

Leaf trichome production and responses to defoliation and drought in *Arabidopsis lyrata* (Brassicaceae)

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Leaf trichomes can protect plants against herbivory and drought, but can be costly to produce. Theory suggests that selection for reduced costs of resistance may result in the evolution of inducible defences. We quantified variation in tolerance to drought and defoliation, and tested the hypotheses that (a) tolerance is associated with cost, (b) leaf trichome production increases tolerance to drought, and (c) trichome production is increased in response to defoliation and drought stress in *Arabidopsis lyrata* (Brassicaceae). Eight maternal half-sib families were exposed to two watering regimes and four defoliation treatments in a factorial design. Tolerance to drought varied among families and was inversely related to leaf size, but was not related to trichome density. Family mean performance in the low-watering treatment tended to correlate negatively with that in the control treatment. Trichome production was not induced by defoliation or drought stress. The results suggest that there is genetic variation in tolerance to drought in the study population, that tolerance to drought is associated with a cost, and that trichome production does not increase tolerance to drought in *A. lyrata*.

Key words: *Arabidopsis lyrata*, Brassicaceae, defoliation, drought, stress, trichome production

Introduction

Plants have evolved a multitude of defence mechanisms against abiotic and biotic stress factors such as drought, heat, and herbivory (e.g. Karban & Baldwin 1997, Stowe *et al.* 2000, Agrawal & Fishbein 2006). Resistance

traits, i.e., traits that are associated with reduced damage, range from plant surface characteristics (e.g. trichomes and spines) to chemical defences, whereas tolerance traits, i.e. traits that contribute to sustained fitness in the face of damage, range from compensatory growth to increase in photosynthetic activity in remaining parts (Tiffin

2000). Some traits act as defences against several stress factors (e.g. Karban & Baldwin 1997). Plant populations vary genetically in tolerance to drought (e.g. Link *et al.* 1999, Sandquist & Ehleringer 2003), and in resistance and tolerance to herbivory (e.g. Agrawal *et al.* 1999, Tiffin & Rausher 1999, Fornoni & Núñez-Farfán 2000, Mutikainen *et al.* 2000), but the traits underlying variation in resistance and tolerance are commonly poorly known.

Theory suggests that allocation to defence can be costly, and costs of tolerance (e.g. Simms & Triplett 1994, Koskela *et al.* 2002), and resistance to herbivory have been detected in several systems (Bergelson & Purrington 1996, Strauss *et al.* 2002). Inducible defences may have evolved as a mechanism to reduce costs of defence (Karbon & Baldwin 1997). Inducible defences are activated upon damage, and may involve increases in secondary metabolites (Agrawal 2000), reduced plant nutritional value (Bi *et al.* 1997) and increased trichome density (e.g. Traw & Dawson 2002, Dalin & Björkman 2003, Abdala-Roberts & Parra-Tabla 2005).

Leaf trichomes are multi- or unicellular hairs that can be either non-glandular or glandular, and may protect plants against herbivory (e.g. Mauricio 1998, Valverde *et al.* 2001, Handley *et al.* 2005), UV-radiation and drought (Ehleringer 1984, Skaltsa *et al.* 1994, Espigares & Peco 1995). Leaf trichome production and density vary genetically and are negatively related to damage from insect herbivores in several members of the Brassicaceae, including *Arabidopsis thaliana* (Mauricio & Rausher 1997, Handley *et al.* 2005), *Brassica rapa* (Ågren & Schemske 1994), and *Sinapis arvensis* (Roy *et al.* 1999).

We conducted a greenhouse experiment to quantify variation in tolerance to drought and defoliation, and to determine whether (1) tolerance is associated with a cost, (2) tolerance to drought is positively related to leaf trichome density, and (3) trichome production is induced by drought or defoliation in the outcrossing perennial herb *Arabidopsis lyrata* ssp. *petraea*. This taxon is polymorphic for trichome production, with trichome-producing and glabrous morphs (Kärkkäinen & Ågren 2002, Kärkkäinen *et al.* 2004). In addition, number of trichomes varies quantitatively among trichome-producing

plants (Kärkkäinen & Ågren 2002). Earlier studies have suggested that trichome production is subject to divergent selection (Kärkkäinen *et al.* 2004), and that the production of trichomes is correlated with reduced damage from insect herbivores in natural populations (Kivimäki *et al.* 2007, Løe *et al.* 2007).

We mimicked the damage caused by insect herbivores and subjected plants to drought stress to address the following questions: (1) Is there among-family variation in tolerance to drought or defoliation? (2) Is tolerance associated with a cost? (3) Does tolerance to drought increase with increasing trichome density? (4) Does drought or defoliation induce trichome production?

Material and methods

Study species

Arabidopsis lyrata ssp. *petraea* (Brassicaceae; syn. *Arabis petraea* and *Cardaminopsis petraea*) is a self-incompatible perennial herb closely related to *A. thaliana* (Price *et al.* 1994, Koch *et al.* 1999, Koch *et al.* 2008). The species has a scattered distribution in Europe, from Iceland to southern Germany and Alps (Jalas & Suominen 1994). In Scandinavia, it is found in western Norway and in a limited area along the coast of eastern Sweden (Hultén 1971). Leaf trichomes in *A. lyrata* are non-glandular and form up to five branches, whereas trichomes on inflorescences are predominantly unbranched. Controlled crosses have shown that a single gene explains most of the variation in trichome production in *A. lyrata* (Kärkkäinen & Ågren 2002), and glabrousness has been found to be associated with mutations in a regulatory gene homologous to *GLABROUS1* identified in *A. thaliana* (Kivimäki *et al.* 2007).

Experimental design

Seeds of *A. lyrata* were collected from a population located at Stubbsand, in the province of Ångermanland, on the east coast of Sweden (63°13'05''N, 18°58'02''E). The population is polymorphic for trichome production with a frequency of glabrous plants of about 40%

(Kärkkäinen *et al.* 2004). For the present study, we used seeds from eight open-pollinated maternal families, four families with a glabrous mother and four with a trichome-producing mother. The study was conducted in the greenhouse of the University of Jyväskylä.

We planted altogether 100 seeds per family. Seeds were planted in pots (5 × 5 × 5 cm) filled with peat and vermiculite (proportion 1:1). Two-four seeds were planted in each pot. After planting, the seeds were stratified under plastic covers in a cold room (+5 °C) for three days, and then transferred to the greenhouse at 12 hours of light (+22 °C daytime/+15 °C nighttime). The plastic covers were removed after germination had occurred. Three weeks after stratification we transplanted the seedlings into new pots, one seedling per pot. The pots were randomized into twenty blocks, each block consisting of 32 plants (four plants from each of the eight maternal families). The plants were watered every 5th day.

To examine the effects of drought and defoliation on plant performance and trichome production, we changed the watering regime, and applied defoliation treatments three weeks after transplantation. Two watering treatments (control and low) were randomly allocated to ten blocks each. In the control, plants continued to be watered every 5th day, which was enough to keep the plants from showing any symptoms of drought stress. In the low water treatment, plants received water every 10th day, which caused the plants to develop clear signs of drought stress. Within each block, we assigned each of the four plants of a given maternal family to one of four defoliation treatments (0%, 15%, 25% or 50% leaf area removed with scissors). All fully-developed leaves were subject to the defoliation treatment. Twenty days after defoliation the ventilation system of the greenhouse broke down and the plants experienced approximately +48 °C for 48 hours. To reduce the effect of the high temperature, all plants were watered. After the return to the programmed temperature the experimental watering regimes were resumed. All the control plants survived the temperature stress, but 23% of the plants in the low-watering treatment died within the following week. This constituted about a third of the total mortality observed in the low-water treatment during the

experiment (*see* Results).

To quantify the effects of the experimental treatments on plant survival, growth and trichome production, we scored the plants once before the experimental treatment (two weeks after transplantation), and twice after the experimental treatments had been applied (seven and nine weeks after transplantation). At each census, we recorded the survival and rosette diameter (to the nearest mm) of all experimental plants. For two fully expanded leaves per plant, we recorded the number of trichomes, and the length and width of the leaf blade and of the petiole, respectively. Trichome density was quantified using the expression:

$$\text{Density} = \text{TN}/A$$

where TN is the trichome number on the upper side of the leaf and A is the leaf area, estimated as follows:

$$A = \pi(\text{LL}/2)(\text{LW}/2) + \text{PL} \times \text{PW}$$

where LL is the leaf length, LW is the leaf width, PL is the petiole length, and PW is the petiole width.

At the final census, at the end of the experiment, we in addition collected the aboveground parts of all surviving plants, dried them at 75 °C for 24 hours and weighed them to the nearest mg.

Statistical analyses

To determine whether families varied in survival, we first ran a logistic regression analysis (Proc Logistic in SAS; SAS 1999) with survival as the dependent variable and family, watering, defoliation, and block nested within watering as our independent variables. We also included the family × watering regime, family × defoliation, watering regime × defoliation and family × watering regime × defoliation interactions into the analysis. Mortality was observed in the Low-watering treatment only, so we further analysed which factors effected survival in Low-watering treatment with logistic regression including family, defoliation, block and the family × defoliation interaction as independent variables.

We used mixed model ANOVA (Proc Mixed in SAS) to examine whether the effects of defoliation and drought on rosette diameter, above-ground mass of surviving plants, and cumulative fitness (0 for dead plants, and aboveground mass for survivors) varied among families. These models included family, watering regime and defoliation (fixed effects) and block (nested within watering regime; random effect) and the family \times watering, the family \times defoliation, the watering \times defoliation, and the family \times watering \times defoliation interactions as independent factors. A significant family \times watering or family \times defoliation interaction would indicate among-family variation in tolerance to drought and defoliation, respectively. A significant watering \times defoliation interaction would indicate that the effects of the watering and defoliation treatments were not additive, while a significant three-way interaction would indicate that the combined effects of watering and defoliation varied among families.

To explore possible mechanisms underlying among-family variation in tolerance to drought, we further examined whether tolerance was related to trichome density or leaf size. For each maternal family, tolerance to drought was quantified as the difference in “cumulative fitness” between control and low-watering treatment. The effects of density of leaf trichomes and leaf size on tolerance to drought were examined with multiple regression analysis based on maternal family-means of leaf characteristics recorded at the first census, prior to the experimental treatment.

We used mixed-model ANOVA (Proc Mixed in SAS) to examine whether drought or defoliation influenced number of leaf trichomes or trichome density. These models included watering regime, family and defoliation (fixed effects), and block (nested within watering regime, random effect) and all possible interactions as independent variables.

Results

Among-family variation in morphology

Leaf size, ($F = 17.5$, $P < 0.001$) trichome number per leaf ($F = 6.7$, $P < 0.001$) and trichome density ($F = 6.5$, $P < 0.001$) varied among maternal fami-

lies five weeks after sowing, i.e., prior to the initiation of experimental treatments (ANOVAs, which included maternal family and block as independent variables). Mean leaf size varied from 0.84 to 1.62 cm² ($n = 8$, maternal-family means). About 14% of all plants were completely glabrous ($n = 635$). Five families included both glabrous and trichome-producing individuals, while three families consisted of trichome-producing plants only. Including the glabrous plants, the mean number of trichomes produced per leaf ranged from 31.7 to 81.5, and the mean trichome density ranged from 2.3 to 6.7 trichomes per cm² ($n = 8$ maternal-family means). Number of trichomes per leaf and trichome density varied significantly among maternal families also if only trichome-producing plants were considered (data not shown).

Tolerance to drought

Drought affected the relative survival, above-ground biomass of survivors, and cumulative fitness of the different families as indicated by significant family \times watering regime interactions in ANOVA models (Tables 1 and 2; Figs. 1 and 2). In the low-water treatment, survival varied from 10% to 57% ($\chi^2 = 5.2$, $df = 1$, $P < 0.02$; median 25%), whereas almost all plants survived in the high-water treatment (Fig. 1A). Family mean cumulative fitness in the low-water treatment tended to correlate negatively with that in the control treatment ($r = -0.65$, $P = 0.078$, $n = 8$). Multiple regression based on family means indicated that tolerance to drought was negatively related to mean leaf size (partial regression coefficient: $b = -0.964$, $P < 0.03$, Fig. 3), but not significantly related to mean trichome trichome density ($b = -0.391$, $P = 0.26$). Moreover, there was no evidence that survival of trichome-producing plants was higher than that of completely glabrous plants in the low-water treatment (29%, $n = 277$ vs. 43%, $n = 42$, $\chi^2 = 3.2$, $P = 0.07$; data pooled across maternal families).

Effects of defoliation on plant performance

Defoliation reduced plant growth temporarily

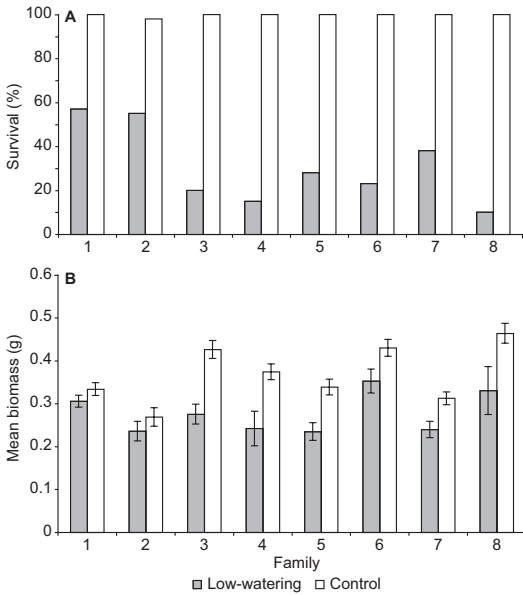


Fig. 1. Performance of eight maternal families of *Arabidopsis lyrata* in the low-water and control treatments in a greenhouse experiment: (A) survival and (B) mean \pm 1 SE aboveground dry mass of survivors at the end of the experiment.

(significant effect on rosette size four weeks after the experimental defoliation; $F_{3,337} = 8.58$, $P < 0.0001$), but did not significantly affect survival in the low-water treatment ($\chi^2 = 0.8$, $df = 1$, $P = 0.37$), or final size among survivors (rosette diameter at end of the experiment, $F_{3,335} = 0.96$, $P = 0.41$ and aboveground mass; Table 1). The family \times defoliation interaction was not statistically significant in analyses of

Table 1. Effects of family, watering regime, defoliation and block nested within watering regime on biomass of survivors examined with mixed-model ANOVA.

Source	df	MS	F	P
Family	7	0.057	6.13	<0.0001
Watering	1	0.322	16.8	0.0007
Defoliation	3	0.013	2.15	0.2935
Family \times Watering	7	0.018	1.99	0.0553
Family \times Defoliation	21	0.010	0.86	0.6402
Watering \times Defoliation	3	0.007	0.56	0.6415
Family \times Watering \times Defoliation	19	0.009	0.78	0.7306
	Estimate	SE	Z	P
Block (Watering)	0.0018	0.0008	2.23	0.0127
Residual	0.011	0.0009	12.93	<0.0001

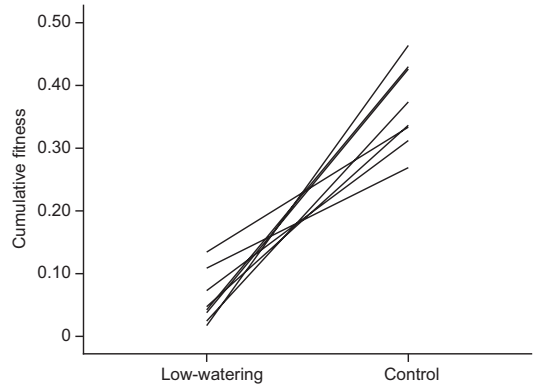


Fig. 2. Mean cumulative fitness of eight maternal families grown at two watering levels (low water vs. control). Cumulative fitness was coded as zero for plants that died and was estimated as aboveground dry mass at the end of the experiment for survivors.

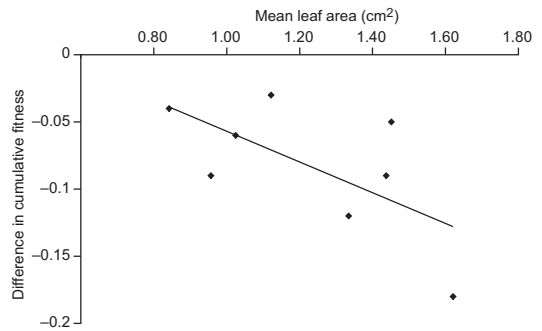


Fig. 3. Relationship between tolerance to drought (quantified as the difference in cumulative fitness between low-water treatment and control) and mean leaf size prior to the experimental treatment (maternal-family means).

Table 2. Effects of family, watering regime, and block nested within watering regime on cumulative fitness (set to zero for plants that died and quantified as aboveground dry mass for plants that were alive at the end of the experiment) examined with mixed-model ANOVA based on family \times block least-square-means.

Source	df	Den df	F	P
Family	7	126	3.45	0.002
Watering	1	18	213.2	<0.0001
Family \times Watering	7	126	14.8	<0.0001
	Estimate	SE	Z	P
Block (Watering)	0.0015	0.0007	2.29	0.01
Residual	0.004	0.0005	7.49	0.0001

survival in the low-water treatment, and no two-way or three-way interaction was statistically significant in analyses of rosette diameter four weeks after defoliation, and rosette diameter and aboveground mass at the end of the experiment, which indicates that tolerance to defoliation did not significantly vary among families or watering treatments.

Effects of mechanical damage and drought on trichome production

Number of trichomes per leaf and trichome density varied among maternal families four weeks after defoliation ($F = 3.0$, $P < 0.0001$) and at the end of the experiment ($F = 4.5$, $P < 0.0001$), but were not affected by mechanical damage or drought neither four weeks after defoliation (Mechanical damage: number of trichomes per leaf $F = 1.5$, $P = 0.22$; trichome density $F = 2.1$, $P = 0.10$; Drought: number of trichomes per leaf $F = 3.6$, $P = 0.11$, trichome density $F = 1.4$, $P = 0.25$) nor at the end of the experiment (Mechanical damage: number of trichomes per leaf $F = 0.22$, $P = 0.88$; trichome density $F = 0.41$, $P = 0.71$; Drought: number of trichomes per leaf $F = 0.32$, $P = 0.58$; trichome density $F = 0.68$, $P = 0.42$).

Discussion

The results of the present study indicate that there is genetic variation in tolerance to drought in the studied *Arabidopsis lyrata* population and suggest that tolerance to drought is associated with a cost. However, there was no evidence that tolerance to drought increases with increasing leaf trichome density or that trichome production is induced by drought or defoliation.

Tolerance to drought

Tolerance to drought varied significantly among the eight maternal families tested, and family-mean performance in the low-water treatment tended to correlate negatively with that in the control treatment ($P = 0.078$). This suggests that the study population may respond to selection

for increased tolerance to drought, but also that tolerance to drought may be associated with a cost that could contribute to the maintenance of genetic variation in this trait (cf. Strauss & Agrawal 1999). One obvious caveat is that the study was based on variation among maternal families collected in the field, which means that among-family variation in performance may be influenced not only by additive genetic variance but also by maternal genetic and environmental effects (Roach & Wulff 1987). To explore further the magnitude of genetic variance in tolerance in *A. lyrata*, ongoing experiments make use of experimentally produced paternal half-sib families.

Leaf trichomes can protect plants against drought by reducing absorption of solar radiation, which in turn reduces the heat load and minimizes the need for transpirational cooling (Ehleringer & Björkman 1978, Espigares & Peco 1995). However, although a substantial proportion of plants were completely glabrous and mean trichome density varied significantly among maternal families, there was no evidence that resistance to drought increases with increasing density of leaf trichomes in *A. lyrata*.

In addition to trichome density, tolerance to drought can be related to plant traits such as shoot and root morphology, root/shoot ratio (Passioura 1983), leaf wax production (Jordan *et al.* 1984), the leaf area to volume ratio (Givnish 1979) and leaf area *per se* (Lambers *et al.* 1998). Small leaves have thinner boundary layers. Because heat transport is inversely related to boundary layer thickness, plants with small leaves should have an advantage under hot and dry conditions (Lambers *et al.* 1998). Consistent with this prediction, tolerance to drought was negatively related to leaf size in the present study. Future work should assess the relative importance of morphological and physiological traits potentially influencing variation in tolerance to drought in *A. lyrata*.

Effects of defoliation on plant performance

Defoliation reduced rosette size temporarily in both watering treatments, but did not affect plant

size at the end of the experiment, indicating that the defoliated plants were able to compensate for the lost biomass. Compensatory regrowth following herbivory should contribute to tolerance, but is more likely under benign than under harsh environmental conditions (Lennartsson *et al.* 1998, Stowe *et al.* 2000, Piippo *et al.* 2005). The results suggest that the experimental conditions in both watering treatments were sufficiently benign for plant growth to allow recovery from the effect of defoliation. In contrast, field experiments indicate that levels of herbivory similar to those employed in the present study can result in both reduced size and flowering propensity in natural populations of *A. lyrata* (Løe 2006, A. Puentes & J. Ågren unpubl. data). The effect of leaf damage on plant performance should depend on the magnitude of damage and plant resource status, but also on the timing and frequency of damage in relation to plant development (Maschinski & Whitham 1989). Additional studies are needed to explore the effects of damage during different stages of plant development and of repeated damage.

Effects of drought and defoliation on trichome production

Trichome production is associated with reduced damage from insect herbivores in *A. lyrata* (Løe *et al.* 2007), but we found no support for the hypothesis that trichome formation is increased in response to defoliation or drought. Trichome production was not affected by defoliation and was not higher in the low-water treatment than in the control. By contrast, leaf damage has been found to induce trichome production in several species including the closely related *A. thaliana* (Traw & Bergelson 2003). The failure to detect an induced increase in trichome production in response to leaf damage may be related to the way damage was inflicted. It has been suggested that mechanical defoliation may not induce plant defences as efficiently as damage caused by herbivores (Haukioja & Neuvonen 1985, Karban & Baldwin 1997). However, consistent with the present findings, damage by the diamondback moth, *Plutella xylostella* did not induce trichome production in *A. lyrata* in another experiment.

(Sletvold *et al.* 2010). Taken together, the results suggest that trichome production is a constitutive resistance trait in *A. lyrata*.

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