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## Low Inbreeding Depression and High Plasticity under Abiotic Stress in the Tall Morningglory (*Ipomoea purpurea*)

Chase M. Mason, Dorothy A. Christopher, Ashley M. Rea, Lauren A. Eserman, Alex J. Pilote, Nicholas L. Batora, and Shu-Mei Chang\*

Weeds represent a major cause of agricultural losses worldwide. Most weeds share a common set of life history characteristics that predispose them to weediness, two of which are self-compatibility, which allows for ease of colonization through reproductive assurance, and high trait plasticity, which allows for tolerance of a wide variety of environments and abiotic conditions. However, self-fertilization typically comes at the cost of inbreeding depression. This study investigates the role of inbreeding depression and trait plasticity under abiotic stress in the tall morningglory, a widespread self-compatible agricultural weed in the southeastern United States. Results show very little inbreeding depression in this species, likely due to purging of deleterious alleles through repeated founder events in agricultural landscapes. In contrast, abiotic stress induced substantial plasticity in ecophysiological traits, reproductive traits, and biomass allocation. In terms of performance, drought sharply impacted reproduction but not vegetative growth, and nitrogen limitation sharply impacted both. These findings are applicable to the control of weedy morningglory and underscore the usefulness of evolutionary ecology to weed management.

**Nomenclature:** Tall morningglory, *Ipomoea purpurea* (L.) Roth.

**Key words:** Abiotic stress, drought, ecophysiology, inbreeding depression, common morningglory, nitrogen limitation, reproduction.

Weeds represent the largest biotic loss potential for crop production worldwide (34%), much larger than animal pests or pathogens (Oerke 2006). Mechanical and chemical control efforts currently make the efficacy of weed management quite high, reducing actual weed-attributable losses to approximately 10% (Oerke 2006). The success of weed control over the past century has been in large part due to the widespread use of herbicides, and the ongoing evolution of herbicide resistance poses a huge threat to successful weed management (Busi et al. 2013; Heap 2014). Evolution itself has not been a major focus of weed studies (Baucom and Holt 2009), despite the fact that key life history traits predispose particular species to weediness (Baker 1965, 1974; Kuester et al. 2014), and weeds evolve rapidly under agricultural and weed management practices that impose strong selection (Baucom and Holt 2009). A better understanding of the evolutionary dynamics of weedy species can inform effective weed management, especially in the face of widespread herbicide resistance (Baucom and Holt 2009; Neve et al. 2009). Here we focus on two potentially important aspects of weedy plant

evolution—inbreeding depression and the response to abiotic stress.

Worldwide, approximately 90% of extant plant species are hermaphroditic (Richards 1997), and approximately 40% of those species are self-compatible (Fryxell 1957). Self-compatibility confers substantial evolutionary benefits to an individual because self-compatible plants can produce progeny even in the absence of pollinators or conspecifics (Baker 1955). This assured reproduction is one trait thought to predict weediness in annuals, as it allows for rapid colonization of new environments and robustness to weed elimination efforts and resulting population bottlenecks (Baker 1974). However, along with the benefits of self-compatibility come the risks of potential inbreeding depression, the reduction in fitness often experienced by progeny that are the product of repeated self-fertilization or mating between closely related individuals. Inbreeding depression results from increased homozygosity, and can occur via either the unmasking of deleterious alleles (the dominance hypothesis) or the loss of heterosis (the overdominance hypothesis) (Charlesworth and Charlesworth 1999). These effects have been well documented in nature, and are a central explanation for the evolutionary origins of self-incompatibility and other mechanisms that promote cross-fertilization (Barrett 2002; Lande and Schemske 1985; Lloyd 1992). Agricultural

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weeds are subject to repeated founder events and population bottlenecks, making them potentially susceptible to high levels of inbreeding depression. When evaluating the impact of inbreeding depression in plants the focus is typically on overall plant fitness, but the phenotypic effects of inbreeding depression can manifest at any point during the plant life cycle, from germination (e.g., Richards 2000), through vegetative growth (e.g., Brennan et al. 2005; Teixeira et al. 2008), to final reproduction (e.g., Busch 2004; Krebs and Hancock 1990). Understanding how inbreeding depression is phenotypically expressed represents one angle to understanding the long-term evolutionary dynamics of self-compatible weed populations.

Abiotic stress is another major force that limits plant growth and reproductive success. Baker identified high trait plasticity and tolerance of a wide range of environmental conditions as predictive of weediness (Baker 1974), and environmental heterogeneity is widespread in both agricultural systems and similar ruderal habitats. Two universal plant requirements are water and nitrogen, and limitation by these two primary resources is acknowledged as a major driver of plant ecology and evolution (Bloom et al. 1985; Chapin 1980; Chapin et al. 1987; Reich 2014). Limitation by nitrogen or water availability reduces plant growth and results in integrated stress responses and plastic shifts in plant traits across multiple plant organ systems (Chapin 1991; Chapin et al. 1987; Donovan et al. 2014). These abiotic stresses also limit plant reproduction, though the effects on male and female reproductive function have been shown to differ by the type of stress (Galen 2000; Hayes et al. 2005; Lau and Stephenson 1993; Maad and Alexandersson 2004). Understanding trait plasticity and tolerance to drought and low-nitrogen stress represents a second angle to understanding the long-term evolutionary dynamics of major agricultural weeds.

Tall morningglory is one of the top ten worst agricultural weeds in the southeastern United States, and is particularly problematic for the production of corn, cotton, peanut, and soybean (Baucom et al. 2011; Webster and Coble 1997). Native to Mexico and Central America, tall morningglory was likely introduced to the southeastern United States around 300 years ago and has since become naturalized and widespread throughout the region (Fang et al. 2013; Glover et al. 1996; McDonald 1991). This annual twining vine exhibits many of Baker's weediness characteristics (1974), including rapid and choking growth, continuous and very

high seed output, and self-compatibility (Chaney and Baucom 2012). Plants flower indeterminately, with tubular flowers that open in the early morning and senesce by early afternoon (Brown and Clegg 1984). Flowers are hermaphroditic with delayed selfing, with stamens that elongate continuously throughout the day and thus allow for selfing or outcrossing (Smith and Rausher 2007). In natural populations, tall morningglory has selfing rates of approximately 30% (Ennos 1981). Each flower has an ovary with three locules, producing up to two gravity-dispersed seeds per locule (McDonald 1994). This combination of narrow flowering period per flower, self-compatibility, and gravity-dispersed seeds generates the potential for widespread inbreeding (both selfing and biparental inbreeding) in weedy populations (Chang and Rausher 1999). Additionally, the fact that morningglory is able to reduce yields in a variety of crops grown under very different agronomic regimes (e.g., in both irrigated and rain-fed cropping systems, as well as in both highly fertilized crops like cotton and corn and minimally fertilized crops like soybeans and peanuts) indicates that this species is capable of broad trait plasticity and tolerance of a variety of abiotic variation. Here we address four primary questions:

1. How severe is inbreeding depression in weedy tall morningglory?
2. Does the magnitude of inbreeding depression vary with environmental conditions?
3. How tolerant of abiotic stress is tall morningglory?
4. How plastic are key ecophysiological and reproductive traits under abiotic stress?

## Materials and Methods

**Experimental Design and Plant Growth.** Morningglory seeds used for this study came from lines established for a different experiment to examine the genetic basis of pollen size (Chang, unpublished data). These lines were originally started from seeds collected from two populations located on the edges of agricultural fields in Oconee County, Georgia, USA. Because the pedigree of every individual was recorded through the subsequent five generations, we were able to calculate the inbreeding coefficient of each seed used in this study. Twenty-four ovule parent plants were used to produce the following three types of progeny: selfed, biparentally inbred with a related individual, or outcrossed to an unrelated individual. This design generated three

classes of inbreeding coefficients: high for selfed plants (0.52 to 0.56), intermediate for biparentally inbred plants (0.05 to 0.21), or zero for mating between unrelated plants. For each of the 24 ovule parents, three selfed seeds, six biparentally inbred seeds, and three outcrossed seeds were selected for inclusion, for a total of 288 seeds.

Seeds were scarified and sown into individual cells of a seedling tray filled with Fafard 3B peat mix (Fafard Inc., Anderson, SC). Seedlings were transplanted at the emergence of the first true leaf into 6-inch pots filled with river sand, and placed in the greenhouse under natural sunlight with day and night temperatures set to 27.7 and 23.3°C, respectively. Plants were arranged in a nine-block Latin square design (three rows across three benches) to control for spatial environmental variation in the greenhouse, with three blocks assigned to each treatment group (control, drought, and low nitrogen). There were 32 pots within each block, each containing four seedlings (one selfed, two inbred, one outcrossed) from eight different ovule parents. Seedlings were distributed across treatment blocks such that each ovule parent had four seedlings randomly assigned to one of the three blocks of each treatment. Locations of pots within each block were fully randomized. Plants were initially watered twice daily to field capacity with automatic drip irrigation, and fertilized twice weekly with half-strength Hoagland's solution (Hoagland and Arnon 1950). There was only one mortality among all 288 plants, a selfed plant in the control treatment that died within a week of being transplanted. After 3 wk to allow plants to establish, treatments were initiated. Control plants continued to receive the initial watering and fertilization regime. Low-nitrogen treatment plants continued to receive twice-daily watering by drip irrigation, but the twice-weekly fertilization was changed to a modified Hoagland's solution with 1/20 the nitrogen of the control (1/40 of full-strength Hoagland's solution), in order to induce nitrogen limitation. For drought-treatment plants, the twice-daily automatic irrigation was stopped and plants were checked each morning for loss of leaf turgor and rewatered to field capacity with half-strength Hoagland's solution only when wilted, so as to impose recurring water limitation via pot dry-down without inducing nitrogen limitation or causing mortality. This resulted in an average of  $4.1 \pm 1.1$  (mean  $\pm$  SD) wilting events per plant during the course of this experiment, with the average number of days between wilting events ranging between

around 13 when plants were small to around 4 by the end of the experiment (Figure S1; <http://dx.doi.org/10.1614/WS-D-15-00005.S1>).

### **Ecophysiological and Biomass Measurements.**

Because biomass measurements require destructive sampling, we designated plants from only half of the ovule parents for harvesting for biomass quantification ( $n = 143$ , hereafter "biomass plants"). The remaining plants from the other half of the ovule parents were kept alive longer to allow reproductive-trait assessment and the quantification of seed fitness ( $n = 144$ , hereafter "fitness plants"). Most ecophysiological traits were assessed on both groups ( $n = 287$ ); however, some traits were assessed on subsets of plants based on inbreeding type, as detailed below.

Ecophysiological measurements were taken 2.5 wk after treatments were initiated, after several new leaves developed on all plants. Drought-treatment plants were rewatered the day prior to measurements and the day of measurements, such that all plants were well-watered during trait assessment. Gas exchange measurements were made at 2,000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity and 400 ppm  $\text{CO}_2$  with a LI-6400 Portable Photosynthesis System (Li-Cor Biosciences, Lincoln, NE), in order to assess photosynthetic rate ( $A_{\text{area}}$ ) and stomatal conductance ( $g_s$ ) on the most recently fully expanded leaf of each plant. The gas exchange leaf was tagged and excised the following morning before dawn, when leaves were maximally hydrated. Leaves were then weighed with a digital scale to obtain leaf fresh mass and scanned on a flatbed scanner to obtain digital images from which leaf area, perimeter, major and minor axis, circularity, and solidity were calculated with ImageJ freeware (Schneider et al. 2012). The leaf major axis reflects the widest part of the leaf, whereas the leaf minor axis reflects the width of the axis perpendicular to the major axis. Leaf circularity is a measure of roundness, where a value of one reflects a perfect circle and values approaching zero reflect increasing elongation, and leaf solidity reflects the ratio of leaf area to leaf convex area. Chlorophyll content was assessed with a SPAD-502 meter (Konica-Minolta, Tokyo, Japan) as an average of five measurements taken on different parts of the leaf. Leaf toughness was assessed with a digital penetrometer (Chatillon Force Measurement Products, Largo, FL) as the amount of force (in grams) required to puncture the leaf lamina, and measurements were taken on the left and right side of the leaf and averaged. Trichome density was

assessed on the gas exchange leaf by counting the number of trichomes in a standardized 0.25 cm<sup>2</sup> area in the center of the leaf. The gas exchange leaf was then dried at 60°C for 72 h in a forced-air drying oven, weighed for dry mass, and ground into a fine powder with a Spex8000 ball mill (Spex Sampleprep, Metuchen, NJ). This powder was used for carbon and nitrogen analysis, performed at the University of Georgia Analytical Chemistry Laboratory with Micro-Dumas Combustion (NA1500, Carlo Erba Strumentazione, Milan, Italy), yielding leaf nitrogen concentration ( $N_{\text{mass}}$ ), leaf carbon concentration ( $C_{\text{mass}}$ ), and the carbon-to-nitrogen ratio (C:N). Leaf tannin activity was quantified via the radial diffusion method (Hagerman 1987), in which sample leaf extracts are compared to a tannic acid standard (C<sub>76</sub>H<sub>52</sub>O<sub>46</sub>, CAS No. 1401-55-4) for protein precipitation capacity. Leaf water content ( $W_{\text{m}}$ ) was calculated as the ratio of the mass of water present in the fresh leaf to the dry mass of the leaf (Shipley et al. 2006). LMA was calculated by dividing leaf dry mass by leaf area, mass-based photosynthetic rate ( $A_{\text{mass}}$ ) was calculated by dividing  $A_{\text{area}}$  by LMA, and instantaneous water use efficiency (iWUE) was calculated by dividing  $A_{\text{area}}$  by  $g_s$ . Chlorophyll content, fresh and dry mass, water content, leaf area and shape descriptors, toughness, and trichome density were assessed on all plants ( $n = 287$ ). Due to feasibility and cost, gas exchange traits, leaf nutrients, and tannin activity were assessed on only selfed and outcrossed plants ( $n = 143$ ).

All biomass plants were harvested 6.5 wk after treatments were initiated, with whole-plant green leaf area determined with a LI-3100 conveyor belt leaf area meter (Li-Cor Biosciences). Harvested plants were divided into leaves, stems, roots, and reproductive parts, and were dried at 60°C for 72 h in a forced-air drying oven before being weighed to quantify total plant biomass and mass fractions of each organ type.

**Reproductive Measurements and Fitness Estimation.** Date of first flower was recorded for all plants ( $n = 287$ ). All other reproductive traits were assessed on fitness plants only ( $n = 144$ ). Once all plants were in flower (approximately 4 wk after treatments were initiated), the number of flowers produced per plant each day was recorded for 2 wk to estimate relative flower production. During this period, a target of seven flowers per plant were assessed for floral morphology in the early mornings, though some plants (especially in the low-

nitrogen treatment) produced fewer flowers and were thus sampled for fewer. Digital calipers were used to measure corolla height, corolla width, pistil height, and height of the tallest anther. To assess pollen production, anthers from two flowers (10 anthers total) were placed together in a 1.5 ml tube with 750  $\mu\text{l}$  of ethanol (70% v/v) to preserve the pollen grains. Pollen grains were counted with a Beckman Multisizer II Coulter counter (Beckman Coulter, Inc., Fullerton, CA). To assess floral pigment concentrations, three flowers were collected per plant. Each flower was placed in a 1.5 ml tube and placed into a -20°C freezer for storage. Total anthocyanins and carotenoids were extracted with a method modified from Schemske and Bradshaw (1999) and Janik et al. (2007). Corolla tissue was homogenized, and 750  $\mu\text{l}$  of ethanol (50% v/v) was added per 0.2 g of homogenized floral tissue. Samples were allowed to mix at room temperature for 20 min, and were then centrifuged for 10 min at 4,000 rpm. Absorbance of anthocyanins is highly dependent upon pH, with highest absorbances at pH near 1 (Giusti and Wrolstad 2001), so 60  $\mu\text{l}$  of the resulting supernatant were mixed with 750  $\mu\text{l}$  of ethanol (50% v/v) and 36  $\mu\text{l}$  of 1 M HCl to obtain a solution of floral pigments with a pH close to 1. Absorbance was measured at 520 nm for total anthocyanins and 480 nm for total carotenoids with a spectrophotometer.

Five flowers per fitness plant were hand-pollinated with pollen collected from another plant of the same treatment group. Flowers were emasculated the evening prior as needed to prevent self-pollination. All fruits from these hand pollinations, mostly immature, were removed from plants 8 wk after treatments were initiated, and were dried at 60°C for 48 h. Dry mass of immature fruits was measured, along with the number of immature seeds per fruit and total seed mass. A fitness metric was calculated as the product of the total number of flowers produced in the 2 wk observation period and the average number of seeds produced per fruit as a rough representation of the relative reproductive potential for each plant. Fitness plants were then harvested to determine total plant biomass and mass fractions in the same manner as for biomass plants. The duration of the entire experiment from germination through the final harvest was 80 d.

**Statistical Analysis.** All statistical analyses were performed in JMP Pro version 11 (SAS Institute, Cary, NC). Univariate ANOVAs were used to assess the effects of treatment, breeding type, treatment by

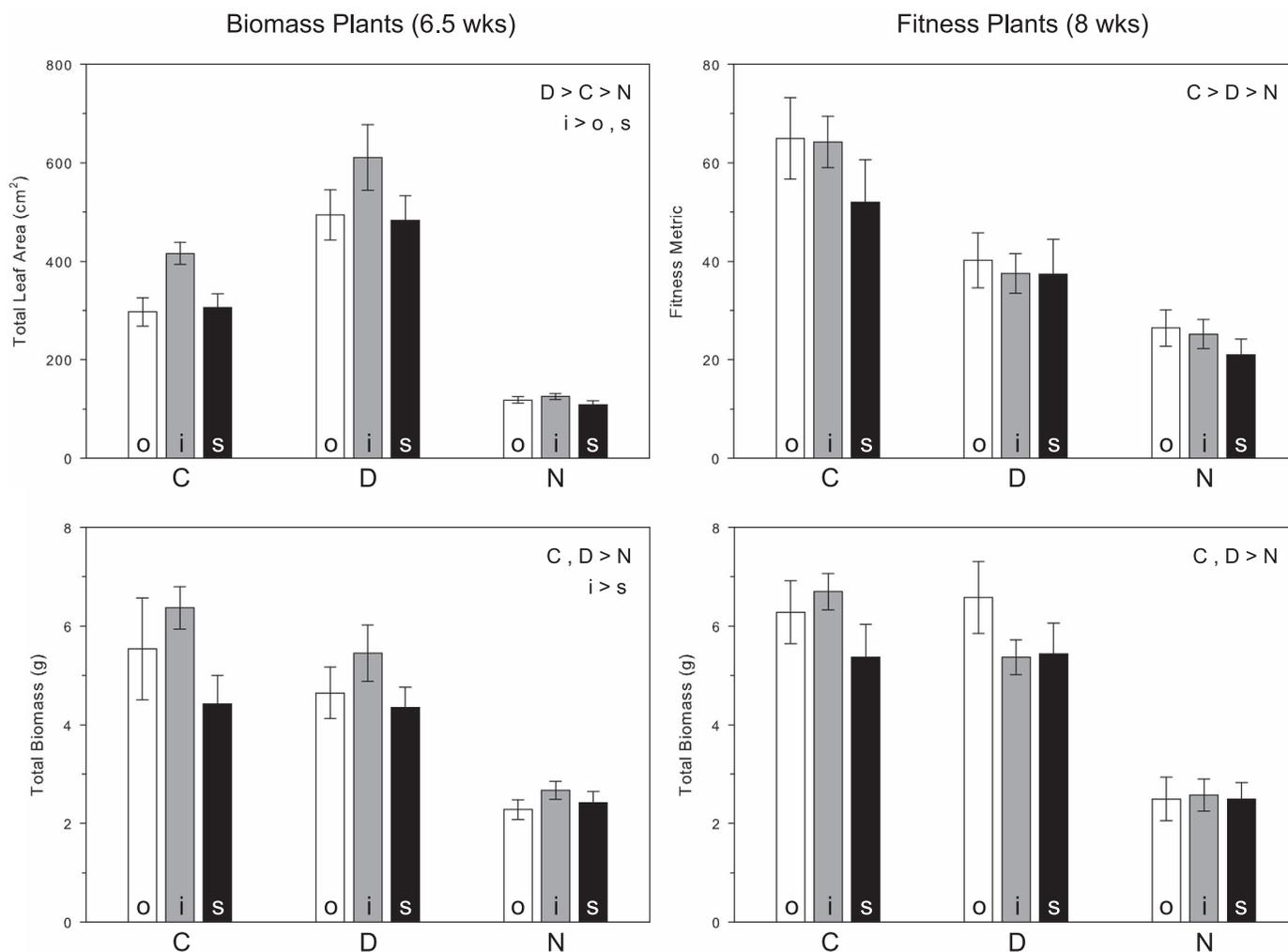


Figure 1. Differences in performance traits across treatments and breeding types. Left column, total leaf area and total biomass assessed on biomass plants at 6.5 wk; Right column, fitness metric and total biomass assessed on fitness plants at 8 wk. Bars reflect means for outcrossed (o), biparentally inbred (i), and selfed plants (s) across each of the three treatments (C, control; D, drought; N, low nitrogen). Error bars represent standard errors. Inset letters at the top right of each panel indicate significant differences among treatments and breeding types as determined by Tukey test.

breeding type interaction, block factors (row and bench), and ovule parent. Block factors were used to account for potential spatial environmental gradients in the greenhouse, while ovule parent was used to account for both maternal effects and a major aspect of plant genotype. Tukey post hoc tests were used to assess group differences for significant model effects. Residuals for all trait models were found to be unimodal and roughly normally distributed. Given the large number of traits assessed in this study, false discovery rate was implemented to correct for multiple comparisons (Benjamini and Hochberg 1995). This method has been strongly advocated in recent years as preferable to simple or sequential Bonferroni correction for applications in ecology and evolution (Pike 2011; Verhoeven et al. 2005). All data used for analyses can be found in Supplementary Appendix S1 (<http://dx.doi.org/10.1614/WS-D-15-00005.S2>).

## Results

**Treatment and Inbreeding Effects on Performance Traits.** Performance traits were strongly affected by treatment, but to a lesser extent by breeding type. The low-nitrogen treatment group performed the poorest, with the lowest total biomass, total leaf area, and fitness (Figure 1). Plants in the control and drought treatment groups had comparable total biomass at both sampling dates, but despite this similarity in size the control group had significantly higher fitness (Figure 1). Interestingly, the drought group had a far higher total leaf area than the control group (Figure 1). Breeding type did not have a significant effect on fitness or total biomass at 8 wk, though biparentally inbred plants had significantly higher total biomass than selfed plants at 6.5 wk, as well as significantly

higher total leaf area than selfed or outcrossed plants (Figure 1).

**Treatment and Inbreeding Effects on Biomass Allocation.** Allocation among the four organ types considered was strongly altered by treatment, and to a lesser degree by breeding type. Interestingly, at 6.5 wk the leaf mass fraction, stem mass fraction, and root mass fraction were all significantly affected by treatment but not breeding type, while the reproductive mass fraction was significantly affected by breeding type but not treatment (Table 1). Reproductive mass fraction was significantly higher in outcrossed plants relative to either selfed or biparentally inbred plants at 6.5 wk (by Tukey test), but this effect was no longer detectable at 8 wk (Table 1). By that time, reproductive mass fraction instead had a significant effect of treatment like the other mass fractions (Table 1).

Treatments induced strong plastic differences in organ mass fractions. Root mass fraction was by far the most variable, with the low-nitrogen group having roughly three times the allocation to roots of the drought group at 6.5 wk (Figure 2). The low-nitrogen group was thus significantly higher than the control group in root mass fraction, which was in turn significantly higher than the drought group at 6.5 wk (Tukey test). Accordingly, the inverse pattern was seen for leaf and stem mass fractions (Figure 2), where the drought group was significantly higher than the control group, which was in turn significantly higher than the low-nitrogen group for both fractions (Tukey test). Reproductive allocation was not significantly different among treatment groups, however (Table 1). At 8 wk, the low-nitrogen group remained the highest in root mass fraction and lowest in stem and leaf mass fractions (Figure 2), and was significantly different from the other treatments in allocation to these three organs (Tukey tests). The control and drought groups were no longer significantly different in root or stem allocation, but the drought group remained significantly higher in leaf mass fraction (Figure 2; Tukey tests). The drought group was thus significantly lower in reproductive mass fraction than the control group, indeed so much so that it was not significantly different from the low-nitrogen group in allocation to reproduction (Figure 2; Tukey test).

**Treatment and Inbreeding Effects on Ecophysiological and Reproductive Traits.** Treatment significantly affected almost every ecophysiological trait, resulting in plastic differences in all traits

except leaf carbon content and tannin activity (Table 1). By contrast, breeding type significantly affected only a handful of traits, including LMA, leaf nitrogen content ( $N_{\text{mass}}$ ), the C:N ratio, and the leaf minor axis (Table 1). These significant differences were small in magnitude, with leaf minor axis differing between inbred and selfed plants by an average of 1.5 mm, or 2.5% of the average width of the leaf. Likewise,  $N_{\text{mass}}$ , C:N ratio, and LMA had small differences among breeding types of under 6%. Differences due to treatment were typically much larger (Figure 3). Relative to controls, drought group plants had significantly higher photosynthetic rate by mass ( $A_{\text{mass}}$ ), instantaneous water use efficiency (iWUE), water content ( $W_m$ ), nitrogen content ( $N_{\text{mass}}$ ), and chlorophyll content, as well as significantly lower carbon content ( $C_{\text{mass}}$ ), C:N ratio, LMA, trichome density, solidity, and overall smaller leaves as evidenced by lower dry mass, leaf area, and major and minor axis (Figure 3; Tukey tests). Low-nitrogen group plants, relative to controls, had significantly higher LMA, toughness, trichome density, and C:N ratio, and significantly lower photosynthetic rate by both area ( $A_{\text{area}}$ ) and mass ( $A_{\text{mass}}$ ), lower stomatal conductance ( $g_s$ ), instantaneous water use efficiency (iWUE), nitrogen content ( $N_{\text{mass}}$ ), water content ( $W_m$ ), chlorophyll content, circularity and solidity, as well as overall smaller leaves as evidenced by lower fresh and dry mass, leaf area, perimeter, and major and minor axis (Figure 3; Tukey tests).

As with ecophysiological traits, treatment significantly affected almost all reproductive traits, inducing plastic differences in all traits except the average seed number per fruit. Breeding type, by contrast, only significantly affected corolla width, though groups were not found to be different with a Tukey test. Relative to controls, drought group plants had significantly lower total anthocyanins and carotenoids per mass of corolla, as well as lower average pollen count, though larger average mass per fruit and per seed (Figure 3; Tukey tests). Low-nitrogen group plants also had significantly lower total anthocyanins and carotenoids per mass of corolla, as well as overall smaller flowers, as evidenced by shorter anther and pistil height and well as shorter and narrower corollas (Figure 3, Tukey tests). Number of flowers was significantly lower in the drought group relative to controls, and significantly lower than that in the low-nitrogen group (Tukey test), mirroring fitness differences overall.

Table 1. Univariate ANOVA results for the 46 assessed traits, with P values listed for whole models as well as whether whole models were significant after implementing the false discovery rate (FDR). For models significant under the FDR, P values for individual effects are listed: treatment (Trt), breeding type (BrdType), treatment-by-breeding-type interaction (Int.), row, bench, and ovule parent. When P values are significant ( $P < 0.05$ ), they are presented in bold. F statistics can be found in Table S1 (<http://dx.doi.org/10.1614/WS-D-15-00005.S1>).

Trait	Model	FDR?	Trt	BrdType	Int.	Row	Bench	Ovule
$A_{\text{area}}$	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.6230	0.7345	< <b>0.0001</b>	0.0775	0.8720
$A_{\text{mass}}$	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.8376	0.2392	< <b>0.0001</b>	0.9262	0.0814
$g_s$	< <b>0.0001</b>	Yes	<b>0.0011</b>	0.1322	0.2476	< <b>0.0001</b>	<b>0.0005</b>	0.0759
iWUE	<b>0.0025</b>	Yes	<b>0.0058</b>	0.4325	0.6694	<b>0.0058</b>	<b>0.0251</b>	0.2035
Chlorophyll content	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.7321	0.5537	< <b>0.0001</b>	<b>0.0106</b>	0.6129
Water content	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.5755	0.0861	<b>0.0100</b>	<b>0.0023</b>	<b>0.0017</b>
LMA	< <b>0.0001</b>	Yes	< <b>0.0001</b>	<b>0.0041</b>	0.1276	< <b>0.0001</b>	< <b>0.0001</b>	<b>0.0108</b>
$N_{\text{mass}}$	< <b>0.0001</b>	Yes	< <b>0.0001</b>	<b>0.0470</b>	0.3588	0.0519	0.7257	0.1444
$C_{\text{mass}}$	0.7828	No	—	—	—	—	—	—
C:N ratio	< <b>0.0001</b>	Yes	< <b>0.0001</b>	<b>0.0212</b>	0.1284	0.1239	0.2979	0.1213
Fresh mass	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.1022	0.3881	<b>0.0001</b>	<b>0.0391</b>	0.7868
Dry mass	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.0979	0.2496	< <b>0.0001</b>	<b>0.0011</b>	0.5616
Leaf area	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.0903	0.7804	<b>0.0004</b>	0.0609	0.5449
Perimeter	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.4747	0.6454	<b>0.0073</b>	0.2622	0.7423
Major axis	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.1038	0.4725	<b>0.0004</b>	0.0669	0.5560
Minor axis	< <b>0.0001</b>	Yes	< <b>0.0001</b>	<b>0.0391</b>	0.9052	< <b>0.0001</b>	<b>0.0136</b>	0.5561
Circularity	<b>0.0006</b>	Yes	<b>0.0006</b>	0.1427	0.8628	0.4335	0.2668	<b>0.0172</b>
Solidity	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.1859	0.9227	0.9749	0.3058	<b>0.0009</b>
Toughness	<b>0.0011</b>	Yes	< <b>0.0001</b>	0.7888	0.0789	<b>0.0007</b>	0.7894	0.8186
Trichome density	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.7508	0.5814	0.5796	0.1568	<b>0.0359</b>
Tannin activity	0.1614	No	—	—	—	—	—	—
Days to first flower	< <b>0.0001</b>	Yes	<b>0.0083</b>	0.0944	0.7637	0.0758	<b>0.0220</b>	< <b>0.0001</b>
Total leaf area (6.5 wk)	< <b>0.0001</b>	Yes	< <b>0.0001</b>	<b>0.0182</b>	0.5287	0.6517	0.4367	0.2381
Total biomass (60.5 wk)	< <b>0.0001</b>	Yes	< <b>0.0001</b>	<b>0.0303</b>	0.7942	<b>0.0113</b>	<b>0.0025</b>	0.1671
Leaf mass fraction (6.5 wk)	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.5041	0.6276	<b>0.0011</b>	0.1978	0.4789
Stem mass fraction (6.5 wk)	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.2981	0.7666	0.7875	0.8710	<b>0.0071</b>
Root mass fraction (6.5 wk)	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.3122	0.7190	0.5324	0.4486	0.5485
Reproductive mass fraction (6.5 wk)	<b>0.0109</b>	Yes	0.0938	<b>0.0050</b>	0.6779	0.7659	0.5230	<b>0.0088</b>
Total biomass (8 wk)	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.7385	0.4852	< <b>0.0001</b>	0.4799	0.1764
Leaf mass fraction (8 wk)	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.2320	0.9766	<b>0.0299</b>	0.2274	<b>0.0305</b>
Stem mass fraction (8 wk)	<b>0.0248</b>	Yes	<b>0.0160</b>	0.3371	0.3705	0.5810	<b>0.0279</b>	0.4419
Root mass fraction (8 wk)	<b>0.0020</b>	Yes	< <b>0.0001</b>	0.4137	0.9806	0.1174	0.1546	0.4332
Reproductive mass fraction (8 wk)	<b>0.0031</b>	Yes	< <b>0.0001</b>	0.4605	0.2767	<b>0.0454</b>	0.1540	0.1965
Anthocyanin content	< <b>0.0001</b>	Yes	<b>0.0010</b>	0.8717	0.6279	<b>0.0029</b>	0.3493	<b>0.0002</b>
Carotenoid content	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.7287	0.7439	<b>0.0003</b>	0.1809	<b>0.0003</b>
Pistil height	<b>0.0046</b>	Yes	<b>0.0027</b>	0.1019	0.5915	0.0672	0.1190	0.2566
Corolla height	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.4607	0.7870	0.7732	<b>0.0377</b>	<b>0.0247</b>
Corolla width	<b>0.0012</b>	Yes	<b>0.0009</b>	<b>0.0359</b>	0.0792	0.3531	0.5682	<b>0.0158</b>
Average anther height	<b>0.0009</b>	Yes	<b>0.0018</b>	0.1363	0.4298	<b>0.0314</b>	0.7646	<b>0.0404</b>
Average pollen size	< <b>0.0001</b>	Yes	<b>0.0262</b>	0.3823	0.7465	0.9055	0.9852	< <b>0.0001</b>
Average pollen count per anther	<b>0.0040</b>	Yes	<b>0.0025</b>	0.0753	0.2539	0.2449	0.2634	0.2981
Average fruit mass	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.6412	0.4214	0.3958	0.6982	<b>0.0114</b>
Total no. flowers (2 wk)	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.4352	0.9686	< <b>0.0001</b>	<b>0.0321</b>	0.7075
Average seed no. per fruit	0.2311	No	—	—	—	—	—	—
Average seed mass	<b>0.0063</b>	Yes	< <b>0.0001</b>	0.2588	0.5247	0.0888	0.4923	0.1484
Fitness metric raw	< <b>0.0001</b>	Yes	< <b>0.0001</b>	0.3951	0.8956	<b>0.0005</b>	<b>0.0237</b>	0.7524

## Discussion

**Minimal Inbreeding Depression across Environments in Tall Morningglory.** The effects of inbreeding on *I. purpurea* were either statistically nonsignificant or very small in magnitude. At the performance trait level, the only significant differences among breeding types were that inbred plants had

significantly higher total leaf area and total biomass than outcrossed or selfed plants at 6.5 wk (though not at 8 wk). This runs counter to expectations, as total leaf area and total biomass have been found to decrease with inbreeding in a number of species (Birmingham and Brody 2011; Carr and Dudash 1996; Carr and Eubanks 2002; Cheptou et al. 2000;

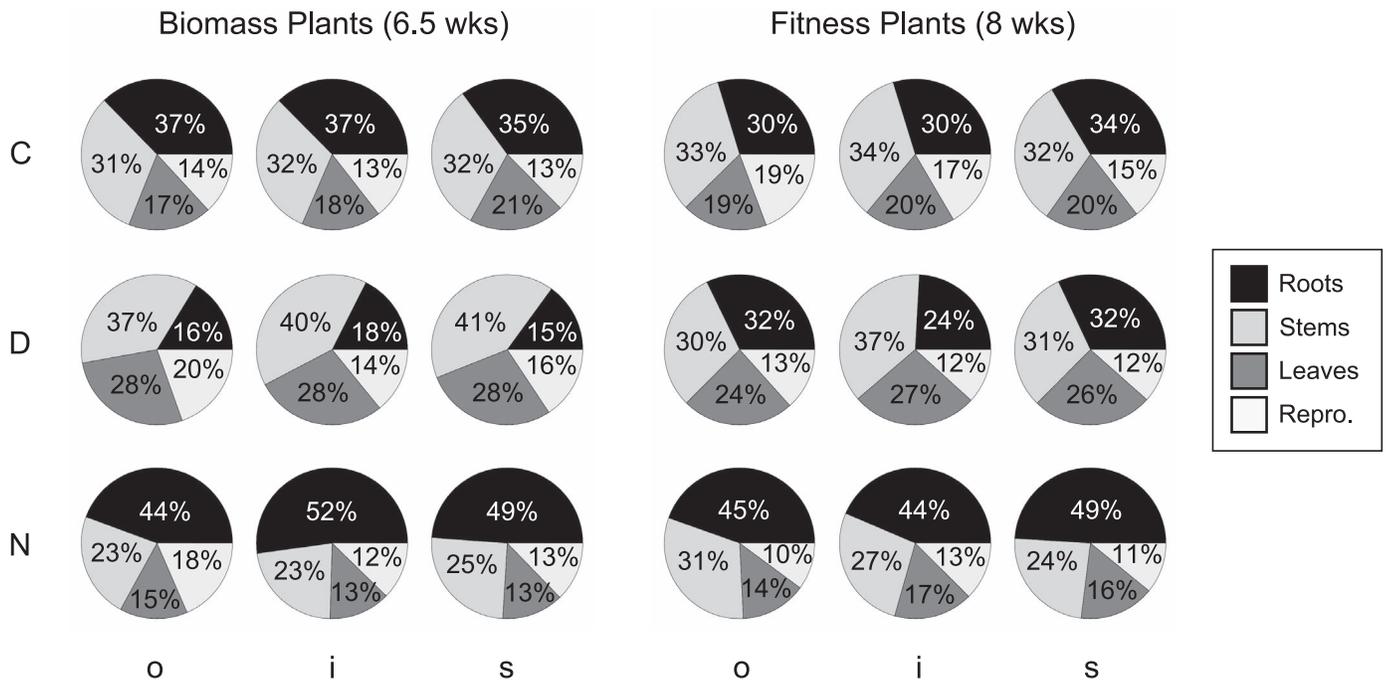


Figure 2. Differences in biomass allocation across treatments and breeding types. Left three columns, biomass allocation as assessed on biomass plants at 6.5 wk. Right three columns, biomass allocation as assessed on fitness plants at 8 wk. Percentages reflect mean mass fractions of roots, stems, leaves, and reproductive tissues (see legend), for each combination of treatment (C, control; D, drought; N, low nitrogen) and breeding type (o, outcrossed; i, biparentally inbred; s, selfed). Percentages may not sum to 100% due to rounding.

Mooney and Mcgraw 2007; Waller et al. 2008). Additionally, the lack of significant breeding type effects on fitness, total leaf area, or biomass at 8 wk highlight that the magnitude of inbreeding effects can vary over the life cycle of a plant (Husband and Schemske 1996; Willis 1999). Similarly, the biomass allocated to reproductive organs in selfed and inbred plants was significantly lower than outcrossed plants at 6.5 wk, but at 8 wk this effect was not present. This change suggests that what little inbreeding depression is present in this species is caused by many weakly deleterious mutations that do not act equally across all stages of the life cycle (Husband and Schemske 1996). The small but significant inbreeding effects on leaf traits seen here in tall morningglory resulted in narrower leaves with slightly higher leaf nitrogen and slightly lower LMA and C:N ratio. Although statistically significant, the higher leaf nitrogen content in selfed tall morningglory found here did not result in significantly increased photosynthetic rates, or seemingly any other ecophysiological or performance effect. The only significant inbreeding effects on reproductive traits were slightly more narrow corollas.

The minimal inbreeding depression in tall morningglory found here is consistent qualitatively with results from a previous study on the same species, which found only moderate levels of inbreeding depression in selfed progeny under field

conditions (Chang and Rausher 1999). This minimal level of inbreeding depression is perhaps not surprising for a facultatively selfing weedy species like tall morningglory. The low impact of inbreeding also makes sense in light of the modern history of this species, which likely experienced bottlenecks and inbreeding that may have purged most existing genetic load during its introduction and expansion throughout the southeastern United States. Additionally, with frequent colonization of new areas, selfing is likely to occur at a much higher rate than the reported average of 30% (Ennos 1981), providing the conditions for effective purging of genetic load through inbreeding depression.

Quantitatively, we found a much smaller magnitude of inbreeding depression in all traits measured here than the 12.5% reduction in male fitness and 24% reduction in female fitness previously reported in tall morningglory (Chang and Rausher 1999). There are several possible explanations for the discrepancy between our results and the previous study. First, the seed source differs between studies, and seeds used in this study may have come from populations that have purged more of their genetic load than the source population used by Chang and Rausher (1999). Second, because our study was carried out in the greenhouse, plants did not

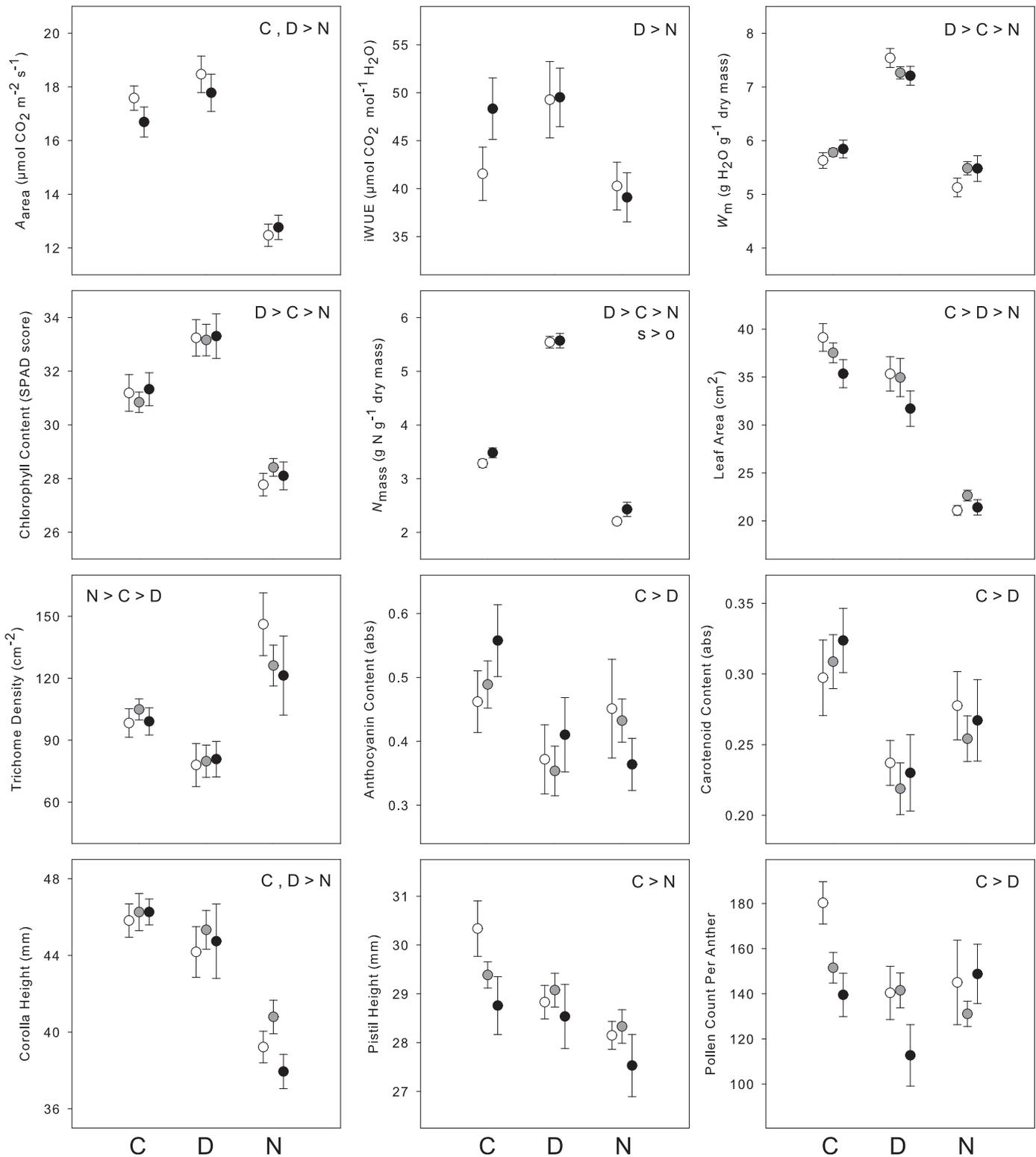


Figure 3. Differences in selected ecophysiological and reproductive traits across treatments and breeding types. Points reflect means for outcrossed (open circles), biparentally inbred (grey circles), and selfed plants (black circles) across each of the three treatments (C, control; D, drought; N, low nitrogen). Error bars represent standard errors. Inset letters at the top right or left of each panel indicate significant differences among treatments and breeding types as determined by Tukey test.

experience reproductive interactions with pollinators, and the weather conditions that usually dictate the end of their reproductive season were also absent. Because of these effects, inbreeding de-

pression might be underestimated in our study relative to field estimates. Regardless of the reasons for this quantitative discrepancy, both studies suggest that that inbreeding depression has little

effect on the performance and reproductive success of this species.

**Tolerance of and Plasticity under Drought and Nutrient Stress.** Performance traits were strongly affected by treatment in this study. Plants in the low-nitrogen group generally had the lowest performance (total biomass, total leaf area, and fitness) of all three treatments. Plants in the low-nitrogen group also showed leaf ecophysiological traits indicative of a more resource-conservative leaf economic strategy supporting slower growth (Reich et al. 1997; Wright et al. 2004). The effect of water limitation, however, did not mirror the effect of nitrogen limitation, as might be expected from traditional plant physiology literature (Chapin et al. 1991). On average, droughted plants produced leaves with ecophysiological traits indicative of a more resource-acquisitive leaf economic strategy supporting faster growth (Reich et al. 1997; Wright et al. 2004). Droughted plants also showed greater water use efficiency than control plants due to lower stomatal conductance and higher photosynthetic activity. These results, combined with comparable total biomass in droughted plants, may indicate that drought escape and drought avoidance strategies are not mutually exclusive in tall morningglory, and that there is no evidence of a tradeoff between growth and drought resistance (Blum 1988; Mitra 2001). Additionally, the far higher root mass fraction in the nitrogen group relative to drought or control plants suggests that tall morningglory root allocation is more plastically responsive to low-nutrient conditions than to drought.

We expected plants subjected to drought to have lower total leaf area and lower leaf nitrogen content than control plants, as it has been shown that under favorable conditions higher leaf area is needed to maximize photosynthesis and thus relative growth rate (Zlatev and Lidon 2012). However, higher leaf area also increases the surface area for potential transpirational water loss at the whole-plant level, and so can be disadvantageous under drought conditions. We therefore expected plants subjected to drought to have lower total leaf area than control plants. However, the opposite effect was observed, with drought plant leaves having greater total leaf area. Drought plants additionally had higher leaf nitrogen content than controls. Both increased leaf area and leaf nitrogen content may result from the inherently high mobility of nitrogen in soils. In contrast to control plants, which were watered to field capacity twice per day with an automatic drip

irrigation system, drought plants were only watered to field capacity when wilted (Figure S1; <http://dx.doi.org/10.1614/WS-D-15-00005.S1>), which likely resulted in decreased leaching of nitrogen from the soil in drought treatment pots. This may have led to increased nitrogen availability in drought plants relative to controls, but this is a similar effect to what might happen in a fertilized agricultural field under reduced rainfall conditions (Meisinger and Delgado 2002). Nitrogen availability is known to increase allocation to aboveground biomass of plants (Ding et al. 2005; Uhart and Andrade 1995), which may have led to the greater total leaf area and reduced root mass fraction observed in drought treatment plants despite reduced water availability.

Drought has been observed to accelerate the completion of the life cycle in many annuals to ensure that seed is set before the end of the favorable growing period (Aronson 1989; Fox 1990; Newman 1965), and therefore we expected to find an accelerated rate of flower development in the drought treatment. However, date to first flower for plants in the drought group was not significantly different from controls, suggesting that tall morningglory does not respond to drought by accelerating reproduction. Conversely, the small flowering delay observed in the low-nitrogen group is likely due to their small leaf size and low total leaf area, as nitrogen deficiencies during the early stages of plant growth could hinder the initiation and development of leaves and flowers. A delay in flower production due to nitrogen limitation has been observed in several species (Halse et al. 1969; Ma et al. 1997), as has a reduction in both the rate of flower production and the total number of flowers produced (Gardner and Tucker 1967; Reid and York 1958).

The less brightly colored flowers produced in the nitrogen and drought treatments would likely result in significant effects on pollinator visitation in the field. Work on pollination biology in tall morningglory has demonstrated that lighter-colored floral morphs have decreased pollinator visitation and lower outcrossing rates compared to darker-colored morphs (Brown and Clegg 1984). The primary pollinators of tall morningglory are bumblebees (Brown and Clegg 1984), and it has furthermore been demonstrated that decreased floral anthocyanin and carotenoid content is associated with reduced bee visitation (Schemske and Bradshaw 1999). In our study, plants under drought treatment had lower pollen count but higher average mass per fruit and seed. This could indicate that

drought affects male fitness more than female fitness in the hermaphroditic tall morningglory. Droughted plants possibly produced larger seeds and fruits because of their higher total leaf area and photosynthetic rate, as greater carbon fixation during seed filling would allow for greater investment in carbon-rich seeds and fruits. Alternatively, given the observed reduction of the number of flowers in droughted plants, a fixed reproductive investment would be expected to result in larger fruits and seeds under a size–number tradeoff (Sakai 2000). The lower pollen count per anther, however, is less expected because drought has been shown to affect the process of seed production more than pollen production in several species (Galen 2000; Maad and Alexandersson 2004). In contrast to this, our results suggest that tall morningglory pollen development is more dependent on access to water than access to nitrogen.

The observed large plasticity in ecophysiological, reproductive, and biomass allocation traits under abiotic stress in this study is consistent with Baker's conception of characteristics that explain weediness (1974). Here we find that chronic drought stress reduces fitness but not overall biomass, indicating that the water limitation imposed affects reproduction disproportionately more than vegetative growth. This is consistent with the fact that in the southeastern United States this species is regularly subjected to intense summer heat and drought (Coberly and Rausher 2003), and must resist summer drought in order to flower from late summer through autumn. The fact that nitrogen limitation reduces both growth and fitness so sharply also makes sense given morningglory's aggressive weediness in agricultural fields subject to fertilization, where selection would favor enhanced growth and competitiveness (Chaney and Baucom 2012, 2014). Interestingly, plasticity in biomass allocation appears to play a large role in stress response in this system, as it is strongly labile under abiotic stress. The directions of shifts seen are largely consistent with a recent meta-analysis of environmental control of biomass allocation across plants generally (Poorter et al. 2012). This flexibility in growth form with variation in resource availability fits with the profile of morningglory as an aggressive weed (Baker 1974; Chaney and Baucom 2012), allowing the species to successfully grow and reproduce in a wide variety of environments.

### **Implications for Management of Morningglory.**

Overall, treatment effects were much stronger than

inbreeding effects in the tall morningglory. While drought and low-nitrogen conditions significantly reduced fitness and altered trait values, inbreeding had little effect on fitness or the measured traits, and the magnitude and expression of inbreeding depression was not strongly affected by abiotic stress in this study. The results of this study have several implications for management of morningglory as an agricultural weed. First, the lack of major impacts of inbreeding depression indicates that morningglory populations are likely to rebound quickly and successfully from small numbers of individuals, and thus be robust to control efforts that attempt to reduce population size. Second, the robustness of vegetative performance to drought stress indicates that morningglory is likely to have larger competitive effects on the performance of drought-sensitive crops in drought years versus non-drought years, even while the reproduction of morningglory will itself be limited by reduced water availability. This suggests that there are potential benefits to targeting control of severe infestations (e.g., with herbicides) during drought years relative to non-drought years, as such efforts would serve to reduce competition to crops made vulnerable by water limitation while simultaneously exploiting drought-reduced morningglory reproduction to shrink future population sizes. Third, the strong reductions seen in vegetative and reproductive performance of morningglory under nitrogen limitation may indicate that weedy populations of morningglory in southeastern agricultural landscapes have not experienced selection for increased tolerance of low soil nitrogen, and consequently populations in fallow or unfertilized fields may be prime targets for control.

The results of this study suggest that both a lack of inbreeding depression and high trait plasticity under abiotic stress contribute to the inherent weediness of tall morningglory in the southeastern United States. The recent rise of herbicide resistance in this species (Baucom and Mauricio 2010) underscores the value of understanding evolutionary processes in agricultural weeds for practical purposes, as well as the usefulness of weed systems for the advancement of evolutionary ecology.

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