Fading of wound-induced volatile release during *Populus tremula* leaf expansion

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Abstract

The release of stress-driven volatiles throughout leaf development has been little studied. Therefore, we subjected poplar leaves during their developmental stage (from two days to two weeks old) to wounding by a single punch hole, and measured online the wound-induced volatile organic compound emissions. Our study shows that the emission of certain volatile compounds fades with increasing leaf age. Among these compounds we found lipoxygenase products (LOX products), acetaldehyde, methyl benzoate, methyl salicylate, and mono- and sesquiterpenes.

In parallel, we studied the fading of constitutive emissions of methanol during leaf maturation, as well as the rise in isoprene constitutive emission during leaf maturation and its relationship to leaf photosynthetic capacity. We found highly significant relationships between leaf chlorophyll content, photosynthetic capacity, and leaf size during leaf ageing.

As the level of constitutive defences increases with increasing leaf age, the strength of the volatile signal is expected to be gradually reduced. The higher elicitation of volatile organic compound emissions (especially LOX products) in younger leaves could be an evolutionary defence against herbivory, given that younger leaves are usually more subjected to infestation and herbivory.

1 Introduction

Plants release a part of their assimilated carbon into constitutive (not related to periods of stress) volatile organic compound (VOC) emissions (Grote et al. 2013b; Niinemets et al. 2013). Isoprene is the major compound emitted in mature leaves of many tree species, including *Populus tremula*. Isoprene is constitutively synthesized as a product of the

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methylerythritol phosphate (MEP) pathway in isoprene-emitting plants (Sharkey et al. 2008), which is directly related to photosynthetic activity through the Calvin cycle and DMADP (dimethylallyl diphosphate) pool. Based on the close relationship between isoprene synthesis, isoprene emission, and photosynthesis (Loreto and Sharkey 1990; Monson and Fall 1989), leaf isoprene emissions may then vary within the same shoot together with leaf age because of the increasing photosynthetic capacity (Centritto et al. 2004) and chlorophyll content during leaf growth (Rasulov et al. 2014). By extension, the constitutive emission of other volatiles may also vary during leaf development.

Populus genus has acropetally development, which means that the youngest leaves are found at the tip of the shoot, and the leaf age and size increase as we move downwards. Therefore, another important factor for leaf constitutive emissions would be the fact that cell elongation and division occurs more intensively in younger and expanding leaves than in more mature and fully grown leaves. Pectin demethylation during cell wall expansion is a likely source of methanol emission (Fall and Benson 1996; Galbally and Kirstine 2002), and therefore we may find differences in constitutive methanol emission levels in neighbouring leaves within the same shoot.

Plants also emit VOCs when subjected to biotic and abiotic stress, so-called stress-driven emissions (Grote et al. 2013b). For example, as a consequence of mechanical damage, poplar leaves emit a rapid burst of volatiles lasting ca. 5 min (Portillo-Estrada et al. 2015b). The volatile blend is mainly composed of green leaf volatiles, methanol and acetaldehyde. The damage leads one to assume a loss of carbon to the plant as well as a decrease in the photosynthetic activity and a loss of water balance in the leaf. There are studies on the quantitative effect of the degree of damage to wound-induced volatile emissions in *P. tremula* mature leaves (Brilli et al. 2011; Fall et al. 1999; Portillo-Estrada et al. 2015b), but up to present, there is little experimental data on leaf constitutive volatile emissions linked to the leaf ontogenetic level, and no studies yet on the potentially different wound-induced volatile emissions throughout young leaf development.

We used *P. tremula* shoots with leaves of increasing age during leaf expansion. We measured both online leaf photosynthetic capacity and constitutive volatile emissions in leaves of increasing age under optimal growth conditions. This was followed by mechanical wounding using a hole puncher to measure the wound-induced responses as a function of leaf age.

We hypothesize that: (1) constitutive isoprene emission is positively related to photosynthetic activity, which also means that it increases with leaf growth; (2) constitutive methanol emission is maximum in younger and smaller leaves; and (3) wound-induced volatile emissions will be linked to leaf age during expansion presumably because of the different physiological activity during leaf development.

Material and methods

Plant material

We used root suckers of 15-20 leaves from a naturally established *Populus tremula* population at the campus of the Estonian University of Life Sciences (58.39° N, 26.70° E, elevation 41 m). All of the shoots used for this experiment are clones from the same tree, thus minimizing genetic variation effect among replicates. The shoots, grown in the field in natural conditions and natural soil, were cut under water and always transferred to the laboratory in the morning around 9:00. The shoots underwent a period of adaptation to the measurement conditions by keeping them at room temperature beneath a 500 W halogen lamp providing a quantum flux density of ca. 350 μmol m^{-2} s^{-1} at leaf level. Measurements were performed in leaves that had an area ranging between 3.2 to 65.2 cm^2 and corresponded to the first (ca. 1-2 days old) to the ninth (ca. 14 days old) leaf position from the tip of the shoot, respectively (Fig. 1b).

Experimental setup

The experimental setup and procedure were similar to the experiment in Portillo-Estrada et al. (2015b). We measured leaf net CO_2 assimilation rate by enclosing the leaves in a standard 8 cm^2 leaf cuvette (3010-S of Walz GFs-114 3000) of a GFS-3000 gas-exchange system (Walz GmbH, Effeltrich, Germany). A LED array/PAM-fluorimeter 3055-FL (Walz GmbH) was used for leaf illumination with a saturating quantum flux density of 500 μmol m^{-2} s^{-1}. Leaf temperature was kept constant at 25 °C. The cuvette was flushed with ambient air at a flow rate of 750 μmol s^{-1}. Air humidity was maintained at a constant level (16000 ppm H_2O, approx. 60% relative humidity) and CO_2 concentration was 400 μmol mol^{-1}.

Simultaneously with photosynthesis, constitutive leaf volatile emissions were measured online by a PTR-TOF-MS (proton-transfer-reaction time-of-flight mass spectrometer) model 8000 (Ionicon Analytik GmbH, Innsbruck, Austria). The details of the measurement principle, parameters and calibration are described in Portillo-Estrada et al. (2015b). We measured 26 relevant volatile molecules emitted by poplar leaves constitutively and after mechanical wounding (see the detailed list in Table 1 in Portillo-Estrada et al. (2015b)). Among these, the group of lipoxygenase products (LOX products) included C_5 and C_6 compounds derived from the oxidation of linoleic acid, present in the cell membranes (see biosynthetic pathways in Fall et al. (1999) and Fall et al. (2001)).

Once the leaves had reached steady levels of CO_2 exchange, water vapour, and isoprene emission at saturating light level, we averaged three consecutive measurements of CO_2 exchange as an estimate of photosynthetic capacity (A_{max}). Isoprene, methanol, and acetaldehyde emission levels at maximum photosynthetic capacity were estimated by averaging a series of data of ca. 2-3 minutes during the steady state. A total of 45 leaves of different age were measured for constitutive VOC emissions.

The dataset of constitutive isoprene emission through leaf expansion was fitted to an "exponential rise to maximum" equation where the theoretical values would achieve a maximum during leaf maturity (Eller et al. 2012; Sun et al. 2013). Following the same principle (Rasulov et al. 2014; Sun et al. 2013), photosynthetic capacity was fitted to the

same function type. As for constitutive methanol emission, we used a negative exponential equation knowing that during leaf maturity there is also a basal level of methanol emission (Eller et al. 2012) and that other authors found a higher emission at the top of poplar shoots (Nemecek-Marshall et al. 1995).

Leaf wounding

Volatile emissions induced by wounding with a hole puncher were tested in leaves of increasing age: first nine leaves from the shoot top. The punch hole area was 19.07 ± 0.15 mm^2, and its perimeter 15.48 ± 0.06 mm, which was used to express the volatile emission rates per unit wound length (mm). We used the punch hole procedure to perform the mechanical damage because of the rapidness of the wounding treatment and its high replicability in producing wound edges of given length (Portillo-Estrada et al. 2015b).

The volatile emissions induced during the few minutes following the wounding were integrated and expressed by wound length. Seven minutes of emission data were enough to record the first emission burst of volatiles related to the wounding, after which the emission levels came back to pre-wounding values.

The datasets of wound-induced volatile emission though leaf expansion were fitted to a negative exponential equation assuming that the emission of the volatiles of study was positive or close to zero during leaf maturity, as shown by some studies (Brilli et al. 2011; Portillo-Estrada et al. 2015b).

Leaf area and leaf age

Once leaf cuvette measurements were finished, the leaf petiole was removed and the leaf area measured by scanning the leaf blade. Leaf area picture processing occurred as in Portillo-Estrada et al. (2015a) to minimize errors in the leaf area estimation due to shadows in the leaf picture.

Leaf age was estimated from leaf area after modelling the data published by Rasulov et al. (2015) on the evolution of the leaf area during leaf expansion of clonal *P. tremula* individuals used for this experiment. The data was fitted to a sigmoidal function ($r^2 = 0.998$, $P < 0.001$):

$$L_{area} = 1.062 + \left(\frac{52.167}{1 + e^{-\left(\frac{L_{age} - 7.575}{2.399} \right)}} \right), \quad \text{(Eqn 1)}$$

and then inversed to a logit function to estimate the leaf age (L_{age}, in days) based on the leaf area (L_{area}, in cm^2):

$$L_{age} = 7.575 - 2.399 \times ln \left(\frac{52.167}{L_{area} - 1.062} - 1 \right). \quad \text{(Eqn 2)}$$

Leaf chlorophyll content

Circular leaf discs of 1 cm diameter were taken by a cork borer and stored at -80 ℃ for further analysis of leaf chlorophyll content. Leaf discs were ground with Precellys 24 tissue homogenizer (Bertin Technologies, France) in Precellys lysing kits for soft tissue CK 14 (2 mL plastic tubes with 1 mm diameter plastic balls inside) at 0 ℃ in 100 % acetone with added calcium carbonate. The extracts were then centrifuged and filtered through a 0.45 μm PTFE membrane filter. Leaf chlorophyll content was determined with an Agilent Technologies 1200 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) using a linear gradient of acetone concentration in water as in Opris et al. (2013).

Leaf chlorophyll content (mmol m^{-2}) increase through leaf age was fitted with the same equation type than photosynthetic capacity, knowing that leaves will achieve a maximum of chlorophyll content at maturity (Rasulov et al. 2014).

Results

Photosynthesis and constitutive volatile emissions throughout leaf expansion

We confirmed a highly significant ($r^2 = 0.88$ and $P < 0.001$; multiple linear regression analysis) three-way interaction between leaf age (L_{age}, in days), leaf chlorophyll content (L_{Chl}, in mmol m^{-2}) and net photosynthetic capacity (A_{max}, μmol m^{-2} s^{-1}) at a constant saturating photosynthetic photon flux density:

$$A_{\text{max}} = -1.38 + 0.54 \times L_{\text{age}} + 17.88 \times L_{\text{Chl}}. \quad \text{(Eqn 3)}$$

The equation was also significant ($r^2 = 0.84$ and $P < 0.001$) when accounting for leaf area (L_{area}, in cm^2) instead of leaf age:

$$A_{\text{max}} = -2.72 + 0.0451 \times L_{\text{area}} + 27.36 \times L_{\text{Chl}}. \quad \text{(Eqn 4)}$$

As leaves expanded, leaf chlorophyll content raised to a level of 0.4 to 0.5 mmol m^{-2} (ca. 40 to 50 μg cm^{-2}) (Fig. 1a) and leaf photosynthetic capacity attained a level of 10-15 μmol m^{-2} s^{-1} (Fig. 1c) following the trend of an "exponential rise to maximum" function in both cases. Photosynthetic capacity linearly increased as leaf chlorophyll content increased during leaf development (Fig. 1d).

When applying the same light and temperature conditions to *P. tremula* leaves of increasing age, the constitutive volatile emissions of isoprene increased exponentially to a maximum, reached after 10-15 days of development (Fig. 2a). Leaf isoprene emission positively correlated with the net CO_2 assimilation rate (Fig. 2b). Methanol emission rates were higher as leaves were younger and smaller (Fig. 2c). We also found a vague relationship ($r^2 = 0.31$; $P = 0.001$) between leaf methanol emission rate and leaf photosynthesis (Fig. 2d).

We could visually identify a threshold at one week of leaf development (typically a leaf blade of 5.5 cm in length at the midrib level, maximum 5 cm in width, leaf area of ca. 20

cm^2, and 7 days of development) where the exponential character of the relationships (Fig. 1a and c) turned to an almost steady response against the *x*-axis (leaf area) also showing larger variability in leaf chlorophyll content and photosynthetic capacity. In accordance with that, leaf chlorophyll a+b content of the youngest leaves (smaller than 20 cm^2) was smaller, 0.307 ± 0.032 mmol m^{-2} (or 27.4 ± 2.9 µg cm^{-2}, average $\pm$ SE), than for the more mature leaves (larger than 20 cm^2), 0.466 ± 0.010 mmol m^{-2} (or 41.6 ± 0.9 µg cm^{-2}) ($P < 0.0001$, two-tailed Student's *t*-test) (Fig. 1a). Leaf net CO_2 assimilation was also significantly ($P < 0.0001$) smaller in younger leaves, 5.7 ± 0.7 µmol m^{-2} s^{-1}, than in larger and more mature leaves, 10.88 ± 0.39 µmol m^{-2} s^{-1} (Fig. 1c).

Concerning the constitutive volatile emissions, isoprene emission rates were lower ($P < 0.0001$) in younger leaves, 2.60 ± 0.49 nmol m^{-2} s^{-1}, than in older leaves, 6.24 ± 0.43 nmol m^{-2} s^{-1} (Fig. 2a). Contrarily, leaf methanol emission rate during the first week of leaf expansion (leaf area smaller than 20 cm^2) was significantly ($P < 0.0001$) higher, 5.43 ± 0.17 nmol m^{-2} s^{-1}, than in more mature leaves, 4.09 ± 0.13 nmol m^{-2} s^{-1} (Fig. 2c).

The changes of leaf chlorophyll content, photosynthetic capacity, and constitutive emissions of isoprene and methanol with increasing leaf area (cm^2) can be found in Figure S1, Suppl. 1.

Wound-induced volatile emissions throughout leaf expansion

Mechanical damage (leaf punch) was performed to leaves of increasing leaf age (i.e. increasing leaf size), and the subsequent induced volatile emissions measured.

The sum of all volatiles emitted during the post-wounding period was larger in younger leaves than in further developed leaves (Fig. 3a), and similarly occurred in LOX products (Fig. 3b) and acetaldehyde (Fig. 3c) emissions. Contrarily, leaf age did not have an effect on methanol wound-induced emission (Fig. 3d). At a much smaller level (lower than 1 pmol mm^{-1} cut length, i.e. three orders of magnitude smaller than LOX products), the wound-induced emissions of methyl benzoate (Fig. 3e), methyl salicylate (Fig. 3f), monoterpenes (Fig. 3g), and sesquiterpenes (Fig. 3h) presented significant relationships with the leaf age, following an exponential decay function in all cases.

The relationships of leaf wound-induced emissions with leaf area can be found in Figure S2, Suppl. 1.

Discussion

Leaf chlorophyll content, photosynthesis, and isoprene emission throughout leaf ontogeny

In previous studies on hybrid poplar, leaf chlorophyll content and photosynthetic capacity were observed to increase rapidly during early leaf development (Rasulov et al. 2014; Reich 1983), both parameters being positively interrelated (Reich 1983). Similarly to Reich (1983), we found that linear fitting was the best option to model the increase of the light-saturated photosynthesis over rising chlorophyll content (Fig. 1d).

Leaf net photosynthesis raised exponentially during leaf ageing and reached a maximum value at ca. 10 and 15 $\mu mol\ m^{-2}\ s^{-1}$ in mature leaves. These CO_2 assimilation levels as well as the concomitant isoprene emissions during leaf ontogeny (Fig. 2a) are in accordance with the findings of Sun et al. (2013) during poplar canopy development, confirming Hypothesis 1. Moreover, the observed isoprene emission levels (Fig. 2a) were comparable to those reported by Loreto et al. (2007) at different leaf positions from the apex (also studying the effects of leaf ontogeny) at ambient CO_2 concentration and at a temperature of 25 °C.

The correlation between isoprene emission and photosynthesis rates (Fig. 2b) is well known throughout scientific literature, especially when one considers the instantaneous responses of isoprene emission and photosynthesis to changes in light intensity (e.g., Grote et al. 2013a; Monson 2013). Contrarily to light manipulation experiments where isoprene emission is related to photosynthesis, different relationships can be observed across long-term environmental gradients (Rasulov et al. 2015) and throughout leaf ontogeny (Niinemets et al. 2015; Rasulov et al. 2014). In fact, throughout leaf lifespan, isoprene emission is typically induced somewhat later than positive net photosynthesis rates are observed (Harley et al. 1994; Rasulov et al. 2014). In our case, we measured the maximum photosynthetic capacity at saturating light level and associated maximum isoprene emissions at a given leaf age (Fig. 2a, b). Clearly, isoprene emission and photosynthesis rate were positively correlated, but we nevertheless want to emphasize that using leaves of different age and shoots integrates random effects that make the observed relationship somewhat scattered (Fig. 2b).

Despite the precise knowledge on the isoprene biosynthetic pathways, the evolutionary and ecological reasons of leaf isoprene emission are still unclear, but different plausible hypotheses have been proposed (Sharkey et al. 2008; Vickers et al. 2009). Isoprene emission is responsive to leaf temperature and sensitive to light input, but ultimately related to isoprene synthase activity (Kuzma and Fall 1993) and dimethylallyl diphosphate pool size (Niinemets and Sun 2015), that we hypothesize smaller in younger leaves, as observed by Rasulov et al. (2014).

Constitutive methanol emissions during leaf development

Methanol emission has been shown as plant waste product that, due to its solubility in water and high volatility, is unavoidably emitted (Peñuelas and Llusià 2004). Despite that, Peñuelas et al. (2005) suggested a potential ecological role in mediating plant-insect interactions after measuring large emission amounts caused by caterpillar feeding (Peñuelas et al. 2005). In addition, a controlled mechanical wounding experiment on mature poplar leaves showed that methanol emission can constitute 15% of wound-induced emissions and is highly correlated with the degree of damage (Portillo-Estrada et al. 2015b).

As proposed by several authors, constitutive methanol emission peak is closely related to leaf expansion. This is due to pectin demethylation during cell wall expansion (Harley et al. 2007; Karl et al. 2003). In our study, constitutive methanol emission consequently peaked at the earliest developmental stage (Fig. 2c), when the growth rate was presumably at its highest (Sun et al. 2013). This has been also found by other studies (Fares et al. 2010) and confirms Hypothesis 2. There was also a weak relationship between constitutive methanol

emission and photosynthesis (Fig. 2d), most probably driven by the simultaneous leaf expansion and net assimilation rate (Fig. 1c).

Wound-induced volatile emissions related to leaf age

Total volatile emissions after wounding were constituted mainly by LOX products, acetaldehyde and methanol emissions (Fig. 3). The level of wound-induced emissions was dependent on leaf ontogenetic level in many cases, which confirms Hypothesis 3.

Methanol, as well as emitted as a product of leaf expansion, it can be emitted due to oxidative stress. Such conditions occur upon leaf wounding (Brilli et al. 2011; Loreto et al. 2006; Portillo-Estrada et al. 2015b). However, we did not find a higher elicitation of methanol emission in younger leaves and it was steady throughout leaf expansion (Fig. 3c). The cause could be related to the previously mentioned methanol high volatility and solubility in water. For that reason, methanol emission is directly related to stomatal conductance, preventing the formation of a stock within the leaf. Therefore methanol pool size in the cytosol and interstitial spaces of leaf mesophyll could be always small regardless of leaf age. This makes inevitably high methanol emission in young leaves certainly *de novo* emission (not originated from a stock). In conclusion, upon leaf wounding and breakage of leaf tissues, no additional methanol would be released from any stock in younger leaves.

Acetaldehyde is one of the most frequently identified oxygenated compounds emitted from leaves (Monson 2013). It could be related to leaf wounding in the way that the pool of acetyl-CoA may react with C_6 aldehydes synthesized upon wounding (in the LOX product blend) to form C_6 acetates, and acetaldehyde could leak from the acetyl-CoA pool during this reaction (Graus et al. 2004). The question lies in why younger leaves could hold a bigger acetyl-CoA or pyruvate pool, which are precursors of acetaldehyde emission. Young leaves have been associated with higher growth respiration (Loreto et al. 2007), dark respiration (Rasulov et al. 2014) and mitochondrial activity (Dickmann et al. 1975) than older leaves. Therefore the higher activity could explain a higher acetyl-CoA and pyruvate content in younger poplar leaves, making the response to wounding greater through acetaldehyde emission.

LOX products were emitted in higher amounts by younger leaves. Linoleic acid is the precursor of LOX products (Fall et al. 1999; Fall et al. 2001), and it is found in cell membranes. In principle, young leaves could have smaller content of linoleic acid per unit area in comparison to older leaves, which are thicker and supposedly have more membranes per area unit. However, the content of lipoxygenase isozymes in young leaves is by far higher than in older leaves (Saravitz and Siedow 1995). This suggests that the higher enzymatic activity may therefore drive the higher response in younger leaves, as we observed (Fig. 3b). In ecological terms, a higher LOX product emission is beneficial for younger leaves in keeping herbivores away, because young leaves with thinner cell walls (Tosens et al. 2012) lack constitutive mechanical defences and also contain less non-volatile defensive metabolites such as condensed tannins (Kursar and Coley 1991). In addition, young leaves have been shown to be more heavily consumed by herbivores (Dudt and Shure 1994; Johnson et al. 1984; Southwood et al. 1986). Nevertheless, we must acknowledge that the results of our study cannot be fully extrapolated to a herbivory situation. Bricchi et al.

(2010) found significant differences in leaf responses to mechanical wounding in comparison to real herbivory: membrane polarization and volatile blend composition; most probably due to the lack of herbivore-derived oral secretions in mechanical wounding.

At a much lower level, monoterpenes, sesquiterpenes, and methylated benzenoids as MeBe and MeSa were emitted after leaf wounding. Their emission level depended also on leaf age. The emission of LOX products is an ubiquitous response across species and stresses, whereas the emission of volatile mono- and sesquiterpenes and benzenoids such MeSa are stress dependent, reflecting selective activation of genes after the stress event (Copolovici et al. 2014; Possell and Loreto 2013). Monoterpenes and sesquiterpenes were most likely emitted as a result of the exposure of the non-specific pools of these volatiles in leaf lipid and liquid phases (Niinemets and Reichstein 2002) to the ambient upon leaf wounding, and therefore should not reflect *de novo* emissions. Newly synthesized isoprenoids normally peak in the subsequent hours to days after wounding (Erb et al. 2015). Their accumulation in leaf structures (especially in conifer needles) protects the plant against herbivory by decreasing leaf palatability (Dicke and Baldwin 2010) and may help to seal leaf wounds (Loreto et al. 2008). But poplar leaves do not have specialized structures to store terpenoids. However, a basal synthesis level could happen in poplar leaves, and according to our results, could be greater in younger leaves.

The synthesis and emission of volatile hormones such as MeBe and MeSa have been found to attract natural predators during plant feeding as a defence response to plant herbivory (War et al. 2011; Zhao et al. 2010) as well as repel parasites themselves (Snoeren et al. 2010). However, the peak of synthesis of MeBe and MeSa normally occurs hours to days after the wounding (Niinemets and Monson 2013; Staudt et al. 2010), but our results then suggest that there could be a minimal basal synthesis forming a small pool of these compounds in younger leaves. Our results are in agreement with findings that in general, young leaves of *Populus* spp. have a greater capacity for biotic stress-dependent induction of monoterpene emission than older leaves (Brilli et al. 2009).

Fares et al. (2010) also found a higher level of stress-induced volatile emission in younger leaves when exposed to oxidative stress (2-week exposure to 80 ppb of ozone). In particular, they found a higher amount of LOX products in younger leaves. In accordance to our results, the level of stress-induced methanol emission did not depend on leaf age during poplar leaf expansion. Our study evidences an effect of leaf age on stress-induced volatile emissions in poplar. More research is needed to characterize this relationship across other species.

Conclusion

This study provides evidence of major variation in wound-induced volatile release through leaf expansion. However, the key implication of the study is that even for the same species and tree shoot, volatiles released in response to the elicitor of given strength can vastly vary in dependence on leaf ontogeny. As the level of constitutive defences increases with leaf age, the strength of the volatile signal is expected to be gradually reduced. The higher elicitation of BVOC emissions (especially LOX products) in younger leaves could be an evolutionary defence against herbivory, given that younger leaves are usually more subject to infestation

and herbivory. Further studies are needed to test whether herbivory also causes similar BVOC emission level changes through leaf development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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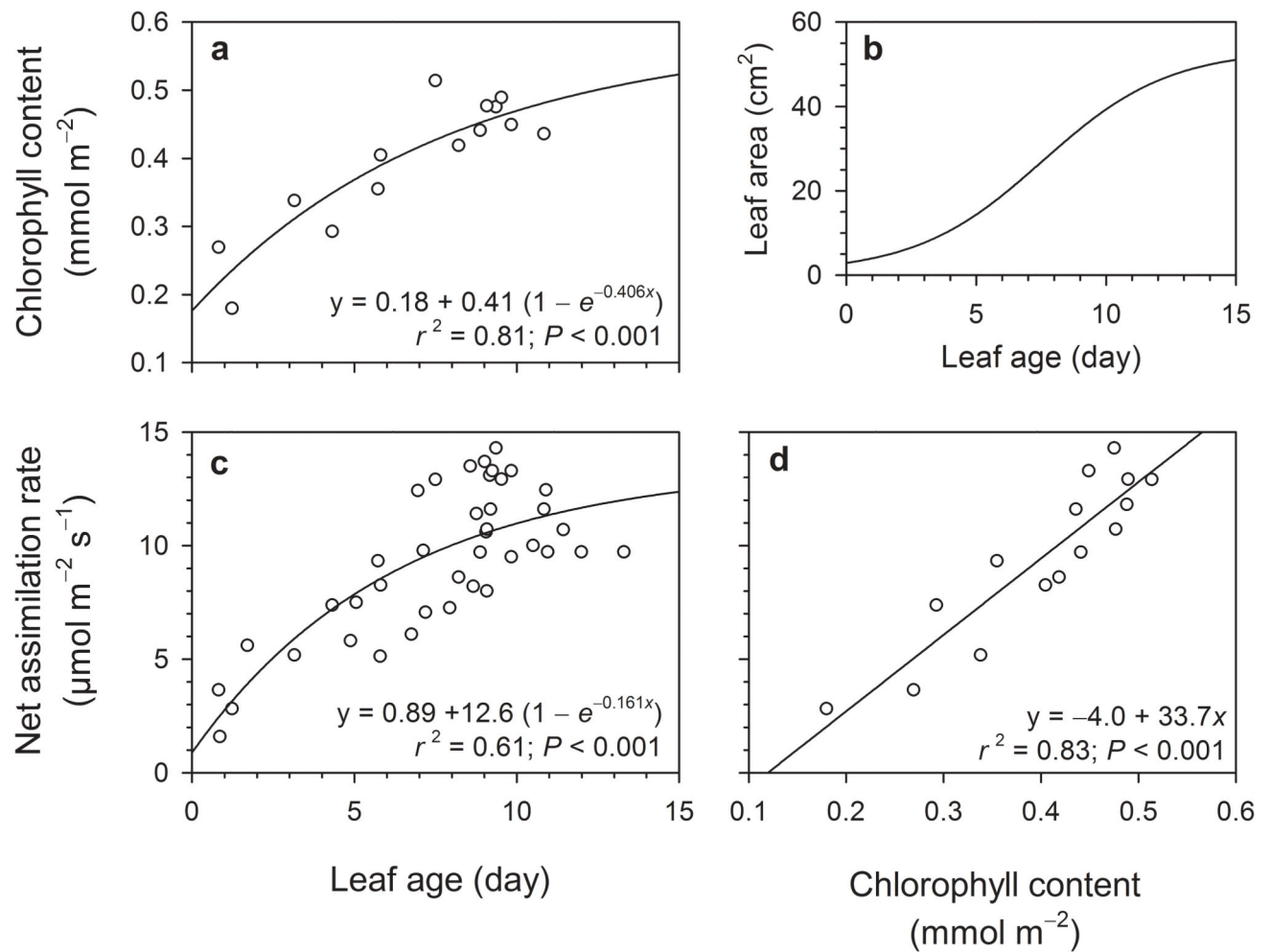
Figure 1.

Ontogenetic changes in (a) the chlorophyll a+b content, (b) leaf area, and (c) photosynthetic capacity in leaves of temperate deciduous broad-leaved poplar (*Populus tremula*). Panel (d) shows the linear relationship between leaf photosynthetic capacity and chlorophyll content. In (b), leaf size increases with increasing leaf age according to a logistic (or sigmoidal) relationship (see Sun et al. (2013) and Rasulov et al. (2015) for characteristic growth curves of poplar leaves with 5 cm^2 leaves being ca. 2 days old, and 50 cm^2 leaves 14 days old.
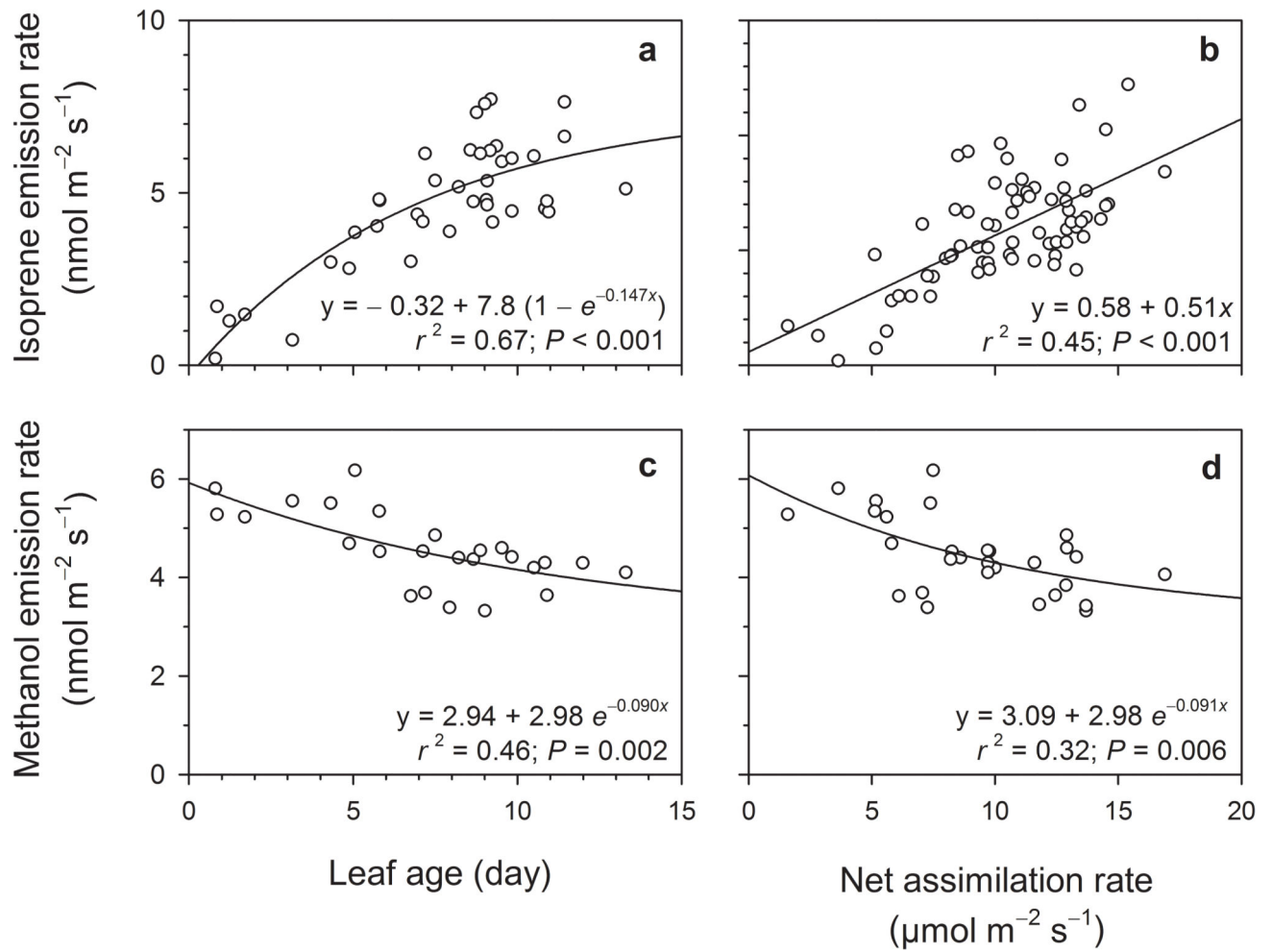
Figure 2.
Leaf isoprene and methanol emission rates in relation to (a, c) the leaf age and (b, d) leaf photosynthetic capacity in leaves of *Populus tremula*.
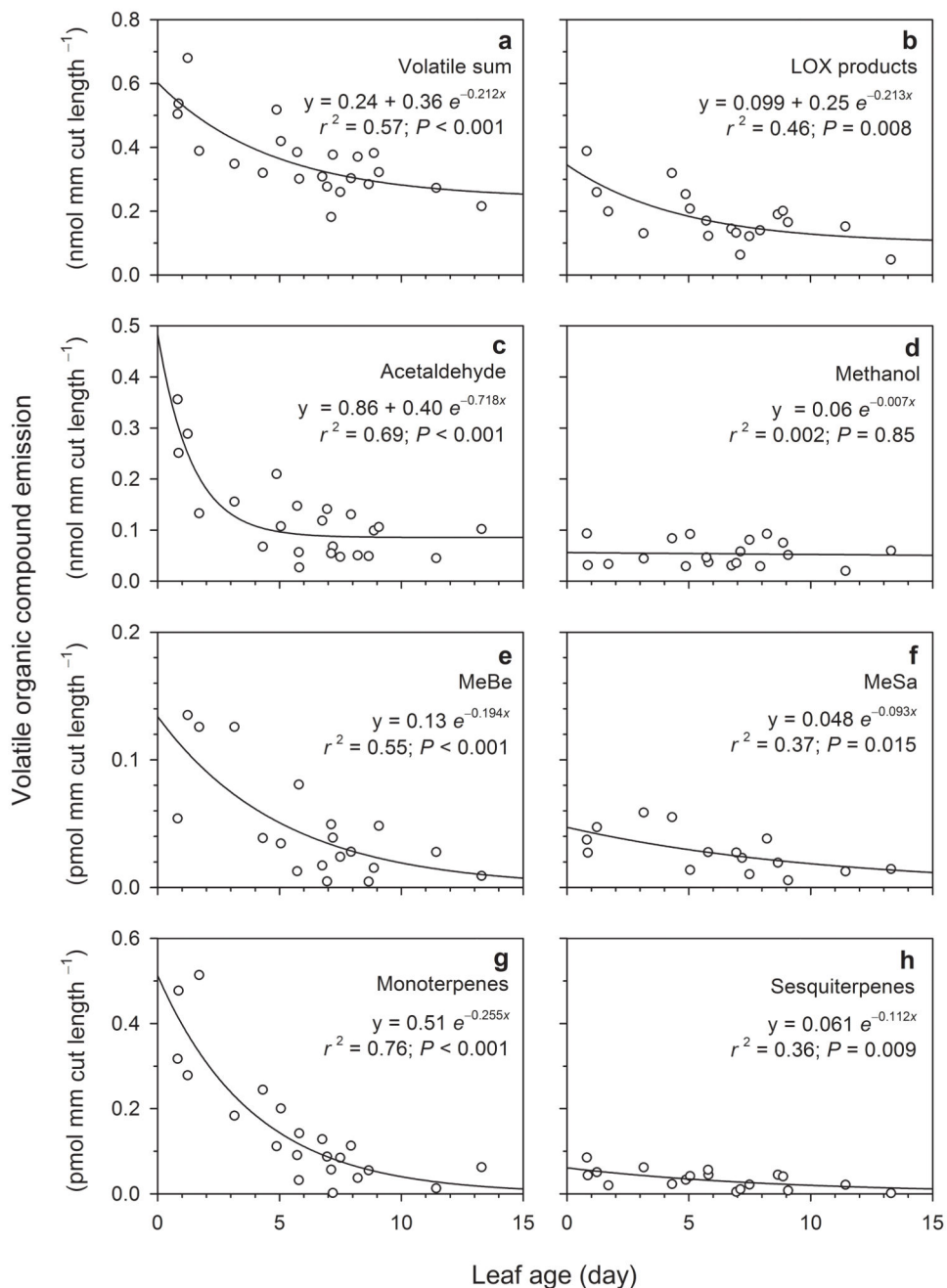
Figure 3.
Correlations of the integrated volatile emissions released by leaf wounds to leaf age *Populus tremula* leaves. The wounds were produced by a single punch hole (15.48 mm edge length). In (a), the sum of volatiles includes 26 relevant compounds and in (b), LOX products include C_5 (pentenyl family) and C_6 (hexenal and hexanal families). For full list of compounds we refer to Portillo-Estrada et al. (2015b). MeBe (e) stands for methyl benzoate, and MeSa (f) for methyl salicylate.