Production of glandular trichomes responds to water stress and temperature in silver birch (*Betula pendula* Roth) leaves

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ABSTRACT

Silver birch (*Betula pendula* Roth) allocates substantial resources into the production of glandular trichomes. If these trichome can protect trees from temperature and water stress, their production would be expected to increase under these conditions. We studied how glandular trichome density and number in the leaves of 2-year-old silver birch plantlets respond to single and combined treatments of elevated temperature (+1 °C) and three different levels of soil moisture (low, normal, and excess watering). Moreover, we quantified the seasonal variation in trichome density in mature long-shoot leaves of young, greenhouse-grown silver birches.

Our results demonstrate clear differences between responses of glandular trichomes on different leaf surfaces. On the adaxial leaf surface, both drought and elevated temperature reduced the production of glandular trichomes. Interestingly, this response was absent in plants subjected to the combined treatment. Glandular trichome production on abaxial leaf surface increased considerably in leaves produced during the growing season, reflecting a seasonal trend. Maintaining strong seasonal increase in trichome production of abaxial surfaces even in low-water conditions suggests an important, though still unknown, role for abaxial glandular trichomes. In silver birch stems those trichomes are strongly responsible for herbivore defense.

Keywords: glandular trichomes, drought, elevated temperature, seasonal variation, silver birch

1. Introduction

Current climate-change models for the boreal and hemiboreal forest zone on the Northern Hemisphere predict an increase in the frequency of hot days (Field et al. 2014). Increasing temperature in the boreal zone could lead to increased growth of forest trees (e.g. Kellomäki & Väisänen 1997), but also leads to higher evaporation. This in turn decreases the soil water content, exposing plants to drought during the growing season (Jylhä et al. 2009). Increased precipitation in northern Eurasia is expected to occur mainly in winter months (Field et al. 2014), decreasing its potential to alleviate drought. Water shortage induces stomatal closure in leaves, stopping the evaporational cooling and increasing leaf temperatures (Grant et al. 2006). Co-occurring high temperatures and low soil moisture levels modify the physiological functioning and optimal allocation of carbon into new structural components in trees compared to the situation under single stress (Niinemets 2010). Glandular trichomes – uni- or multicellular projections of epidermis, which are able to produce and secrete exudates (Wagner 1991) – affect the physical and chemical characteristics of the leaf surface, an interface regulating the transportation of water and heat between the leaf and the environment (Gutschick 2012). Thus, glandular trichomes may have a role in acclimation to adverse temperature and soil moisture conditions through their effects on evaporation and thermal dissipation, as has been shown for non-glandular trichomes, hairy leaf surface structures with no secretory activity, in a perennial shrub, *Encelia farinosa* A. Gray ex Torr., and two tree species, *Olea europaea* L. and *Mallotus macrostachyus* (Miq.) Müll. Arg. (Sandquist & Ehleringer 2003; Guerfel et al. 2009; Kenzo et al. 2008).

Silver birch (*Betula pendula* Roth) is a pioneer species, common both in naturally regenerated and cultivated forests across Northern Europe (Hynynen et al. 2010) and Russia (Zyryanova et al. 2010). Although silver birch is likely to benefit from increasing summer temperatures in many parts of its range (e.g. Lavola et al. 2013), the combination of drought and high temperature may also cause stress. Glandular trichomes of silver birch accumulate triterpenoids and flavonoid aglycones (e.g.

Valkama et al. 2003), which are excreted on the surface of expanding leaves (Laitinen et al. 2002, Valkama et al. 2004). Both types of compounds contribute to the hydrophobicity of cuticular waxes (Keinänen & Julkunen-tiitto 1998), decreasing cuticular permeability to water (Barnes et al. 1996).

Since trees in the Northern Hemisphere – including the economically and ecologically important silver birch – will experience increasing temperatures and prolonged periods of adverse soil moisture conditions in the future, it is important to understand the role of each structural component of leaves for acclimation and adaptation of trees. This understanding is essential also for tree breeding, in which genotypes with high tolerance traits and superior growth are preferred. Since non-glandular trichomes can protect plants against excessive temperature and drought (e.g. Sandquist & Ehleringer 2003; Guerfel et al. 2009; Kenzo et al. 2008) it is interesting to study whether the glandular trichome production responds to elevated temperature and altered soil moisture. Increased production would suggest that glandular trichomes are part of the acclimation strategy of silver birch to these stress factors.

Trichomes are produced either in the bud, prior to the development of the leaf epidermis (Valkama et al. 2004), or also in mature leaves (Maffei et al. 1989). The number of protodermal cells differentiating into trichomes determines the final number of trichomes per leaf. In young silver birch leaves, trichome density declines as epidermal cells enlarge and move trichomes farther away from each other until the leaves have fully expanded (Valkama et al. 2004). This suggests that trichome density depends on the initial number of differentiating epidermal cells as well as the final area of fully expanded leaves. Therefore, treatments affecting leaf area are bound to have an effect also on trichome density.

We investigated the effects of soil moisture and increased air temperature on the number and density of glandular trichomes on both the upper (adaxial) and lower (abaxial) surface of long-shoot leaves of two-year old silver birch plantlets. Graphical vector analysis was used to identify true responses in trichome production from changes in trichome density caused by changing leaf area. We hypothesized that the production of glandular trichomes in silver birch is adjusted in response to a changing environment. More specifically, as leaf water and thermal economics are intertwined, there may be interactive effects of elevated temperature and soil moisture on the number and density of glandular trichomes. If glandular trichomes are important for regulating temperature and water economics of silver birch leaves, we expect the production to increase in response to these stresses. If not,decreased availability of photosynthates caused by stress may decrease the carbon allocation into glandular trichomes, decreasing the trichome numbers.

2. Materials and methods

2.1 The experimental setup and leaf sampling

Eight silver birch genotypes (4, 8, 12, 14, 18, 19, 23, 26) were randomly selected from a stand of silver and downy birch (*B. pubescens* Ehrh.), regenerated naturally after logging in 1979 in Punkaharju, Finland (61°48'N, 29°18'E). The eight genotypes were micropropagated from cuttings 30 cm in length, sampled from the upper third of parental trees. Cultivations were started from buds on woody plant medium (WPM), with BAP (6-benzylaminopurine, 0.9 mg l^{-1} at the initial medium, 0.2 mg l^{-1} thereafter) and IBA (indole-3-butyric acid, 0.2 mg l^{-1}) to induce root formation. The plantlets were grown in greenhouses at the Suonenjoki Research Nursery (Natural Resources Institute Finland, Suonenjoki Unit, Finland, 62°38'N, 27°03'E) following standard nursery protocol. The plantlets, moved to 7.5 l pots (MCI26, Schetellig Oy, Vantaa, Finland) filled with nursery peat (Novarbo Metsätaimiturve B1F, Novarbo Oy, Eura, Finland) in early May 2011, were randomly assigned to the different treatments 6 June 2011 (average height 44.0 ± 0.1 cm). The plant material and study set-up is described in detail in Possen et al. (2015).

The experiment consisted of three replicates (blocks), each containing six plots with different treatments (full factorial design, combining two temperature and three watering levels). Plots were systematically arranged into the blocks so that heating and watering levels alternated. Each plot contained four plantlets for each of the 8 genotypes with randomized position within plot, and was surrounded by a row of shelter plants. Three infrared heaters per plot (CIR110, Frico AB, Göteborg, Sweden, 1000W, wavelengths >800 nm) were placed above the plots with elevated temperature treatment ('H' for Heated in figures). The plots without temperature treatment ('A' for Ambient in figures) received no heaters, but were fitted with wooden dummy heaters of the same size to mimic possible shading effects. Warming lasted 12 weeks, starting after the transfer of the plantlets to the greenhouses (6 June 2011) and ending when the plantlets had dropped their leaves in autumn (24 October 2011).

The height of the heaters and dummies was adjusted regularly to ensure a distance of 1.0 m between the heaters and the top of the plantlets. Throughout the whole experiment, the temperature in both elevated and ambient-temperature plots was monitored every 15 minutes at 1.2 m below the heaters (i.e. 20 cm under the top of the plantlets) using thermocouples and temperature sensors (Hobo H08-032-08, Onset, Bourne, MA, USA). The increase in air temperature in the heated plots was on average +0.88 °C, and thus close to the target of + 1 °C. However, according to leaf temperature measurements by Possen et al. (2015), leaf surface temperatures in the ambient temperature and warming plots differed on average by +1.9 °C, which is comparable to an earlier field study in which a similar warming system was employed (Riikonen et al. 2009).

In order to study the effect of water availability, three watering treatments were established: low watering (volumetric water content, VWC, 20-30 %, 'L' in figures), normal watering (VWC 40-50 %, 'N' in figures) and excess watering (VWC >60 %, 'E' in figures). VWC in the low treatment was close to the wilting point of the peat used, while VWC in the excess treatment represents the maximum water-holding capacity of the peat. After the plants were transferred to the greenhouses, VWC was kept close to normal for all plantlets until the start of the watering treatments, when VWC was gradually increased or decreased to the target level within a period of one week. The watering treatment lasted for five weeks (from 11 July until 12 August 2011), after which the VWC was gradually returned to normal for all plantlets within a period of one week. In this way, leaf samples could be collected before, during and after the watering treatment from plantlets already acclimated to increased temperature. The setup aims to mimic the predicted climate conditions in Finland, with infrequent periods of low or high precipitation superimposing consistently higher average temperature.

2.2 Trichome density and leaf area measurements

Silver birch has two types of leaves: the short-shoot leaves, opening rapidly in spring from buds produced in the previous season, and the long-shoot leaves which are produced at the new branches grown during the same season (Maillette 1982). The youngest mature long-shoot leaf on the main stem was sampled from each plantlet for trichome density and leaf area measurements. This leaf was chosen to ensure that the sampled leaves share a similar history of light environments and are of the same physiological age. Sampling took place on 8 July (before the start of the watering treatment, week 28), 11 August (after five weeks of watering treatment, week 33), and 1 September (two weeks after the end of the watering treatment, week 36). From the leaves sampled on week 36, adaxial trichomes were counted only from 6 and abaxial trichomes from 5 genotypes.

Leaf areas (LA) were measured immediately after harvesting using a portable leaf area meter (Li-3000, Li-Cor Inc., Lincoln, Nebraska, USA; Possen et al. 2014, Possen 2015). Then, to evaluate trichome density the leaves were cut in half along the midrib and pressed on a microscope-slide sprayed with glue, one leaf-half for adaxial and the other for abaxial trichome measurements. Glandular trichomes completely visible in the field of view (0.13 cm^2) of a 6.4 magnification stereomicroscope (Zeiss Stemi SV8, Zeiss W10x/25) were counted from halfway between the midrib and leaf edge, between the 3rd and the 4th leaf vein counting from the leaf base. Each microscope view was counted twice and the mean of both counts was used to calculate trichome density (Den) as $\mathrm{Den}\ (\mathrm{cm}^{-2}) = \frac{\text{mean of counts}}{\text{viewed area } (\mathrm{cm}^2)}$. The total number of glandular trichomes per leaf (N) was calculated by multiplying glandular trichome density (Den) with total leaf area (LA), as $\mathrm{N} = \mathrm{Den}\,(\mathrm{cm}^{-2}) * \mathrm{LA}\,(\mathrm{cm}^{2})$.

2.3 Statistical analyses

Linear Mixed Models in the package lmer (Kuznetsova et al. 2016) in R v3.2.2 (R Core Team, 2015) were used to assess the sources of variation in glandular trichome density and (ln-transformed) number, with separate models for adaxial and abaxial leaf surfaces. Some samples with very high variability between counts were removed from the adaxial data, resulting in 378 points of data for adaxial, and 379 for abaxial surface. Various graphs of the model residuals and the Akaike Information Criterion (AIC) were used to model the covariance structure for the data set. The model for the density or ln-transformed number of trichomes in plot *i*, plant individual *j*, genotype *k* and week *l* as

$$y_{ijkl} = \underline{\beta}'\underline{x}_{ijkl} + a_i + b_k^{(1)} w1_{ijkl} + b_k^{(2)} w2_{ijkl} + b_k^{(3)} w3_{ijkl} + c_{ikj} + \varepsilon_{ijkl},$$

where the fixed part $\underline{\beta}'\underline{x}_{ijkl}$ describes the effects of week, temperature, watering and interaction between week and watering and, in the adaxial models, interaction between temperature and watering. The random part includes random constants for the crossed levels of plot (a_i) and for each week separately at the clone level ($b_k^{(1)}, b_k^{(2)}, b_k^{(3)}$; $w1_{ijkl}$, $w2_{ijkl}$ and $w3_{ijkl}$ are binary indicator variables for weeks 1, 2 and 3, respectively) and the nested level for the individual within a plot-clone combination c_{ikj} to the model and, on the other hand, the dependence of observations caused by the grouping of the data to these groups. We assumed that the random effects and residuals are normally distributed, have zero mean and the variance is constant among groups of a given level. The random effects of different levels are uncorrelated but the covariances between $b_k^{(1)}$, $b_k^{(2)}$, and $b_k^{(3)}$ were nonzero. The fixed effects were tested using conditional F-tests using Satterhwaite approximation for the degrees of freedom from the group-specific predictors. Based on conditional F-tests, the non-significant interaction between Heating*Watering (p=0.370) did not improve model fit for abaxial trichome number, and was thus excluded from this model. Additionally, a non-significant 3-way interaction Heating*Watering*Week ($p = 0.226–0.485$ for all models), was excluded from the final models based on conditional F-tests. For significant predictors, Tukey-adjusted multiple comparisons were used for post-hoc tests between individual levels. Linear correlation between leaf area and trichome density was studied by Pearson correlation coefficients in IBM SPSS Statistics for MacIntosh 22.0.0.0 (IBM Corp, Armonk, NY, USA).

Graphical vector analyses (Haase & Rose 1995) were performed for all factors which were statistically significant following from linear mixed models and multiple comparisons. The relationship between concentration (mg g^{-1}) and content (mg), as used by Haase & Rose (1995), is mathematically equivalent to the relationship between density (cm^{-2}) and total trichome number; thus, vector diagrams, previously used for analyzing shifts in nutrients (Haase & Rose 1995) or allelochemicals (Koricheva 1999), allow us to simultaneously consider trichome number and leaf area in a graphic format (Fig. S1)[1]. Vectors were drawn based on relative values, allowing comparisons of successive measurements or among different treatments. Relative values were calculated by dividing the compared values (e.g. density of adaxial trichomes during watering treatment, at week 33) with their reference values (e.g. density of adaxial trichomes before watering treatment, at week 28). In vectors describing the development of trichome density over time, the values at the beginning of the experiment (week 28) were used as the reference values. In vectors describing the effects of treatments, the control treatment (AN) was used as a reference. Some absolute values for leaf area have been partially published in Supplementary tables of Possen et al. (2015).

[1] Supplementary data are available with the article through the the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfr-2017-0036.

3. Results

3.1 Differences between adaxial and abaxial leaf surfaces

In greenhouse-grown silver birches, the mean density of glandular trichomes was 26% higher on abaxial leaf surfaces (351.6 ± 10.6 cm^{-2}; mean ± standard error) when compared to adaxial surfaces (279.3 ± 28.2 cm^{-2} in the control treatment). A similar result was found for the total numbers of trichomes, with 18760 ± 398 on abaxial and 15634 ± 207 on adaxial surface (control treatment). Adaxial trichome density had a slight negative correlation with leaf area in mature long-shoot leaves (Pearson's $r = -0.268$, $p<0.001$), but no correlation was found on abaxial leaf surfaces (Pearson's $r = 0.038$, $p = 0.46$).

3.2 Seasonal variation

The density and number of glandular trichomes changed on both leaf surfaces between consecutive measurements (Table 1, Table S2)[1]. On the adaxial leaf surface, trichome density decreased by 14% between July and August (p=0.04), returning to the level of early July in September. Adaxial trichome number increased by 22 % from July to September (p=0.005). Between July and August, the leaf area increased by 34% without simultaneous increase in trichome production, decreasing the adaxial density (dilution effect, Fig. 1b). The increased production of adaxial trichomes later in the season compensated for the effect of increased leaf area on adaxial trichome density by September (Table 2, Fig. 1b).

During the growing season abaxial trichome number of long-shoot leaves increased by 75% and density increased by 44%. The main increase in abaxial trichome number occurred between July and August (p=0.002), and the effect was caused by increased production of trichomes (Fig. 1d). Long-shoot leaves produced in September had 10 % smaller leaf areas than the leaves produced in

[2] Supplementary table is available at the journal web site.

August, which caused abaxial density to increase ($p<0.001$) even though the production of abaxial trichomes had already ceased (Table 2).

3.3 Effects of soil moisture and increased temperature

Elevated temperature affected adaxial, but not abaxial trichomes. Heating alone (HN) decreased the density of adaxial trichomes by 11 % ($p<0.01$) compared to the control treatment (AN; Fig. 2a). As the leaf area did not change (vector end-point is on the same diagonal in Fig. 2b), this temperature effect was caused by decreased trichome production (Fig. 2b).

Different soil moisture levels also affected adaxial trichomes. At ambient temperature, drought (AL; $p=0.014$) and excess watering (AE; $p=0.026$) decreased adaxial trichome density (Fig. 2a). Excess watering also slightly decreased trichome number ($p=0.018$). As these watering effects on trichomes were accompanied by smaller leaf areas in the respective treatments, the changes in trichome density were brought by decreased trichome production (Fig. 2b).

Soil moisture in pots differed only during the watering treatment, after which it returned to optimal. This caused interactive effects of time and watering on trichome numbers (Table 1, Table S1). In plants treated with optimal and excess watering, adaxial trichome number increased by 23% during the watering treatment (weeks 28–33; Fig. 1a), but did not change in drought-treated plants. This resulted in 23% lower adaxial trichome number in drought-treated plants than in the controls on week 33 (Fig. 1a, $p<0.01$).

The corresponding increases in abaxial trichome numbers were larger: 86% in optimal and 80% in excess watering (Fig. 1c). On abaxial leaf surface, trichome number increased substantially also in drought-treated plants. At the end of the watering treatment on week 33, the abaxial trichome number was 21% lower in low ($p<0.001$) and 12% lower in excess watering treatment ($p=0.002$) compared to the control plants (Fig. 1c). These effects were still visible during week 36 ($p<0.001$), when also the abaxial densities were lower in drought-treated plants ($p<0.001$). Differences between trichome numbers in different soil moisture levels follow the differences in leaf areas: during the five-week watering treatment, leaf area increases above 40% in well-watered (optimal and excess watering) and 17% in drought-treated plants. Trichome density was maintained in all watering treatments, despite the different leaf areas.

Different soil moisture levels and elevated air temperature had interactive effects on adaxial trichomes. Even though the elevated temperature and drought alone decreased trichome density on adaxial leaf surfaces, the drought-treated plants in elevated temperature (HL) had slightly higher number ($p=0.028$) and density ($p<0.01$) of adaxial trichomes compared to drought-treated plants grown in ambient temperature (AL; Fig. 2a).

4. Discussion

4.1 Glandular trichome production responds to soil moisture and elevated temperature on adaxial, but not on abaxial leaf surface

Glandular trichomes affect both physical and chemical characteristics of the leaf surface. In this experiment, production of glandular trichomes was decreased both under adverse soil moisture conditions and elevated temperature, but only on the adaxial leaf surface. On the abaxial side, glandular trichome production was characterised by a strong seasonal increase. Since trichome density and number are a function of leaf area, correct interpretation of our data requires both to be evaluated in conjunction. This, we have achieved through graphical vector analysis.

Drought decreased the production of glandular trichomes on the adaxial leaf surface, but only when no additional heating was involved. By decreasing leaf area, drought also lowered the number of abaxial trichomes, even though the seasonal production of abaxial trichomes was maintained in all treatments. Data from gas exchange measurements in the same study show that stomatal conductance decreased during drought (Possen et al. 2015), reducing the water lost from silver birch leaves. However, moisture is also lost from leaves when stomata are closed (Xu et al. 1995). How much water is lost via nonstomatal transpiration depends on the thickness of the cuticula and the epidermal cells, known to be thinner on the abaxial leaf surface of silver birch (Pääkkönen et al. 1995). Maintenance of abaxial trichome production during drought supports the hypothesis that these glandular trichomes or their exudates could decrease direct transpiration through the abaxial surface. A potential candidate group for this function is triterpenoids, their main product (Keinänen & Julkunen-Tiitto 1998), which could decrease the permeability of abaxial cuticular layer to water due to their lipophilic nature. If this was the case, the generally higher density of glandular trichomes on abaxial leaf side could be seen as an acclimation mechanism to temporary water shortage in silver birch.

Adaxial trichome production was slightly decreased also as a response to excess watering. Even though the targeted > 60% VWC in the excess watering treatment may not have caused severe water-logging stress in this experiment, the adaxial trichome production responsed similarly to excess watering and drought. Water-logging creates hypoxic or anoxic conditions in soil, limiting water uptake in roots and leading to an internal water deficit (Parent et al. 2008). Earlier studies have shown that water-logging events beginning during dormancy or early growing season increase the density of glandular trichomes (on both leaf surfaces) in long shoot leaves of young silver birches, albeit some of this increase may have resulted from a 'concentration effect' caused by a simultaneous decrease in leaf areas (Wang et al. 2015). Contrasting observations may result from timing of water-logging – Wang et al. (2015) exposed silver birch saplings to flooding while they were dormant, or in the first four weeks of the growing season, whereas in our experiment, excessive watering was applied after the plants had grown for 10 weeks in optimal watering conditions. On the other hand, high air humidity decreases the density of glandular trichomes on both leaf sides in young, chamber-grown silver birch plantlets (Lihavainen 2016), consistently with their potential function in decreasing nonstomatal transpiration.

The interactive effect of temperature and watering on glandular trichomes, occurring only on the adaxial leaf surface, is interesting. The combination of drought and elevated temperature did not induce a decrease in adaxial trichome production, as drought or elevated temperature alone did. Earlier studies have shown that mild increases in temperature enhance photosynthesis (Hartikainen et al. 2012). Generally, young silver birches allocate photosynthates to growth and stem biomass (Lavola et al. 2013), which is necessary for success in the fierce competition for light in young forest stands (Hynynen et al. 2010). Increased resources may allow silver birch to maintain adaxial trichome production in the combined treatment. Nevertheless, as we did not measure the size of the glandular trichomes, the amount of photosynthates allocated into trichomes cannot be directly estimated.

The combined effect of drought and elevated temperature would also be expected if the glandular trichomes had a role in the temperature control of the leaves. Our observed decrease in the adaxial trichome production in enhanced temperature could imply that the transpiration cooling sufficiently regulates leaf temperatures in well-watered conditions, but when the increased temperature coincides with drought the cooling effect is restricted due to stomatal closure. Lower transpiration is known to increase leaf temperatures in silver birch (Sellin et al. 2014). For many plants in xeric ecosystems, such as brittlebrush (*Encelia farinosa*), the presence of non-glandular trichomes is essential during drought, because they decrease absorbance of long-wave (thermal) radiation of the leaves and thus lower leaf temperature (Ehleringer & Mooney 1978). It remains to be studied whether the glandular trichomes in leaves of silver birch have a similar role in alleviating high temperature stress, or whether the maximum leaf temperatures achieved in our experiment actually represent a stress in the mild climate typical to boreal and hemiboreal zones.

4.2 Production of glandular trichomes on abaxial leaf surfaces is increased during the season

Silver birch extensively increases the production of abaxial trichomes in leaves produced during the growing season. Adaxial trichome density, on the other hand, decreased with increasing leaf area, supporting the view that no new trichomes were produced on the adaxial leaf surface of 2-year old silver birches. The increase observed in the production of abaxial trichomes may be related to the co-occurring pattern of stress, such as the prevalence of drought later in the season.

Other potential drivers of seasonal change could be herbivory or pathogenic infection levels (Valkama et al. 2005). High density of glandular trichomes in silver birch clones was associated with resistance to birch rust (*Melampsoridium betulinum*) but did not correlate with relative growth rate or pupal mass of autumnal moth (*Epirrita autumnata*) in bioassays (Valkama et al. 2005). Triterpenoid compounds produced by stem resin glands reduce the palatability of silver birch bark to mountain hare (*Lepus timidus*, Laitinen et al. 2004). Artificial defoliation, simulating leaf herbivory, decreased the production of glandular trichomes and increased the formation of leaf hairs in *Betula pubescens* (Rautio et al. 2002).

Sampling the same tree several times per season may trigger defoliation responses in trees. In our experiment, number of sampled leaves was small (three leaves per sampling, or nine leaves per season), decreasing the probability that defoliation affected production of glandular trichomes. Rautio et al. (2002) report a decrease in glandular trichome production in a related species, *B. pubescens*, in response to extensive artificial defoliation. However, no significant decreases in trichome production between successive measurements, which might be caused by artificial defoliation, were observed in our experiment.

To conclude, the number and density of glandular trichomes of silver birch respond to drought, but the response depends on leaf surface. Abaxial trichome production is governed by a strong seasonal increase also in low-watering conditions, implying that abaxial trichomes are necessary for silver birch also during water stress. Abaxial trichomes may participate in the water economics of silver birch leaves, possibly through their effect on nonstomatal transpiration from abaxial leaf surface. Temperature- and moisture-related changes in the number and density of silver birch glandular trichomes may affect the ecological interactions between silver birch and its herbivores or pathogens as the climate changes.

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Table 1. ANOVA summary table showing degrees of freedom in numerator (df1) and in denominator (df2), and F and p values for fixed factors of linear mixed models for glandular trichome density or (ln-transformed) number in the temperature and watering experiment. Interaction of heating and watering did not increase model fit on abaxial trichome models; thus, this term was omitted.

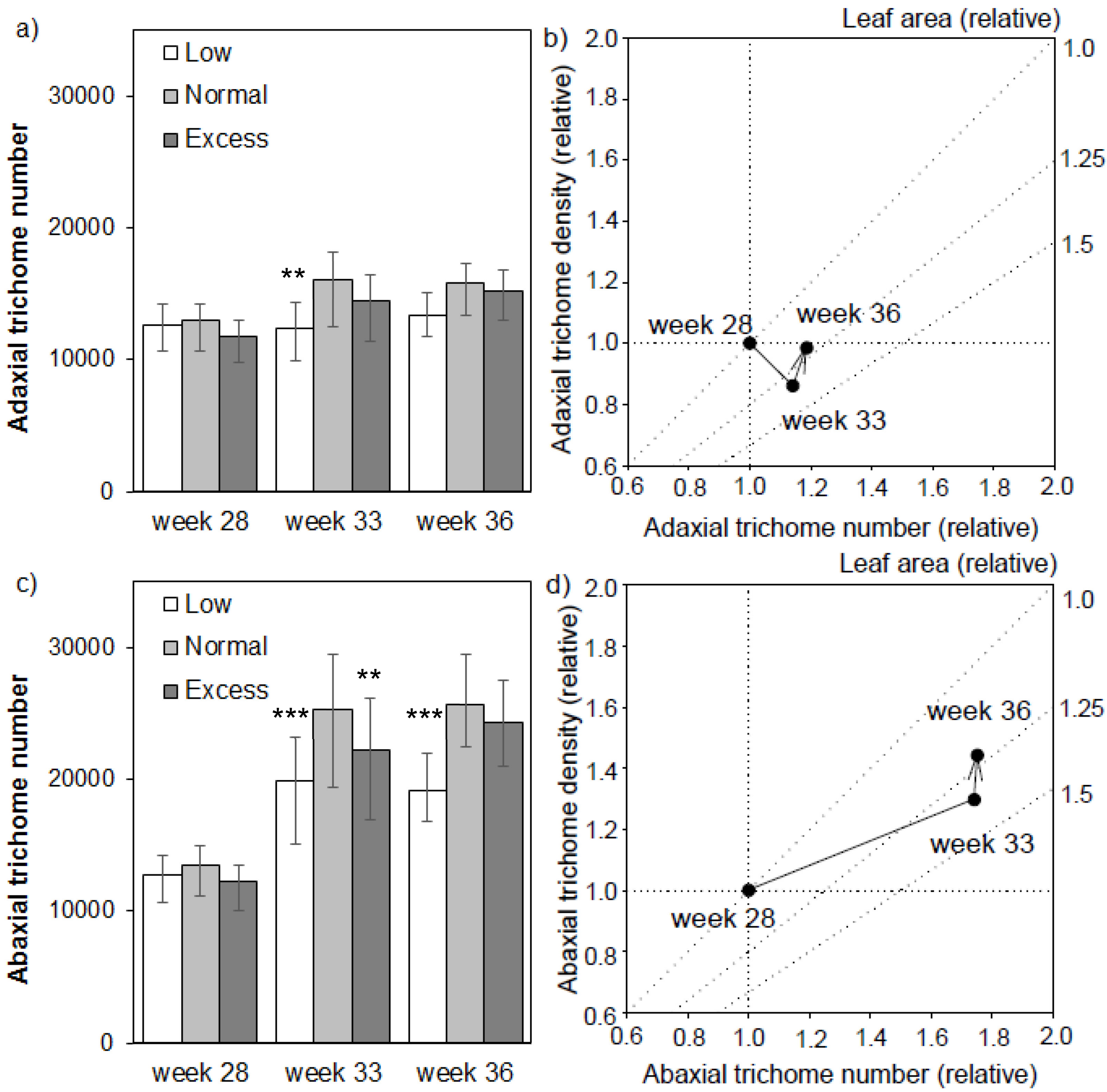
	Adaxial trichomes								Abaxial trichomes							
	Density				ln (Number)				Density				ln (Number)			
Source of variation	df1	df2	F	p	df1	df2	F	p	df1	df2	F	p	df1	df2	F	p
Week	2	11.3	5.987	0.017	2	6.0	10.072	0.012	2	9.3	10.606	0.004	2	4.1	28.110	0.004
Watering	2	117.8	0.063	0.939	2	127.2	6.116	0.003	2	12.5	1.780	0.209	2	14.9	9.281	0.002
Heating	1	116.3	1.689	0.196	1	125.4	0.523	0.471	1	12.0	0.154	0.702	1	14.0	0.854	0.371
Week*Watering	4	239.0	0.804	0.524	4	236.4	3.863	0.005	4	241.0	4.019	0.004	4	236.8	4.077	0.003
Heating*Watering	2	116.4	8.561	< 0.001	2	125.5	5.662	0.004	2	12.0	1.207	0.333	-	-	-	-

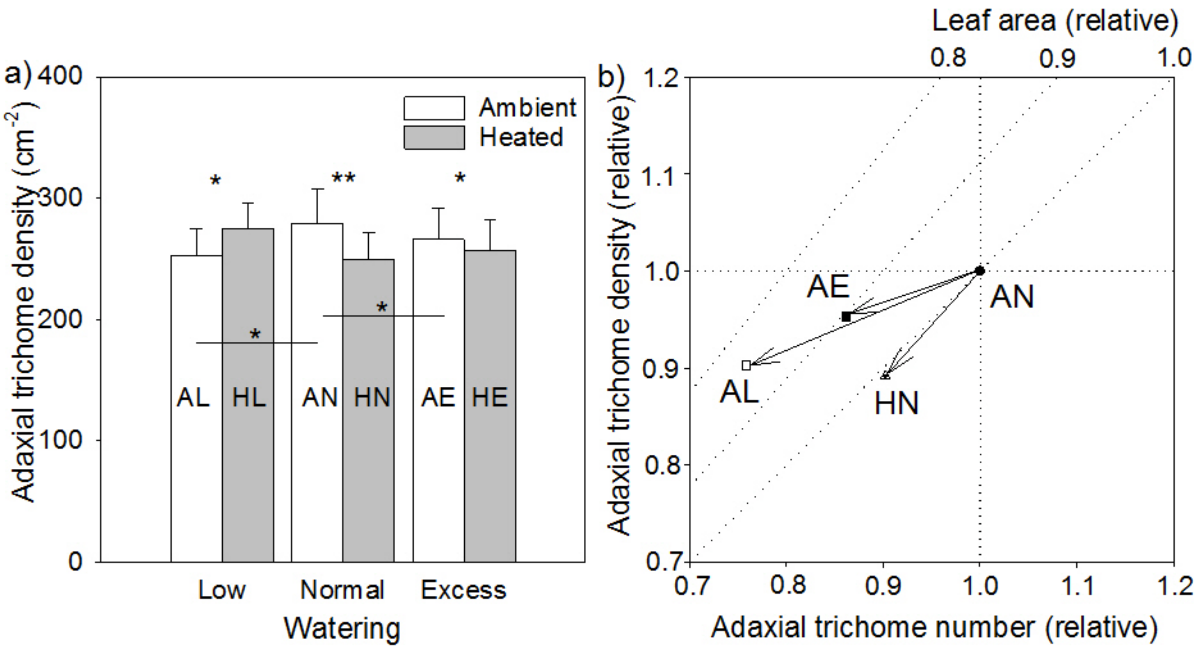
Table 2. Mean (± SEM) trichome density in long-shoot leaves of 2-year-old greenhouse-grown silver birch plantlets grown under different watering levels sampled at different time intervals during the summer 2011.

	Sampling		
	Week 28	Week 33	Week 36
Adaxial trichome density (cm^{-2})			
Low watering	281.09 ± 31.16	240.38 ± 40.16	271.05 ± 38.99
Optimal watering	284.24 ± 42.55	235.51 ± 42.77	273.66 ± 45.99
Excess watering	269.40 ± 37.87	242.14 ± 45.80	276.84 ± 46.34
Abaxial trichome density (cm^{-2})			
Low watering	282.15 ± 33.71	378.93 ± 64.42	395.88 ± 49.11
Optimal watering	297.10 ± 37.16	375.70 ± 57.58	432.15 ± 61.13
Excess watering	285.01 ± 37.85	365.33 ± 61.78	417.99 ± 56.46

Figure 1. Numbers of glandular trichomes per leaf on (a) adaxial and (c) abaxial leaf surfaces in different watering treatments during different times. Asterisks indicate statistically significant differences between watering levels within respective week (**<0.01, ***<0.001). During weeks 28 and 33, averages are based on eight genotypes, whereas on week 36, six genotypes were used for the adaxial and five genotypes for the abaxial mean. Error bars back-transformed 95% confidence intervals obtained by the model .
(b) and (d): Seasonal dynamics in the production of glandular trichomes on adaxial (d) and abaxial (d) leaf surface in mature long-shoot leaves. Endpoints of vectors represent number and density of trichomes in weeks 33 and 36 relative to initial values on week 28. Dashed diagonals display relative changes in leaf areas.

Figure 2. (a) Density of glandular trichomes on adaxial surfaces of mature long-shoot leaves in 2-year old silver birch grown under combinations of three different levels of watering (L=low, N=optimal, E=excess) and two different temperatures (A=ambient, H=elevated). Asterisks above bars indicate statistically significant differences between heated and unheated plants within the same watering treatment. The line on bars shows statistically significant differences between watering levels within a similar heating treatment (* $p<0.05$, ** $p<0.01$). Error bars ± 1 SEM, n=9.
(b) Vector diagram showing the direction and extent of changes in the relative number and density of adaxial trichomes in the treatments that differed statistically significantly from the control treatment (AN). The end-points of the vectors represent the average number and density of adaxial trichomes in the respective treatments relative to the control treatment.


a)
Low
Normal
Excess
Adaxial trichome number
30000
20000
10000
0
**
week 28
week 33
week 36
b)
Leaf area (relative)
Adaxial trichome density (relative)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
1.0
1.25
1.5
week 28
week 36
week 33
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Adaxial trichome number (relative)
c)
Low
Normal
Excess
Abaxial trichome number
30000
20000
10000
0
***
**
***
week 28
week 33
week 36
d)
Leaf area (relative)
Abaxial trichome density (relative)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
1.0
1.25
1.5
week 36
week 33
week 28
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Abaxial trichome number (relative)



a)
Adaxial trichome density (cm^{-2})
400
300
200
100
0
Ambient
Heated
*
**
*
*
*
AL
HL
AN
HN
AE
HE
Low
Normal
Excess
Watering
b)
Leaf area (relative)
0.8
0.9
1.0
Adaxial trichome density (relative)
1.2
1.1
1.0
0.9
0.8
0.7
AE
AN
AL
HN
0.7
0.8
0.9
1.0
1.1
1.2
Adaxial trichome number (relative)

1

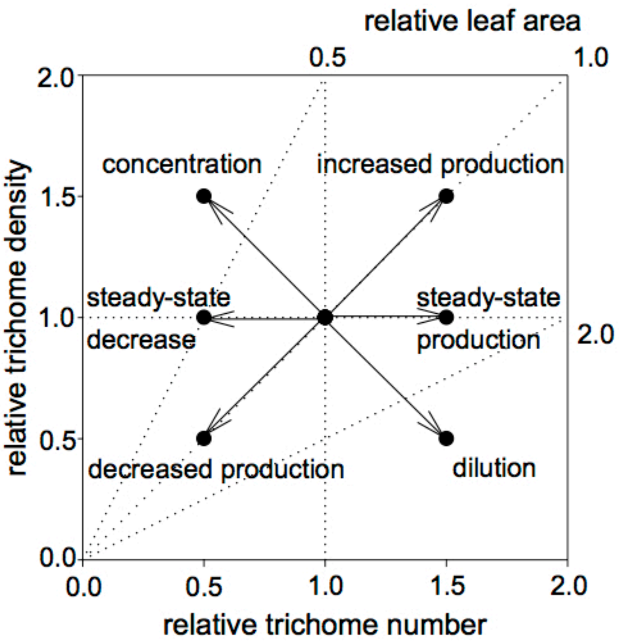


Figure S1. Interpretation of the direction of vectors in graphical vector analyses. Density of glandular trichomes (y-axis) was plotted against their estimated number per leaf (x-axis). In interpretation, both the direction and the length of the vectors are taken into account. The direction of a vector tells if the change among time points or treatments is related to leaf area (dashed diagonals) and the length describes the magnitude of the effect. For example, the dashed diagonal line in the middle of the vector diagram represents the situations where leaf area remains constant (relative leaf area 1.0), but the number of trichomes and thus their density changes.

Table S1. Mean (± SEM) trichome number in long-shoot leaves of two-year-old greenhouse-grown silver birch plantlets grown under different watering levels sampled at different time intervals during the summer 2011.

	Sampling		
	Week 28	Week 33	Week 36
Adaxial trichome number			
Low watering	12679 ± 1945	12338 ± 1971	13397 ± 1585
Optimal watering	13000 ± 2478	16027 ± 3621	15778 ± 3098
Excess watering	11767 ± 2108	14483 ± 2895	15224 ± 2547
Abaxial trichome number			
Low watering	12737 ± 1891	19829 ± 3958	19089 ± 3063
Optimal watering	13511 ± 2402	25242 ± 5045	25687 ± 4887
Excess watering	12267 ± 2051	22225 ± 4250	24341 ± 3633

Table S2. Estimated fixed effect sizes and variance related to random effects according to linear mixed models fitted for density and number of glandular trichomes on both surfaces of long-shoot leaves from 2-year-old, greenhouse grown silver birches. Heating level H corresponds to +1 °C during 12-week temperature treatment, and Low watering levels L and E to low and excess soil moisture maintained for 5 weeks, respectively.

Adaxial density			
Fixed part			
Variable	Estimate (s.e)	df	p
Intercept	302.4 (16.6)	12.06	<0.001
Low watering	-31.4 (12.7)	299.43	0.0155
Excess watering	-28.7 (12.8)	301.59	0.026
Heated	-32.8 (9.5)	117.89	<0.001
Week 33	-48.9 (21.1)	10.39	0.042
Week 36	-12.9 (15.4)	20.40	0.411
Low watering:Week 33	6.4 (15.2)	227.37	0.675
Excess watering:Week 33	22.1 (15.2)	228.40	0.147
Low watering:Week 36	2.9 (16.3)	252.13	0.860
Excess watering:Week 36	23.1 (16.3)	251.20	0.157
Low watering:Heated	54.7 (13.3)	116.68	<0.001
Excess watering:Heated	22.6 (13.3)	116.93	0.092
Random part			
var(a_i)	0.0^2		
var($b_k{}^{(1)}$)	39.1^2		
var($b_k{}^{(2)}$)	64.0^2		
var($b_k{}^{(3)}$)	49.9^2		
corr($b_k{}^{(1)}$, $b_k{}^{(2)}$)	0.60		
corr($b_k{}^{(1)}$, $b_k{}^{(3)}$)	0.82		
corr($b_k{}^{(2)}$, $b_k{}^{(3)}$)	0.95		

var(c_{ikj})	7.7^2		
var(ε_{ijkl})	51.0^2		

ln (Adaxial number)

Fixed part			
Variable	Estimate (s.e)	df	p
Intercept	9.471 (0.070)	16.22	<0.001
Low watering	-0.123 (0.064)	277.82	0.057
Excess watering	-0.155 (0.065)	280.43	0.018
Heated	-0.099 (0.050)	127.18	0.051
Week 33	0.197 (0.100)	10.36	0.076
Week 36	0.208 (0.067)	21.27	0.005
Low watering:Week 33	-0.231 (0.071)	226.77	0.001
Excess watering:Week 33	-0.001 (0.072)	227.89	0.984
Low watering:Week 36	-0.130 (0.077)	247.09	0.094
Excess watering:Week 36	0.062 (0.077)	247.150	0.535
Low watering:Heated	0.238 (0.071)	125.86	0.001
Excess watering:Heated	0.122 (0.071)	126.02	0.087
Random part			
var(a_i)	0.000^2		
var($b_k^{(1)}$)	0.147^2		
var($b_k^{(2)}$)	0.212^2		
var($b_k^{(3)}$)	0.117^2		
corr($b_k^{(1)}$, $b_k^{(2)}$)	0.12		
corr($b_k^{(1)}$, $b_k^{(3)}$)	0.70		
corr($b_k^{(2)}$, $b_k^{(3)}$)	0.76		
var(c_{ikj})	0.088^2		
var(ε_{ijkl})	0.240^2		

Abaxial density

Fixed part			
Variable	Estimate (s.e)	df	p
Intercept	296.7 (16.9)	17.55	<0.001
Low watering	-12.6 (15.7)	33.44	0.426
Excess watering	-12.5 (15.7)	33.44	0.429
Heated	-4.0 (10.2)	14.00	0.704
Week 33	80.9 (40.7)	7.89	0.082
Week 36	157.9 (29.6)	8.96	<0.001
Low watering:Week 33	15.8 (17.0)	226.88	0.352
Excess watering:Week 33	2.2 (17.0)	226.88	0.899
Low watering:Week 36	-47.9 (19.8)	258.18	0.016
Excess watering:Week 36	9.2 (19.6)	257.14	0.640
Low watering:Heated	38.2 (24.7)	11.98	0.147
Excess watering:Heated	21.4 (24.7)	11.94	0.404
Random part			
var(a_i)	14.7^2		

var($b_k^{(1)}$)	32.1^2
var($b_k^{(2)}$)	106.7^2
var($b_k^{(3)}$)	70.6^2
corr($b_k^{(1)}$, $b_k^{(2)}$)	-0.20
corr($b_k^{(1)}$, $b_k^{(3)}$)	0.68
corr($b_k^{(2)}$, $b_k^{(3)}$)	0.59
var(c_{ikj})	24.0^2
var(ε_{ijkl})	59.1^2

ln (Abaxial number)

Fixed part			
Variable	Estimate (s.e)	df	p
Intercept	9.447 (0.070)	14.26	<0.001
Low watering	-0.048 (0.059)	41.35	0.425
Excess watering	-0.096 (0.059)	41.35	0.113
Heated	0.034 (0.036)	13.98	0.371
Week 33	0.616 (0.141)	8.31	0.002
Week 36	0.691 (0.102)	7.03	<0.001
Low watering:Week 33	-0.199 (0.070)	219.69	0.005
Excess watering:Week 33	-0.028 (0.070)	219.69	0.692
Low watering:Week 36	-0.243 (0.082)	250.28	0.003
Excess watering:Week 36	0.026 (0.081)	249.14	0.748
Random part			
var(a_i)	0.040^2		
var($b_k^{(1)}$)	0.149^2		
var($b_k^{(2)}$)	0.241^2		
var($b_k^{(3)}$)	0.092^2		
corr($b_k^{(1)}$, $b_k^{(2)}$)	-0.83		
corr($b_k^{(1)}$, $b_k^{(3)}$)	-0.92		
corr($b_k^{(2)}$, $b_k^{(3)}$)	0.69		
var(c_{ikj})	0.109^2		
var(ε_{ijkl})	0.243^2		