE, Chehab WE, Hayden DM, Tolstikov V, Corwin JA, Kliebenstein DJ, Keasling JD, Dehesh K (2012) Retrograde signaling by the plastidial metabolite MEcPP regulates expression of nuclear stress-response genes. Cell **149:** 1525-1535

Yang D, Ma P, Liang X, Wei Z, Liang Z, Liu Y, Liu F (2012) PEG and ABA trigger methyl jasmonate accumulation to induce the MEP pathway and increase tanshinone production in Salvia miltiorrhiza hairy roots. Physiol Plant **146:** 173-183

Yang L, Li B, Zheng XY, Li J, Yang M, Dong X, He G, An C, Deng XW (2015) Salicylic acid biosynthesis is enhanced and contributes to increased biotrophic pathogen resistance in Arabidopsis hybrids. Nat Commun **6:** 7309

Zheng XY, Zhou M, Yoo H, Pruneda-Paz JL, Weaver Spivey N, Kay SA, Dong X (2015) Spatial and temporal regulation of biosynthesis of the plant immune signal salicylic acid. PNAS **112:** 9166-9173

Zhou N, Tootle TL, Glazebrook J (1999) Arabidopsis PAD3, a gene rquired for camalexin biosynthesis, encodes a putative cytochrome P450 monoxygenase. Plant Cell **11:** 2419-2428

Zhu-Salzman K, Bi J-L, Liu T-X (2005) Moleculare strategies of plant defense and insect counter-defense. Insect Sci **12:** 3-15

10 Supplement

The presented information is meant as supplement for the main part of this thesis. Fig. 10-1 and Fig. 10-2 show data accumulated during the practical part of this thesis that did not find its way into the results part but is needed to support new hypotheses as mentioned in the discussion.

Tab. 10-1: Primers used for RT-qPCR.

Primer	Sequence 5' → 3'
HDS forward	CAG AAT GCG TAA CAC TAA GAC
HDS reverse	GAG AAC CAC CTA CAT ATC CG
DXS forward	TCG CAA AGG GTA TGA CAA AG
DXS reverse	CAG TCC CGC TTA TCA TTC C
ICS forward	GCT TGC AAG AGT GCA ACA C
ICS reverse	AAG CCT TGC TTC TTC TGC TG
APT forward	GTT GCA GGT GTT GAA GCT AGA GGT
APT reverse	TGG CAC CAA TAG CCA ACG CAA TAG

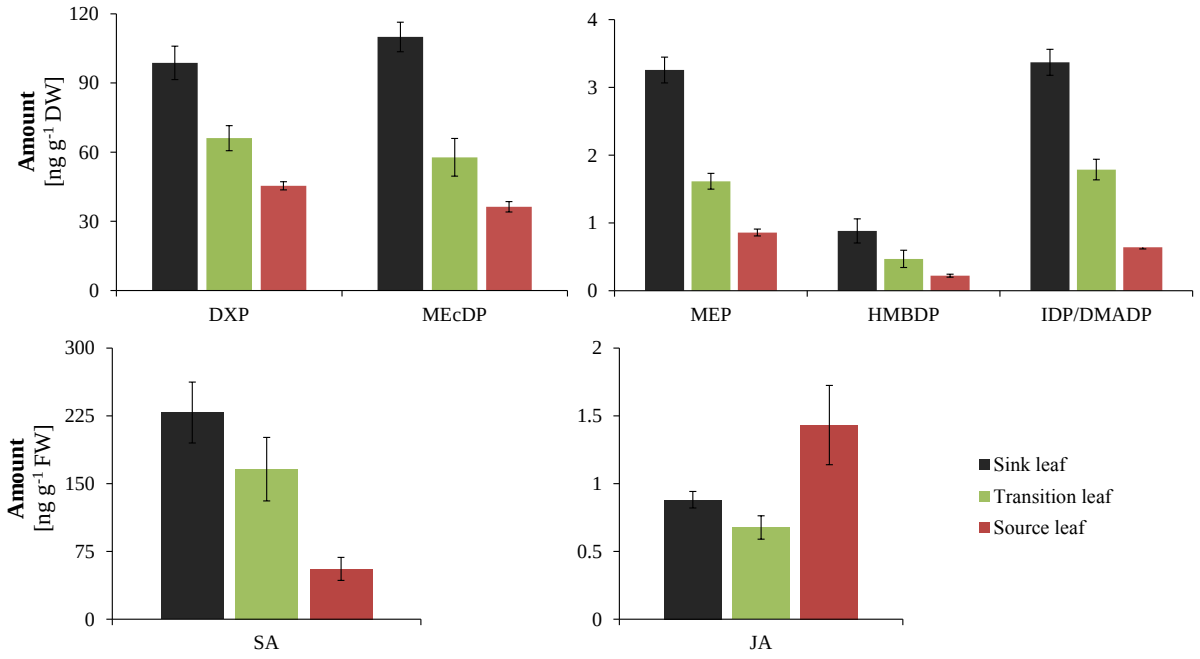

Fig. 10-1: The distribution of investigated metabolites is dependent on leaf age. 40 days old rosette Col-0 plant was divided in sink, source and transition leaves and harvested accordingly. All tested MEP pathway metabolites decrease in concentration with increased leaf age. Salicylic acid (SA) concentration also decreases with increased leaf age. Jasmonic acid (JA) level on the other hand is increased in source leaves. Represented values are means of four replicates for sink and source leaves, only transition leaf values show means of three replicates. Error bars indicate the standard error (SE).

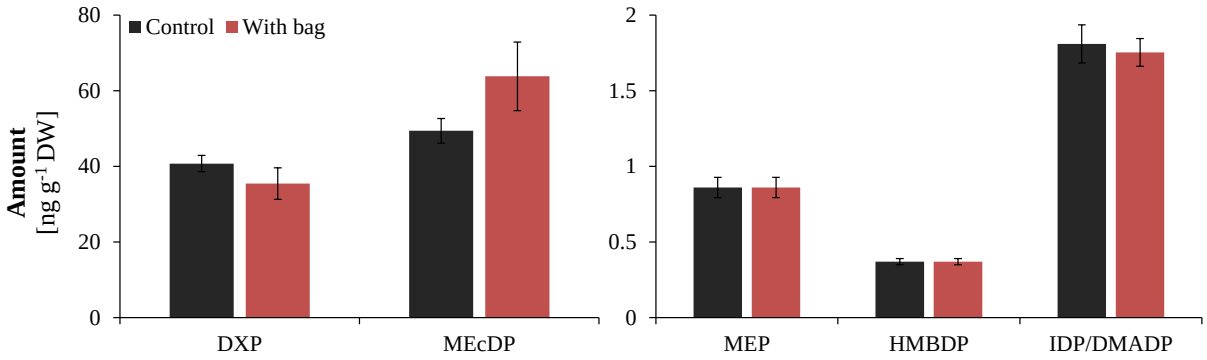

Fig. 10-2: Confining *A. thaliana* Col-0 with bags did not significantly influence MEP pathway metabolites after 24 h of incubation. 40 d old *A. thaliana* rosette plants were confined with the cellophane bag used during experiments and rearing. Only MEcDP seems to be slightly increased, student's t-Test however did not reveal any significance. Values are means of three replicates per treatment. Error bars indicate the standard error (SE).

11 Acknowledgements

In this part I would like to thank Prof. Dr. J. Gershenzon for agreeing to be my supervisor for my T1 and T2 module and also for being the first examiner of this Thesis and allowing me to work in his department. Junior Prof. Dr. S. Sasso I would like to thank for taking up the function of the second examiner.

Furthermore, I would like to thank Dr. S. Mitra for offering this most interesting topic and for her support through any and all up and downs of the practical theoretical site of this work. Dr. L. Wright has been so nice to treat me as a full member of his wonderful group and I am also very thankful for his support and input on my chosen topic of work and any (especially technical) problems which arose. Many thanks also to the MEP pathway group and the entire GER department which provided a friendly and scientific working atmosphere. Jan Günther has kindly read and commented on parts of this thesis and helped to improve my writing.

As already mentioned, Dr. G. Kunert has kindly provided the starting colony of *Myzus persicae* which I worked with. In addition to this she also helped me with statistical and aphid related questions that arouse during my work. Besides aphids my work heavily relied on plants for its success and I would like to thank the Greenhouse Team of the Max-Planck-Institute for chemical ecology Jena for cultivating and maintaining the plants needed for the experiments. I further appreciate the support of the other service groups in our institute such as the house techniques, the IT department and our librarians.

Special thanks to my friends and family for their unwavering support and especially Friedi who agreed to proof read the first drafts of this thesis. Last, but most certainly not least, I would like to thank my partner Sven. He was and is always there for me when I need him and stays patient even if I sent him late night versions to proof read or complain for the n^{th} time about having to discard (read “kill”) superfluous aphids.

Those of you not mentioned and feeling forgotten due to this, do not worry, I am sure that I will remember you five minutes after handing in my thesis.

12 Declaration of Authorship

I hereby declare that the thesis submitted is my own unaided work. All direct or indirect sources used are acknowledged as references. This thesis was not previously presented to another examination board and has not been published.

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