

POPULATION DIFFERENTIATION OF *IMPATIENS CAPENSIS* (BALSAMINACEAE) AT THE RANGE LIMIT

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A plant species that occupies a large geographic range must survive in a variety of environments. *Impatiens capensis*, an herbaceous annual that grows contiguously from the east coast of North America to the Colorado Rocky Mountains, prefers moist environments and is easily susceptible to drought. *Impatiens* populations at the species' western range limit (Colorado) must endure a dramatically different environment than eastern populations do (Rhode Island and Illinois). This study investigated the stress response of a weedy annual at its range limit by examining how regional populations of *I. capensis* morphologically, physiologically, and phenologically adjust to drought. The results suggest that each population has evolved along a different evolutionary axis; each population exhibits a unique strategy for responding to drought stress. Rhode Island populations have evolved along a temporal axis to avoid stress with an accelerated phenology. An Illinois population has evolved along a physiological axis and exhibits a drought-tolerance strategy. Colorado populations have evolved along a morphological axis and tolerate drought. Thus, dealing with drought stress may involve population differentiation along different axes of functional plasticity.

Keywords: drought stress, range limit, stomatal conductance, ABA, population differentiation.

Introduction

For plant species occupying a large geographic range, population persistence can depend on the ability to survive stressful environments (Sultan et al. 1998). Physiological tolerances will often determine species distribution and abundance (Hacker and Bertness 1995); however, the precise traits that limit a species' range are a matter of contention (Farnsworth 2004). Regional differences in light, water, and temperature often present the biggest challenges to widely distributed species. In response to regional heterogeneity, species that occupy a wide geographic range often diversify and exist as ecotypes (Turesson 1922). Such ecotypes have differentiated genetically in order to tolerate the unique conditions of their habitats (cf. Heschel et al. 2004). In this experiment, we explore population differentiation across the range of a weedy annual in response to drought, a ubiquitous stress.

Drought response can involve interplay between leaf desiccation and gas exchange rates (Maherali et al. 2008). Despite the dangers of reaching the permanent wilting point and plasmolysis, some leaf wilting can be advantageous in avoiding photoinhibition (Jones 1992). Wilting of leaves up to 50° below horizontal can reduce leaf temperature by deflecting irradiance without significantly compromising photosynthetic rates (Schulz et al. 1993). A thick, waxy cuticle can also deflect irradiance, reduce the heat load on the plant, and conserve water to prevent severe desiccation and photoinhibition (Robinson et al. 1993; Barker et al. 1997). Furthermore, in-

creased stomatal conductance can reduce leaf temperature by creating a humid boundary layer that absorbs infrared irradiance (IR; Bazzaz 1979). However, prolonged water loss from stomata can lead to severe desiccation and eventually cell death.

There are two general strategies that plants employ when facing drought: avoidance and tolerance. Plants can avoid drought by accelerating growth and maximizing yield before the onset of drought (Geber and Dawson 1990, 1997; McKay et al. 2003; Heschel and Riginos 2005). Plants can tolerate drought stress with morphological and physiological mechanisms. Morphological adjustments include smaller leaves (Caruso et al. 2006), smaller stature (Grace 1977), and thicker cuticles (Barker et al. 1997), as well as an alteration of stomatal density on new leaves (Wu et al. 2010). Plants can physiologically minimize water consumption by closing stomata and decreasing stomatal conductance (Heschel et al. 2002; Caruso et al. 2006). Abscisic acid (ABA) is a mechanism that contributes to these physiological adjustments; foliar ABA, a plant hormone that induces stomatal closure, quickly increases in response to drought cues (Harris and Outlaw 1991). To deal with dry environments, some populations of the weedy annual *Impatiens capensis* (Balsaminaceae) have evolved a greater sensitivity to ABA (Heschel and Hausmann 2001), which results in a higher water use efficiency (WUE) under drought conditions (Heschel et al. 2002). In this experiment, we examined how populations of *I. capensis* deal with drought conditions across its geographic range.

Impatiens capensis is well suited for an ecotype study because it is widely distributed throughout the United States; this species is native to North America and grows contiguously from New England to Colorado, with isolated populations in the Pacific Northwest. *Impatiens* populations at the

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western range limit experience a dramatically different environment than do the eastern populations. Past studies have examined *I. capensis* population differentiation at a local level (Schmitt 1993; Dudley and Schmitt 1995; Lanza et al. 1995; Donohue and Schmitt 1999; Donohue et al. 2000, 2001; Dixon et al. 2001; Heschel and Hausmann 2001; Heschel et al. 2002; Huber et al. 2004; Heschel and Riginos 2005; but see von Wettberg et al. 2008). This experiment explored population differentiation at a regional level, examining *I. capensis* populations from Rhode Island (RI), Illinois (IL), and Colorado (CO).

Located farthest west and highest in elevation of the three sites, Colorado presents a stressful environment with respect to high irradiance and low moisture levels; IR irradiance increases and air humidity decreases at higher elevations (Larcher 2003). Because the Rhode Island, Illinois, and Colorado populations used here grow in forested, riparian habitats, soil moisture does not vary significantly between sites and all sites experience short drought episodes (M. S. Heschel, unpublished data), but Colorado is dramatically less humid than Rhode Island or Illinois. During the growing season, relative humidity (RH) levels in Colorado can average ~30%–40%, whereas in Illinois and Rhode Island they can average ~60%–70%.

In previous studies, populations of *Impatiens* from dry sites have demonstrated drought avoidance as well as tolerance strategies; these strategies utilized physiological, morphological, and/or phenological mechanisms (Heschel and Hausmann 2001; Heschel et al. 2002; Heschel and Riginos 2005). The observed differentiation has been unpredictable but seems to be connected to the maintenance of homeostasis and fitness across a diversity of habitats. By examining population responses to drought at a regional scale, we attempted to determine whether particular drought-response strategies might be shared across this species' geographic range. In particular, we addressed the following question: how has each regional population of *I. capensis* differentiated in response to drought conditions?

Material and Methods

Study Species and Source Populations

Although *Impatiens capensis* is susceptible to drought (Shulz et al. 1993), it occurs across a wide range of light and soil moisture conditions (Waller 1984; Leck 1996; Dixon et al. 2001). Past studies have demonstrated genetic differentiation in stomatal conductance plasticity, water use efficiency (Heschel et al. 2002; Heschel and Riginos 2005), and abscisic acid sensitivity (Heschel and Hausmann 2001). Within and among local populations, variation has also been documented for morphological, life-history, and other physiological traits (e.g., Schmitt 1993; Dudley and Schmitt 1995; Miliakal et al. 1999; Donohue et al. 2001).

To determine whether any observed differentiation had a genetic basis, inbred lines of *I. capensis* were created and used for this experiment. Seedlings were originally collected from wild populations in Rhode Island, Illinois, and Colorado, and inbred lines were developed by single-seed descent for at least four generations in the Colorado College greenhouse.

Because there are a few species of *Impatiens* that have similar morphologies, plants were allowed to chasmogamously flower in order to verify species identity in each population.

The Rhode Island *I. capensis* lines were originally collected in 2003 from two sites in Weetamoo Woods near Tiverton, Rhode Island. Both sites were moist, mixed-hardwood habitats (oak, hickory, maple canopy) located near streams, at sea level. Because of the close proximity of these sites (<1 km apart), they were considered as one population in our study. The Illinois inbred lines were derived from a population in Pekin, Illinois. The conditions at this site were similar to those in Rhode Island but slightly less shaded. The site was a moist, mixed-hardwood habitat (oak, hickory, dogwood canopy) located near a stream, at sea level. The Colorado inbred lines were derived from Fountain Creek Regional Park in Fountain, Colorado, and Boulder Creek in Boulder, Colorado. Both sites were moist, broken woodlands consisting of a cottonwood and willow canopy in a riparian system ~1650 m above sea level. The Colorado sites were treated as one population because no statistical differences were detected between these sites for all measured traits ($P > 0.05$). Moreover, on the basis of historical information (CU Boulder Herbarium), Boulder is likely the source of seeds for both sites.

Stomatal Density and Cuticular Wax Abundance Experiment

Impatiens capensis seeds from three geographic regions (four lines each from RI, IL, and CO) were dark stratified at 4°C for 7 mo. In April 2008, seeds were planted in flats in the Colorado College greenhouse. Plants were allowed to grow under moist greenhouse conditions (temperature, 18°–24°C; RH = 60%) for 2 wk before one fully expanded, mature leaf from each plant was harvested. Light conditions in the greenhouse ranged from ~400–600 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Field Scout Quantum Light Meter, Spectrum Technologies).

In order to compare differences in stomatal density and abundance of cuticular wax, we utilized SEM (Cameron et al. 2002; Teusink et al. 2002). Each harvested leaf was fixed with 2.5% glutaraldehyde, 1.0% paraform, and a 0.1-M phosphate buffer. Four disks were taken from each leaf: one from the leaf tip, one from the leaf base, and two from the middle of the leaf. Of these four disks, two were taken from each side of the leaf midrib. We affixed 100-mesh grids to the dried, mounted, graphite/sputter-coated leaf disks. Each disk was then examined with a low-kV SEM. The number of stomata was counted in four randomly selected grid squares on the abaxial side of each leaf disk. The number of wax crystals was also counted on the five epidermal cells immediately surrounding three pairs of guard cells on each leaf disk (Cameron et al. 2002; Teusink et al. 2002). Stomatal density was calculated by dividing each plant's average number of stomata per grid square by the average area of a grid square (0.04016 mm^2).

Drought Stress Experiment

Seeds from three regions (RI, 18 lines; IL, 15 lines; CO, 18 lines) were dark-stratified in distilled water at 4°C for 5 mo. In July 2009, seeds were planted in flats filled with water-

saturated Fafard Canadian Growing Mix 2. After the planting, all flats were placed under 20-W wide-spectrum grow lights with a 14-h day length and maintained at 65%–75% RH. After 1 wk, the flats were moved into the Colorado College greenhouse.

At 2 wk of age, 102 plants (all lines, two replicates per line) were transplanted into individual plastic pots (100-mm diameter) filled with Fafard Canadian Growing Mix 2. These plants were distributed across six greenhouse trays on three greenhouse benches so that each population was equally represented within each tray or block. Plants were randomly distributed within each tray; each tray (60 cm × 120 cm) held 17 plants and one dummy pot, evenly spaced.

Starting on the day of the transplant, all plants were bottom-watered and fertilized every 3.5 d with 200 ppm Miracle-Gro 24-8-16 (N:P:K) fertilizer (Scotts Horticultural). Greenhouse conditions were warm and moist (temperature, 18°–24°C, reaching 28°–30°C in the afternoon on sunny days; RH = 60%). During this moist period, soil moisture averaged 33% volumetric water content (VWC; TDR Campbell Scientific Hydrosense). Light conditions in the greenhouse ranged from

~400 to 700 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (FieldScout Quantum Light Meter, Spectrum Technologies).

At 4 wk of age, stomatal conductance and leaf temperature were measured on 96 plants. Measurements were taken over the course of two consecutive days between 1100 and 1430 hours on the most recent fully expanded leaf of each plant when light conditions in the greenhouse were ~600–700 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Maximal carbon assimilation rates are reached at 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in *I. capensis*, so light conditions were close to saturating for conductance measurements (Heschel and Riginos 2005). Stomatal conductance measurements (g_{ST} , in $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) were taken with a steady-state diffusion porometer (Decagon Devices Leaf Porometer SC-1) on abaxial leaf surfaces. Leaf temperature ($^{\circ}\text{C}$) was measured at the center of the midrib of the adaxial surface, using an IR thermometer (IT Extech Instruments). (Note: care was taken to measure leaf temperature in the same place on each leaf.)

After these measurements were taken, all plants were watered and fertilized one final time before being submitted to a week-long drought treatment. When all plants were simi-

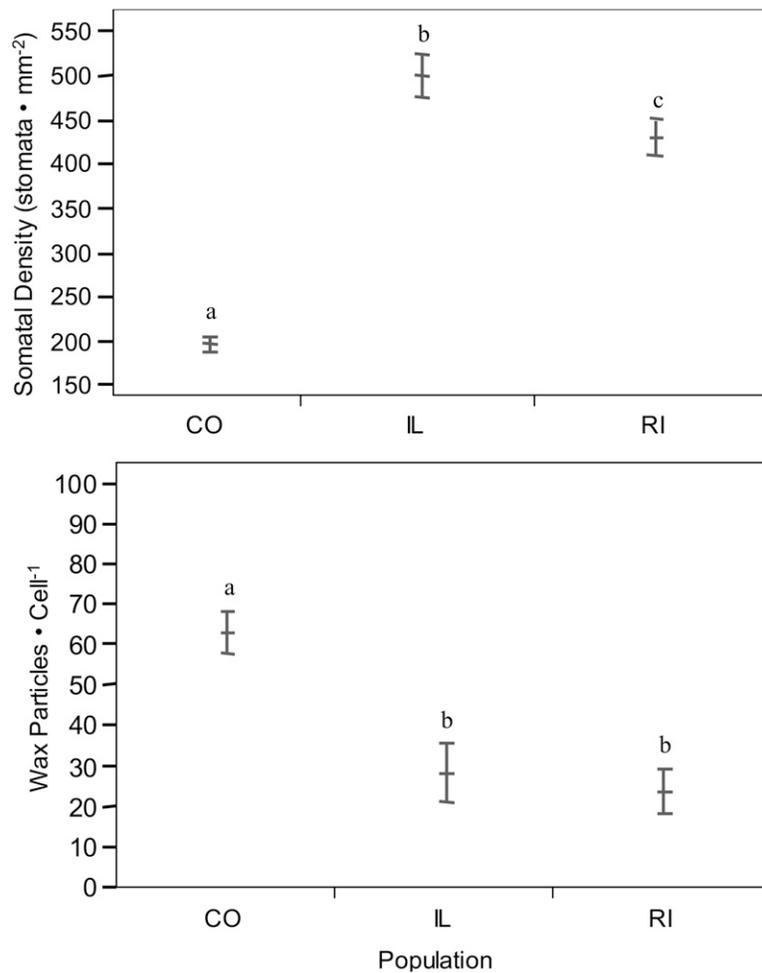


Fig. 1 Mean stomatal density and mean number of wax particles per epidermal cell by population, $\pm 1\text{SE}$. Shared lowercase letters above bars indicate a lack of statistical significance with planned contrasts (t -tests; $P < 0.05$).

Table 1
Repeated-Measures MANOVA for Population, Line (Nested within Population), and Drought Treatment on Physiological Traits Measured in the Drought Stress Experiment

Effect	g_{ST}	Leaf temperature	Growth rate
Population	1.17	4.44*	.78
Line (population)	2.11**	1.69*	1.17
Treatment	150.28***	89.69***	52.65***
Treatment \times population	5.75**	.46	2.22 ⁺
Treatment \times line (population)	1.01	.77	.76

Note. *F* values are reported. g_{ST} = stomatal conductance. Leaf temperature and g_{ST} values are adjusted for time and block.

⁺ $P < 0.10$.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

larly drought stressed at 7 d, the stomatal conductance and leaf temperature measurements were repeated on the most recent fully expanded leaf. During the drought treatment, soil moisture averaged 13% VWC. (Note: independent of soil

moisture, leaf age had no significant effect on gas exchange [see below].) One most recent fully expanded leaf from each of 72 plants (24 from each population) was harvested at the end of the drought period and immediately frozen with liquid

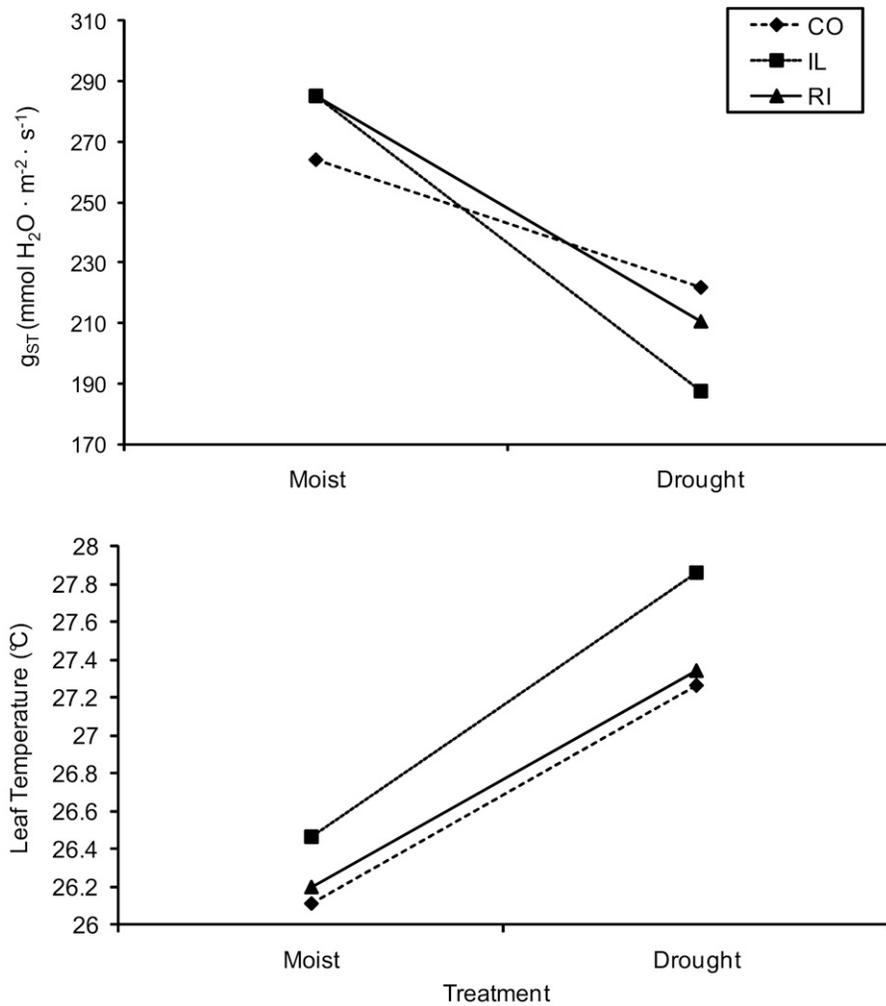


Fig. 2 Mean adjusted stomatal conductance and mean adjusted leaf temperature by population for each treatment during the drought stress experiment.

Table 2

**ANOVA for Population and Line (Nested within Population)
on Morphological and Chemical Traits Measured in the
Drought Stress Experiment**

Effect	[ABA]	Leaf area	Specific leaf area
Population	10.01***	12.80***	48.25***
Line (population)	1.46	1.71*	1.94*
Block	.21	.44	.90

Note. *F* values are reported. [ABA] = abscisic acid concentration.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

nitrogen in preparation for ABA extraction. Thus, ABA was extracted after plants had experienced the drought treatment. All harvested leaves were weighed and then lyophilized for at least 12 h before being ground with 2 mL of an ABA extraction buffer of methanol, butylated hydroxytoluene, and citric acid monohydrate (Boggs et al. 2010). The solution was incubated at 4°C overnight and then centrifuged, and the supernatant was removed and allowed to dry under a hood to allow for additional evaporation. We then removed 100 μ L of supernatant and combined it with 900 μ L of TBS with $MgCl_2$, centrifuged this mixture, and then separated it once more; the supernatant was stored at $-10^\circ C$. ELISA was used to determine the concentration of ABA in each leaf sample (Agdia). Standard curves generated with ABA (mixed isomers, Sigma) were used to determine the mass (ng) of ABA per milligram of leaf fresh mass.

Plant height was measured on all plants at 3.5, 4.5, and 5.5 wk of age to compare growth rates under moist and drought conditions. The area of the most recent fully expanded leaf and its biomass were measured after 7 wk of growth. Specific leaf area (SLA) was calculated by dividing each leaf area by its biomass (Evans and Hughes 1961).

Effect of Leaf Age on Gas Exchange

To assess whether leaf age had a significant influence on gas-exchange measurements, stomatal conductance was measured on leaves that were ~ 1 wk apart, developmentally. In 2008, 12 lines each from Illinois, Colorado, and Rhode Island were planted in flats containing Fafard Canadian Growing Mix 2 in the Colorado College greenhouse. Greenhouse conditions were warm and humid (temperature, 18° – $24^\circ C$; RH = 60%). When plants were ~ 5 wk old, a “new” and an “old” leaf were marked on each plant. The new leaf was the most recent fully expanded leaf; the old leaf was a leaf located one node below the new leaf, on the same plant. Stomatal conductance was measured on both the new and old leaves under moist soil conditions, with light levels ranging from 300 to 600 μ mol photons $m^{-2} s^{-1}$ (FieldScout Quantum Light Meter, Spectrum Technologies). Conductance measurements were taken between 1000 and 1600 hours. ANOVA indicated that stomatal conductance values for new and old leaves were statistically indistinguishable, regardless of population (no significant effect of leaf age on conductance: $F = 0.0014$, $df = 1$, $P = 0.9705$). Therefore, in our repeated-measures

analyses (see below), time was not confounded with drought treatment.

Data Analyses

All statistical analyses were performed in JMP, version 7.0.2 (SAS Institute, Cary, NC). For all ANOVA and MANOVA models, residual variation was examined for normality. One-way ANOVAs were used to examine regional population differences in stomatal density and number of cuticular wax particles. Repeated-measures MANOVAs were used to examine the effects of population, line nested within population, and drought treatment on stomatal conductance and leaf temperature. Line was nested within population and treated as a random effect in analyses. With regard to the response variables, the MANOVA approach to repeated-measures analysis has the advantage of an unstructured variance/covariance matrix. Stomatal conductance and leaf temperature data were adjusted for effects of time and block to account for conductance changes throughout the day and microenvironmental variations within the greenhouse (Heschel et al. 2002). Conductance and temperature were regressed against measurement time and block position; residuals from these linear regressions were added to the conductance and temperature grand means. Contrasts (*t*-tests) were used to examine stomatal conductance and leaf temperature differences among populations within each treatment (drought and moist). Growth rate was adjusted for block (with residual variation; see above) to remove microenvironmental effects, and repeated-measures MANOVA was used to examine population, line nested within population, and drought treatment effects on growth rate. ANOVA was used to determine the effect of population, line nested within population, and block on ABA concentrations, leaf area, and SLA for drought-treated plants.

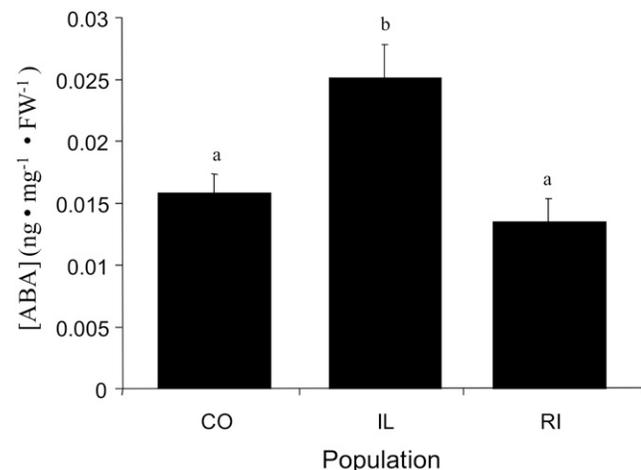


Fig. 3 Mean abscisic acid content ± 1 SE per population at the end of the drought stress experiment. Shared lowercase letters above bars indicate a lack of statistical significance with planned contrasts (*t*-tests; $P < 0.05$).

Results

SEM Analysis of Leaf Surfaces

Population or region was a significant predictor of both stomatal density (one-way ANOVA; $F = 63.77$; $df = 1$; $P < 0.0001$) and cuticular wax abundance (one-way ANOVA; $F = 12.88$; $df = 1$; $P < 0.0001$). Leaves of Colorado plants had lower stomatal density than those of Rhode Island plants, which in turn had lower stomatal density than leaves of Illinois plants (fig. 1). SEM photos revealed a difference in cuticular wax abundance among the three regions. Colorado plants had a greater number of wax particles surrounding their stomata than Illinois and Rhode Island plants (fig. 1). Rhode Island and Illinois populations did not differ in cuticular wax abundance.

Gas Exchange and ABA Responses to Drought Stress

In response to drought conditions, plants from all three regions significantly decreased their stomatal conductance (table 1; fig. 2). A significant population-by-drought treatment effect was also detected. Rhode Island and Illinois plants exhibited the greatest plasticity for stomatal conductance (fig. 2). Under moist conditions, Colorado plants had significantly lower conductance levels than Illinois and Rhode Island plants ($F = 4.45$; $df = 2$; $P = 0.0145$). In contrast, Illinois

plants had significantly lower conductance rates under drought conditions than Colorado and Rhode Island plants ($F = 5.41$; $df = 2$; $P = 0.0061$). Foliar ABA concentration data supported these findings for drought conditions. Colorado and Rhode Island plants did not significantly differ from one another in ABA concentration, but Illinois plants produced higher ABA levels in response to drought (table 2; fig. 3).

Population Differentiation of Leaf Temperature and Area

Population or region and drought treatment had significant effects on leaf temperature (table 1). All populations exhibited higher leaf temperatures during drought conditions (table 1; fig. 2). Also, population differentiation for leaf temperature was evident under drought conditions ($F = 3.78$; $df = 2$; $P = 0.0267$). Colorado and Rhode Island plants had significantly lower leaf temperatures than Illinois plants when drought stressed. Although this pattern was not significant under moist conditions ($F = 1.99$; $df = 2$; $P = 0.1926$), a consistent trend was evident (fig. 2).

Leaf size depended on population in the drought stress experiment (table 2). Colorado plants grew the smallest leaves, Illinois plants had the largest leaves, and Rhode Island plants produced leaves of an intermediate size (fig. 4). Calculating SLA in the drought stress experiment allowed for a comparison of leaf thickness among populations. Colorado leaves

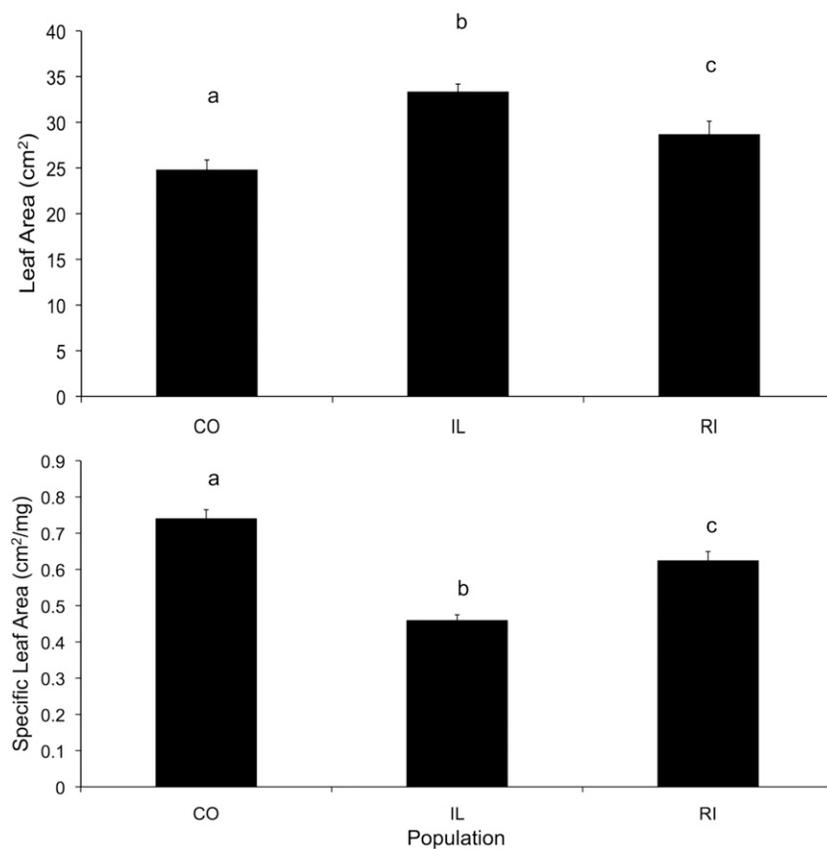


Fig. 4 Mean leaf area and specific leaf area + 1 SE by population at the end of the drought stress experiment. Shared lowercase letters above bars indicate a lack of statistical significance with planned contrasts (t -tests; $P < 0.05$).

were thinner than the leaves of both Illinois and Rhode Island plants, and Illinois plants had the thickest leaves (fig. 4).

Performance Response to Drought Stress

Growth rates measured throughout the drought stress experiment varied significantly by treatment (table 1) and the population-by-treatment interaction was marginally significant (table 1). Weekly growth rates increased all 3 wk, but the Illinois population did not increase its growth rate to the same degree as the other populations after the drought treatment (fig. 5). By the end of the drought treatment, the Rhode Island plants exhibited the fastest growth rate ($F = 3.95$; $df = 2$; $P = 0.0228$). An additional observation made before harvesting the plants was that the Colorado and Illinois populations produced fewer seeds and fruit than did the Rhode Island population (total seeds and fruit harvested per region: for CO, 23 seeds and 12 fruit; for RI, 64 seeds and 27 fruit; for IL, 44 seeds and 20 fruit).

Discussion

This study provides experimental evidence that regional populations of *Impatiens capensis* have differentiated in response to drought stress. Each region has developed a unique evolutionary strategy to deal with a ubiquitous stress, drought. The Rhode Island population exhibited a drought stress-avoidance strategy. The Illinois and Colorado populations exhibited different drought stress-tolerance strategies. Because the inbred lines of each population were grown under the same conditions, the differences observed have a genetic basis and may have resulted from either natural selection in the wild or genetic drift (Endler 1986).

Population Differentiation in Response to Drought

Regional Similarities. Stomatal conductance decreased in all plant populations in response to drought in order to minimize water loss. When drought stressed, plants from all three regions also experienced an increase in leaf temperature. This was potentially due to the thinning of the leaf boundary layer with the closing of stomata (Bazzaz 1979).

Regional Differences. Rhode Island plants avoided drought stress with phenological adjustments. Overall, Rhode Island plants exhibited the most growth: in the week following the drought, Rhode Island *Impatiens* grew significantly faster than plants from the other two regions. Stomatal conductance remained comparatively high for Rhode Island plants under both moist and dry conditions (fig. 3). Due to increased conductance, carbon assimilation rates may have been higher, allowing Rhode Island plants to grow faster than other populations (Heschel and Riginos 2005). These data combined with the observational data that Rhode Island plants flowered first and produced more seeds than the other populations indicated that the Rhode Island populations might have avoided drought stress via an accelerated life history (Geber and Dawson 1990).

In contrast, *Impatiens* in Illinois tolerated drought stress through physiological adjustments. The stomatal conductance response of Illinois plants seemed to be the most plastic (fig. 2). Increased water use plasticity can increase survivorship during late-season droughts (Heschel and Riginos 2005). Reduction of stomatal conductance was potentially important to Illinois *Impatiens* because they also had the highest stomatal density and the largest leaves and therefore may have been more susceptible to severe desiccation through high transpiration rates and water loss. In addition, Illinois plants produced the highest concentration of foliar ABA during the experimental drought. ABA induces stomatal closure, and thus it directly affects stomatal conductance plasticity

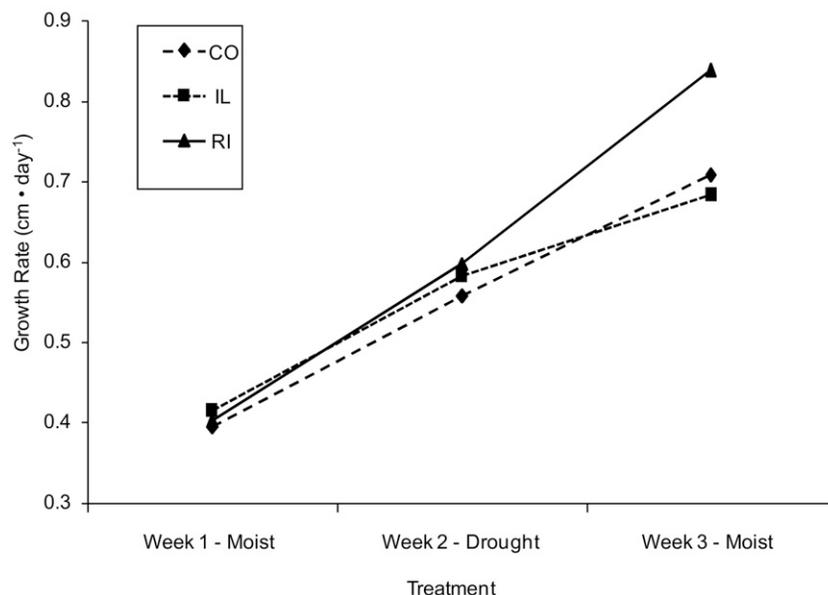


Fig. 5 Weekly mean daily growth rate per population throughout the drought stress experiment. Growth rate was adjusted for block.

(Harris and Outlaw 1991). Increased ABA production can decrease drought response time and increase water use efficiency; however, decreases in transpiration can also lead to an increased thermal load due to a small boundary layer (Heschel and Hausmann 2001).

Under drought conditions, Illinois plants also grew the largest and the thickest leaves. Large leaves present a water-loss challenge to plants in drought conditions, but the Illinois leaves were also thick (increased SLA), which could provide some water storage in hydrenchyma tissue (Larcher 2003). Nonetheless, leaf temperatures were highest for Illinois plants during the drought treatment, indicating that the large leaves and reduced stomatal conductance might have negative consequences for thermal regulation and photoinhibition. This potential cost to photosynthetic capacity might be less important in the shaded woods of central Illinois, where episodic drought is likely to occur later in a growing season.

A variety of morphological mechanisms made the Colorado plants less likely than plants from other regions to incur severe damage from a 1-wk drought. The lower stomatal density and greater waxiness of the Colorado leaves might have reduced water loss also. The plants were relatively smaller in stature than the Illinois and the Rhode Island plants (data not presented), potentially creating a boundary layer around the entire plant instead of around individual leaves (Grace 1977). Additionally, the leaves were small and thin, leaving less surface area for water loss and leaf overheating. These morphological adjustments are consistent with our observations of greater drought tolerance in this population. Interestingly, the Colorado plants did not respond to drought via enhanced physiological plasticity. Colorado *Impatiens* exhibited the least plastic response in stomatal conductance; they also did not produce increased levels of foliar ABA. Past research has indicated that populations of *I. capensis* from dry habitats may be more sensitive to ABA than plants from moist habitats (Heschel and Hausmann 2001). However, this might not be true for Colorado *Impatiens* populations. The ABA concentrations were relatively low and the stomatal conductance remained relatively high, suggesting that Colorado plants are not more sensitive to ABA than plants from the other two regions. Because increased foliar ABA leads to stomatal closure, potentially higher leaf temperatures, and po-

tentially compromised fitness (Heschel and Hausmann 2001), increased ABA sensitivity may not have been selected for in the Colorado populations. A waxier cuticle might have reduced water loss and maintained lower leaf temperature by reflecting irradiance, allowing the Colorado plants to maximize carbon assimilation by maintaining comparatively high stomatal conductance rates in drought conditions.

Conclusions

The drought responses of these geographically distinct *I. capensis* populations revealed three stress strategies. Rhode Island plants evolved on a temporal axis, avoiding stress with an accelerated phenology. Illinois plants evolved on a physiological axis and Colorado plants evolved on a morphological axis, and each employed unique stress-tolerance strategies. These three regional populations have genetically differentiated and might be considered ecotypes; however, it is unclear whether they are adapted to the specific environmental conditions of their native habitats.

For this weedy species, different axes of evolution might drive range expansion and persistence in the face of climate change. Range expansion could be possible with the development of new strategies or with changes in local climate. By evolving a variety of morphological, physiological, and phenological strategies for dealing with stress, natural populations of annual plants might be able to occupy a wide geographic range across a variety of moisture conditions.

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