Home Climate and Habitat Drive Ecotypic Stress Response Differences in an Invasive Grass

Vasiliy T. Lakoba* (ORCID: 0000-0003-4688-3067), Jacob N. Barney1 (ORCID: 0000-0003-2949-5003)

1 School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA 24061

* Corresponding author: vtlakoba@vt.edu
Abstract: Invasive plants and agricultural weeds are a ubiquitous and ever-expanding threat to biosecurity, biodiversity, and ecosystem services. Many of these species are known to succeed through rapid adaptation to biotic and abiotic stress regimes, often in highly disturbed systems. Given the current state of evidence for selection of weedy genotypes via primary physiological stresses like drought, flooding, heat, cold, and nutrient deficiency, we posit that adaptation to land management regimes which comprise suites of these stresses can also be expected. To establish this link, we tested adaptation to water and nutrient stresses in five non-agricultural and five agricultural populations of the invader johnsongrass (*Sorghum halepense*) sampled across a broad range of climates in the USA. We subjected seedlings from each population to factorial drought and nutrient stresses in a common garden greenhouse experiment. Agricultural and non-agricultural ecotypes did not respond differently to experimentally applied stresses. However, non-agricultural populations from more drought-prone and nutrient-poor locations outperformed their agricultural counterparts in shoot allocation and chlorophyll production, respectively. We also found evidence for root allocation adaptation to hotter climates, in line with other C4 grasses, while greater adaptation to drought treatment was associated with soil organic carbon (SOC) rich habitats. These findings imply that adaptation to land use types can interact with other macrohabitat parameters, which will be fluctuating in a changing climate and resource-needy world. We see that invasive plants are poised to take on novel habitats within their introduced ranges, leading to complications in the prevention and management of their spread.

Keywords:

Agricultural weeds; climate adaptation; ecotype; invasive plants; plant stress; rapid adaptation;
Introduction:

Populations of widely distributed species experience a diversity of environments across their ranges, often adapting to major differences in climate, resource availability, competition, trophic interactions, and management regimes (Espeland 2013; Oduor et al. 2016; van Boheemen et al. 2019). This often leads to development of considerable intraspecific variation, which can be an indicator of the species’ local adaptive potential on a continental scale as well as its ability to tolerate habitat and climate change over time (Henn et al. 2018; Razgour et al. 2019).

Local adaptation is the result of spatially heterogeneous selection pressures that give rise to populations with traits not universally present across environmental gradients (Kawecki and Ebert 2004; Leimu and Fischer 2008). Given enough magnitude and duration of such selection, a single species can yield multiple ecotypes which are distinguishable by traits linked with their geographic origin (Leimu and Fischer 2008). In some plants, intraspecific trait divergence has been shown to correlate with microclimate (Carvajal et al. 2017), human land use (Malíková et al. 2016), or biotic interactions like herbivory (Garrido et al. 2012). Traits linked to divergent ecotypes can include biomass production (Matesanz et al. 2012), phenology (Sakaguchi et al. 2019), fitness (Armbruster 2014), cold-hardiness (Lowry et al. 2014), and pollinator relations (Gervasi and Schiestl 2017). Rapid evolution, local adaptation, and ecotypic divergence can readily be seen in invasive plant species which experience a broad spectrum of, often novel, selection pressures across vast ranges (Whitney and Gabler 2008; Oduor et al. 2016). In fact, rapid adaptation and “de-domestication” is often seen in feral crop species and manifests across a variety of their traits. A widely studied system exemplifying this is weedy rice (Oryza sativa f. spontanea), in which (Xia et al. 2011) found evidence for a change in germination niche adapted to novel ruderal habitats, rather than a reversion to a pre-cultivation phenotype.
Intentional selection is not the only way in which agricultural operations facilitate rapid evolution in weedy species. Rapid evolution of invasive plants has, in some cases, been the consequence of attempts at mechanical and chemical control in agroecosystems (e.g., herbicide resistance in *Amaranthus palmeri* (Salas et al. 2016)). Some are also exemplars of the role of management in driving local adaptation (Gao et al. 2018; Gorton et al. 2018). Weedy and invasive plants (i.e., those that exhibit rapid rates of colonization, establishment, and spread) have proven to be excellent models for studying adaptation to climate and competition (Engel et al. 2011; Atwater et al. 2016, 2018). This is, in part, due to the need for considering multiple drivers when studying invasions (Kueffer et al. 2013). Abiotic stresses (e.g., climate, drought, and nutrient stress) are especially pertinent to invasion biology, where release from biotic and abiotic stressors are fundamental to many invasion hypotheses (te Beest et al. 2013; Turner et al. 2014). However, little is known about this trend’s extension to subsidies -- whether elevated nutrient and moisture levels can drive rapid change in phenotypic responses to different climates. We postulate that this may be the case, given other examples in agricultural systems where irrigation performed as a microclimate creator (Li et al. 2008; Cavero et al. 2009). Here we test whether populations of the invasive Johnsongrass (*Sorghum halepense* (L.) Pers., Poaceae) have adapted different stress responses based on their ambient and land use-impacted environments. In this way, we begin to examine whether anthropogenic differences in resource availability are linked with known ecotypes of an invader differing in their response to climatic and edaphic stressors.

Johnsongrass is a perennial grass that is native to parts of the Middle East and is currently a widespread invader in both agricultural and non-agricultural systems worldwide (Warwick & Black 1983). The early introductions of Johnsongrass into North America -- in South Carolina in the 1820s and later in Arizona -- were into agricultural landscapes as a forage crop (Mcwhorter 1971), though it quickly became weedy and was abandoned as a crop. The early invasion syndrome was observed in
agroecosystems, which frequently led to forced abandonment of lands where mechanical removal of Johnsongrass failed (Ball 1902). Johnsongrass populations are mostly found in the southern half of the contiguous United States, with relatively few pioneer populations in the Northeast, Upper Midwest, and Pacific Northwest. Over the course of its nearly 200-year residence in the United States, Johnsongrass has more recently become predominantly an invader of roadside, waste, and other non-agricultural spaces (Sezen et al. 2016). This shift may have been largely driven by chemical control of Johnsongrass in herbicide tolerant cropping systems in the late twentieth century (Sezen et al. 2016).

Across its vast introduced range, Johnsongrass exhibits dramatic genetic and phenotypic variation (Atwater et al. 2016; Sezen et al. 2016). While some of its success across diverse habitats may be explained by phenotypic plasticity, there is reason to believe that different selection pressures among habitats can manifest over time as ecotypic differentiation (Delêtre et al. 2017; Rajakaruna 2018). Such effects have been observed in a range of invasive plants occupying broad novel ranges (Lavergne et al. 2010; Molina-Montenegro et al. 2018). Previous studies of intraspecific variation in Johnsongrass have largely focused on climatic and large-scale geographic origin as predictors of performance (Mcwhorter 1971; Burt 1974). Stimulated by the discovery of genomic patterns over the course of the invasion (Sezen et al. 2016), recent work has examined the differences in stress and competition response between agricultural and non-agricultural populations (Atwater et al. 2018). The findings of differential response based on ecotype identity (i.e., agricultural and non-agricultural) alone are important to the larger question of whether the species is predisposed to generalist adaptability or specialization (Atwater et al. 2016).
Given the observed phenotypic differences in Johnsongrass from agricultural and non-agricultural populations and associated population genetic differences (Atwater et al. 2016, 2018; Sezen et al. 2016), our objective was to test whether ecotypic origin (i.e., agricultural vs. non-agricultural) and environmental selection pressures would predict biomass accumulation and allocation in a common greenhouse experiment during seedling establishment -- the most critical stage in population establishment and invasion (Leguizamón et al. 2011; Postma and Ågren 2016). Specifically, we were interested in the following questions: 1) Does Johnsongrass home climate and soil fertility drive differences in drought and nutrient stress tolerance? and 2) Do Johnsongrass ecotypes vary in their drought and nutrient stress tolerance? Results of this study will be useful in elucidating relative degrees of local adaptation to climatic and habitat types across a large and expanding introduced range of this widespread and advancing invader. More broadly, it would be an uncommon head-to-head comparison of different drivers of local adaptation, while most studies focus on a single driver (e.g. climate, elevation, etc.), possibly addressing important interactive effects on invasive species range expansion.

Methods:

Population Selection

We drew from our collection of >200 Johnsongrass populations representing the full geographic and climate variation of its US range. For this study, we chose from 30 populations that spanned this range, and had been grown for a generation in a common garden to reduce maternal effects, focusing on full representation of the range of mean precipitation as we are examining drought tolerance. We split the set of 30 populations into those collected from agricultural (8) and non-agricultural (22) sites. All populations were plotted in geographic space using ArcMap 10.5.1 (ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.) overlaid with a 30-
year mean annual precipitation (MAP) raster data obtained from PRISM Climate Group, Oregon State University. The MAP values for the non-agricultural populations ranged from 199 to 1430 mm, while MAP for the agricultural populations ranged from 190 to 1481 mm. These were categorized into six “precipitation bands” of equal range: (1) 190-406mm, (2) 406-621mm, (3) 621-836mm, (4) 836-1051mm, (5) 1051-1266mm, and (6) 1266-1481mm. We had viable seed of agricultural and non-agricultural populations in bands 1, 2, 3, 4, and 6. Thus, we arrived at five agricultural populations and five non-agricultural populations which represent the breadth of the precipitation spectrum on which Johnsongrass occurs in the United States (See Fig 1).

Plant Establishment

To release seeds from dormancy, we treated them with commercial strength sodium hypochlorite (Clorox Regular-Bleach, The Clorox Company, Oakland CA) for 4 hours followed by a 1 hour water rinse (Atwater et al. 2018). The prepared seeds were then placed in petri dishes (one dish per population) with four saturated sheets of filter paper (Whatman 1003-055, GE Healthcare, Chicago IL). Upon radicle emergence, seeds were transferred to 6.4 cm diameter, 656 mL tree pots (Stuewe & Sons, Inc., Tangent, Oregon) filled with a 2:1 mixture of washed sand to sifted mineral soil collected locally and allowed to establish for eight days. Individuals from each population were then randomly assigned to one of four treatments: + fertilizer / + irrigation; + fertilizer / - irrigation; -fertilizer / + irrigation; and -fertilizer / - irrigation. Pots were arranged in a randomized complete block design with five replicates, with each block representing a greenhouse bench. Irrigation was applied as 10 mL of water daily, keeping the soil near field capacity. Fertilizer (PowerPak 20-20-20, Southern Agricultural Insecticides Inc., Hendersonville NC) was applied once at time of transplant as a solute in 10 mL of water at a rate of 4 mL/L, which matches the recommended product rate for spray and drench applications. Plants not receiving fertilizer were given 10mL of water at time of transplant. Due to presumed nutrient availability in the mineral soil which made up one third of our
growing media, the ‘-fertilizer’ treatment more likely created a nutrient limitation than a deficiency. The drought treatments (-irrigation) were administered by reducing irrigation to 5 mL per day on day 1 of treatment, and 0 mL irrigation after 7 days of treatment.

**Measurements**

Immediately preceding treatment application, we recorded height to the most recently emerged leaf collar and the number of leaves to account for size asymmetries. Following treatment application, we recorded plant survival, plant height, leaf number, number of culms, chlorophyll content (Chl) and chlorophyll fluorescence ratio (CFR) every seven days. From (Gitelson et al. 1999), the formula for chlorophyll content in mg m^{-2} is

\[ \text{Chl} = 634 \times \frac{F_{735}}{F_{700}} - 391 \]

CFR is the quotient in this equation, or “the ratio of fluorescence or fluorescence emission at 735nm/700nm”. The latter two were measured using CCM-300 Chlorophyll Content Meter (OPTI-SCIENCES, Hudson, New Hampshire, USA). The experiment continued until the most vigorous plants filled their pots with roots, which occurred 28 days after treatment application. At the end of the experiment, presence of rhizome(s) was recorded, and biomass was harvested separately for belowground and aboveground portions and dried at 75C for seven days.
Statistical analyses

To describe climate at the place of origin of each population (hereafter ‘home climate’), we used mean annual precipitation (MAP) and mean annual temperature (MAT) (PRISM Climate Group, 2004), as well as the soil organic carbon content (SOC) (Hengl et al. 2014) as a descriptor of habitat nutrient availability. We chose SOC to represent soil fertility (or nutrient availability) based on findings of strong positive relationships between SOC and N/P/K availability in agroecosystems (Cheng et al. 2016). Following analysis of response variable correlation, we used three independent response variables: total biomass, CFR, and root-to-shoot biomass ratio. The total biomass and root:shoot variables were log_{10}-transformed to meet assumptions of the linear model. Each response variable was analyzed in a mixed effects linear model with block as a random effect and height-at-transplant, MAP, MAT, and SOC as fixed effects, and ecotype (agricultural vs. non-agricultural), fertilization, and irrigation as fixed effects, as well as all possible second order interactions. We then performed backwards model selection, removing non-significant predictors to minimize AICc. The main effects of block, initial height, irrigation treatment, and fertilization treatment were kept in all models. All statistical analyses were performed using JMP Pro, Version 15 (SAS Institute, Inc., Cary, NC).

Results:

Total biomass, root:shoot, and chlorophyll content are correlated with irrigation and/or fertilization

The irrigation and fertilization treatments had strong positive interactive effects on total biomass as well as chlorophyll content (Table 1). Fertilization resulted in a 59% increase in biomass among irrigated individuals (0.96g to 2.35g), but only a 44% increase among unirrigated individuals (0.61g to 1.1g) (Fig 2c). Leaf chlorophyll content increased by only 9% due to fertilization among irrigated plants (306 mg m^{-2} to 338 mg m^{-2}), while fertilization of unirrigated plants yielded a 51% increase in
leaf chlorophyll content (122 mg m$^{-2}$ to 249 mg m$^{-2}$) (Fig 2b). Mean root:shoot of unirrigated populations (0.7) was significantly higher than that of irrigated populations (0.5) (Fig 2a).

**Chlorophyll content response to SOC in home habitat is mediated by ecotype identity**

Leaf chlorophyll content varied between ecotypes as a function of SOC in their home habitat (Fig 3a). Non-agricultural populations from habitats across SOC levels had nearly uniform chlorophyll production. Agricultural populations, however, produced significantly more chlorophyll in populations from home habitats of high SOC. Among populations from the most SOC-poor origins of the US, non-agricultural populations contained as much as 50 mg m$^{-2}$ more chlorophyll than agricultural populations. Among populations whose home habitats had SOC of $>17$g/kg, agricultural populations overtook non-agricultural counterparts in chlorophyll production.

**Chlorophyll content and root:shoot biomass ratio respond to home habitat parameters**

Johnsongrass population differences in root:shoot ratio were affected by home climate, while chlorophyll content differences responded to SOC. We observed a mild, yet significant, positive relationship between home habitat MAT and Johnsongrass root:shoot biomass ratio across populations (Fig 4a). An increase in root:shoot ratio from 0.6 to 0.78 was associated with a twofold increase in home MAT from 11C to 22C, which did not vary between ecotypes.

Root:shoot ratio response of irrigated and unirrigated plants differed based on home habitat MAP (Fig 4b). The root:shoot ratio of Johnsongrass grown with non-limiting soil moisture, increased with higher MAP, while we observed a decreasing root:shoot ratio in droughted Johnsongrass as MAP increased ($p = 0.0147$). In other words, drought produced no significant difference in R:S among plants from the highest precipitation home habitats, while leading to a significant increase (from an average of 0.49 to 0.78) in R:S among plants from the most arid origins.
While Johnsongrass home habitat SOC had a slightly negative effect on chlorophyll content of irrigated plants, it had a positive effect on chlorophyll content in unirrigated plants (see Figure 3b). Thus, the irrigation treatment was responsible for a 67% greater difference in chlorophyll content between samples from SOC-poor habitats (180 mg m\(^{-2}\), on average) than on those from SOC-rich habitats (120 mg m\(^{-2}\), on average).

**Discussion:**

We found evidence for local adaptation in Johnsongrass to home habitat parameters (MAT, MAP, and SOC) as well as differential ecotypic adaptation. Responses to home habitat and ecotype identity interacted with experimentally applied drought and nutrient stresses. MAT was the only home habitat variable found to impact seedling performance (specifically root allocation) on its own, while MAP, SOC, and ecotype identity had interactive effects on plant response. Given earlier studies that find Johnsongrass ecotypic differences in competition (Atwater et al. 2016), stress response adaptation for competitive advantage (Schwinning et al. 2017), as well as abundant intraspecific variation globally (Mcwhorter 1971), our findings of further local adaptation in various seedling traits provide additional evidence for how this species has become so widespread across a large and diverse geographic area. We can now interpret evidence of ecotypic differences in stress response while controlling for other abiotic habitat qualities. Our results suggest that the two Johnsongrass ecotypes, agricultural and non-agricultural, may have differently adapted to nutrient limitation. Our approach in sampling populations of both agricultural and non-agricultural origins along extensive MAP, MAT, and SOC gradients has given us new evidence of Johnsongrass adaptation associated specifically with human land uses. The fact that this effect was mediated by nutrient availability brings us closer to testing its mechanisms in the future. Commonly suggested physiological mechanisms for nutrient stress tolerance in other species include tissue life maximization (Chapin
and microbial symbiosis (Johnson et al. 2010). The latter, with regard to N fixation, has been found in some non-agricultural Johnsongrass populations (Rout et al. 2013).

There are numerous examples of drought tolerance advantages among invasive plants (e.g., Filippou et al., 2014; Vetter et al., 2020) and among C4 invaders in particular (Baruch and Jackson 2005; Fahey et al. 2018). Drought is one of many stresses which is thought to select for invasive species and communities of invaders over native counterparts (Catford et al. 2012). Stochastic stress tolerance in invasive plants is also seen as a key element of their r-selection strategy (Berger and Ludwig 2014). By parsing seedling root and shoot biomass, we were able to examine Johnsongrass seedling drought response in relation to its ecotype identity and home habitat conditions.

Root:shoot biomass ratio is an indicator of plant health, particularly in its extreme values. Ratios approaching zero are indicative of very low root allocation, which signals overall poor health (Thornley 1972; Wilson 1988). Ratios close to 1 (not an absolute rule across species) indicate overall good health. Ratios substantially higher than 1 indicate intensified root allocation, which is a proxy for drought stress response in a vast number of species (Eziz et al. 2017). Increase in root:shoot ratio in response to drought stress was also found in our experiment. Eziz et al’s (2017) meta-analysis found that the root allocation response of perennial herbs, of which Johnsongrass is one, is less drought-sensitive than annual herbs, but more sensitive than that of woody plants. This finding suggests that a plant’s drought sensitivity may be directly related to its degree of investment in perenniating tissues. Specifically, highest sensitivity was linked with no such investment (annuals) and lowest sensitivity corresponded to maximal investment (woody plants), while intermediate drought sensitivity was associated with perennial herbaceous plants. Johnsongrass’s rhizomes are critical in its evasion of and resilience to cold stress (Warwick et al. 1986; Washburn et al. 2013) and could be similarly linked with drought response. However, this could not have been the cause of seedling drought tolerance in our study due to negligible rhizome production at this early life stage.
This suggests that a physiological mechanism -- perhaps even one inherited from its ancestor S. bicolor (Sanchez et al. 2002) -- may be involved in Johnsongrass's drought response.

The positive response of root:shoot ratio to Johnsongrass home MAT is potentially linked to the metabolic physiology of Johnsongrass and, indirectly, its success as an invader. Two global meta-analyses -- one of 7,763 terrestrial ecosystems (Qi et al. 2019) and another of >6,200 forests (Reich et al. 2014) -- found that plants growing in colder climates have a higher root:shoot ratio. However, research on *Sorghum bicolor* (Clark and Reinhard 1991) and *Cleistogenes* spp. (Luo et al. 2013), both C4 grasses like Johnsongrass, shows root:shoot ratio increasing with higher MAT. To explain this, Luo et al. (2013) cite general C-allocation advantages of C4 over C3 plants under high CO2 and high temperature/aridity conditions (Ehleringer et al. 1997). While our study only examined intraspecific variation in a C4 grass, rather than comparing it with C3 species, it stands to reason that the positive relationship between MAT and Johnsongrass root allocation was indicative of adaptation to hotter and drier home habitats. This finding poses the question of whether Johnsongrass’s heightened root allocation impacts its competitive -- and therefore invasive -- ability. Johnsongrass is known to compete with crops (i.e., maize) largely belowground (Acciaresi and Guiamet 2010), which supports the idea that its higher C4-facilitated performance in hotter habitats may translate into a competitive advantage. However, its belowground competitive effect is difficult to test empirically in complex plant communities (Schwinning et al. 2017).

Populations from arid climates responded most strongly to the difference in water availability by allocating significantly more to roots under drought conditions. Compared to mesic home habitat populations, which showed no change in root allocation during drought, arid origin populations show an important plastic response. This suggests that frequent drought stress may have selected for this plastic response in Johnsongrass populations from arid regions, but not their counterparts from places where drought is rare. Studies on other C4 grasses suggest that variation in drought response adaptations may be indicative of the type or duration of drought associated with selection...
in home habitats. For example, (Cardoso et al. 2015) compared drought response in two C4 grasses and found a suite of responses, including plastic increase in root:shoot ratio to be associated with the species that had adapted to intermittent drought, rather than prolonged drought. From the perspective of deep evolutionary time, (Zhou et al. 2018) assert that water limitation (given high atmospheric CO2) was the primary driver of C4 evolution, while today its advantage over C3 is often associated with elevated temperatures (Ehleringer et al. 1997). In a Texas grassland community context, (Wilsey and Polley 2006) found both C3 and C4 grasses (Johnsongrass not among them) to respond plastically to drought, however native grasses had a greater increase in root:shoot ratio than invaders. Schwinning et al. (2017) -- while unable to compare belowground allocation -- also found that Johnsongrass seedlings’ advantage over native competitors was significant aboveground. Thus, given contextual evidence to date, root:shoot plasticity in Johnsongrass from arid, but not mesic, regions is more likely an adaptation linked with general performance in a stressful growing environment rather than competitive advantage.

We saw that SOC-poor origin populations experienced greater differences in chlorophyll content due to irrigation/drought than their SOC-rich origin counterparts. This suggests that habitats with higher nutrient availability may release plants from stresses limiting chlorophyll production, and this is less impacted by drought than in plants from low-SOC habitats. We saw that the irrigation treatment’s interaction with SOC in home habitat mirrored its interaction with the fertilization treatment. In the latter case, the impacts of nutrient limitation were significantly lowered by irrigation. Had we been able to grow the ‘fertilizer’ plants in nutrient-free media, it is more than likely that this relationship would have been conserved, if not strengthened. Considering that agricultural populations increase chlorophyll content with the home SOC gradient, while non-agricultural populations do not, there may be a parallel between drought response and ecotypic adaptation. Specifically, we saw that, with increasing home SOC, unirrigated and agricultural populations produce more chlorophyll, while the chlorophyll content of irrigated or non-agricultural populations does not change. While we did not find a direct interaction between ecotype identity and the irrigation treatment, the combination of
our findings suggests it. Speculatively, the agricultural ecotype’s response to home SOC may reflect higher stress due to less drought adaptation. Meanwhile, the non-agricultural ecotype shows similar unresponsiveness to home SOC as all unirrigated populations -- a condition to which it may be adapted. The interactive effect of irrigation and fertilization on chlorophyll content has been seen in wheat (Dimkpa et al. 2020), while in Sorghum bicolor it may be mediated by microbes aiding in phosphorus acquisition (Kour et al. 2020). Others have found instances of wild and cultivated ecotypes with differentially adapted nutrient uptake and chlorophyll production (Papafilippaki and Nikolaidis 2020). (Kausar and Gull 2019) found different genotypes of Sorghum bicolor to be more tolerant of salinity stress (which inhibited normal nutrient uptake) than others. In fact, much of the research that compares plant ecotypic differences in chlorophyll content does so in the context of tolerance to polluted soil and their phytoremediation (Park et al. 2012; Alirzayeva et al. 2017; Liu et al. 2019). Our comparison of home habitat and ecotype identity as drivers of adaptation is novel, however our approach yielded results similar to others that found ecotypic differences in experimental stress response.

Population differences in stress response at the seedling stages suggest advantages for producing founder populations in new locations. The capacity for these stress adaptations in invasive plants have often been associated with greater spread and persistence (Wen 2015; Yuan and Wen 2018; Abbas et al. 2019). Evolutionary change concurrent with landscape spread has also been linked with higher fecundity and ability to occupy new habitats (Lustenhouwer et al. 2019). We see that no life stage is alone responsible for invasion success, but rather a suite of mechanisms interacts over time to boost the invader’s fitness. Studying seedlings is advantageous for discerning the roles of abiotic stresses to which plants later become less vulnerable, while limiting experiments to this stage may inhibit our ability to understand the invader’s competitive ability, which is dynamic over time. As our interests here were limited to the former, the seedling experiment was most appropriate.
Overall, we found support for differences in drought stress based on home habitat properties – namely, water availability (MAP) and soil fertility (SOC). We did not find evidence for differences in nutrient stress response based on home habitat properties MAT, MAP, or SOC. We found ecotype identity to moderate performance differences based on SOC in home habitat, however, we found no direct relationships between ecotype and MAT, MAP, or the irrigation and/or fertilization treatments. We can use these interpretations to reflect more broadly on long-term trajectories of biological invasions. Human landscape management across fine and coarse scales can have an enormous impact on the distribution (Decker et al. 2012) and adaptation of invaders (Schneider 2006). At the same time, selection for plasticity and “plasticity of plasticity” may be a common trait among invasive species (Funk 2008; Davidson et al. 2011). With land use change and accompanying changes in management regimes in disturbed ecosystems selecting for adaptable lineages, we expect to see similar patterns across taxa. Numerous studies have found nutrient pollution (Crooks et al. 2011), heavy metal pollution (Yang et al. 2007), and urban heat island effect (Battles and Kolbe 2019) to facilitate invasion through the creation of suitable microhabitats, sometimes to the exclusion of other competitors. In particular, landscape novelty and heterogeneity in urbanized areas present great challenges to our understanding of invader adaptation and potential management (Gaertner et al. 2017). In parallel with this work, it will be important to investigate whether selection through land use change has the potential to impact ecotypes’ adaptation to climate change and other habitat modifications.
Acknowledgements:

We acknowledge the contribution of Jeff Mutter to experimental setup, as well as Rebecca Fletcher’s help in harvesting the experiment. We thank Gregory Welbaum and Valerie Thomas for their review of the manuscript. This work was supported by the Virginia Tech College of Agriculture and Life Sciences and the National Institute of Food and Agriculture Global Food Security CAP [2015-68004-23492 to JNB].

Supporting Information:

The raw data is available as supporting information.
Legends:

**Table 1.** Effect tests of each linear model as reduced via backward stepwise selection for optimized AICc. Only effects that remained in at least one of the three reduced models are presented in this table. Total Biomass and Root:Shoot response variables were log-transformed to meet model assumptions. Square brackets around variable names indicate variable locking prior to stepwise selection. Alpha level of significance indicated by *** = 0.0005, ** = 0.005, * = 0.05.

**Figure 1.** The geographic locations in the contiguous United States where Johnsongrass seed for the experiment was collected. Circles and stars indicate ecotype attribution (agricultural vs. non-agricultural). The shading of the background map corresponds to categorical bands of Mean Annual Precipitation, with one agricultural and one non-agricultural population sampled from five of the six bands.

**Figure 2.** (A) The effect (mean +/- SE) of irrigation treatment on root:shoot ratio (p = 0.0008). The interactive effect (mean +/- SE) of irrigation and fertilization on (B) chlorophyll content (p < 0.0001) and (C) total biomass (p < 0.0001).

**Figure 3.** The interactive effect of (A) ecotype identity and soil organic carbon in home habitat on chlorophyll content (p = 0.0395). The interactive effect of (B) irrigation treatment and soil organic carbon in home habitat (p = 0.0359).

**Figure 4.** The effect of (A) mean annual temperature in home habitat on root:shoot biomass ratio (p = 0.0037). The interactive effect of (B) irrigation and mean annual precipitation at home habitat on root:shoot biomass ratio (p = 0.0147). Error bands in both panels represent 95% C.I.
References:

Abbas AM, Rubio-Casal AE, De Cires A, Grewell BJ, Castillo JM. 2019. Differential tolerance of native and invasive tree seedlings from arid African deserts to drought and shade. *South African Journal of Botany* **123**: 228–240.

Acciaresi HA, Guiamet JJ. 2010. Below- and above-ground growth and biomass allocation in maize and Sorghum halepense in response to soil water competition. *Weed Research* **50**: 481–492.

Alirzayeva E, Neumann G, Horst W, Allahverdiyeva Y, Specht A, Alizade V. 2017. Multiple mechanisms of heavy metal tolerance are differentially expressed in ecotypes of Artemisia fragrans. *Environmental Pollution* **220**: 1024–1035.

Armbruster WS. 2014. Floral specialization and angiosperm diversity: Phenotypic divergence, fitness trade-offs and realized pollination accuracy. *AoB PLANTS* **6**: 1–24.

Atwater DZ, Fletcher RA, Dickinson CC, Paterson AH, Barney JN. 2018. Evidence for fine-scale habitat specialisation in an invasive weed. *Journal of Plant Ecology* **11**: 189–199.

Atwater DZ, Sezen UU, Goff V, Kong W, Paterson AH, Barney JN. 2016. Reconstructing changes in the genotype, phenotype, and climatic niche of an introduced species. *Ecography* **39**: 894–903.

Ball C. 1902. Johnson Grass: Report of Investigations Made During The Season of 1901. *USDA Bureau of Plant Industry Bulletin* **11**.
Baruch Z, Jackson RB. 2005. Responses of tropical native and invader C4 grasses to water stress, clipping and increased atmospheric CO2 concentration. Oecologia 145: 522–532.

Battles AC, Kolbe JJ. 2019. Miami heat: Urban heat islands influence the thermal suitability of habitats for ectotherms. Global Change Biology 25: 562–576.

te Beest M, Elschot K, Olff H, Etienne RS. 2013. Invasion Success in a Marginal Habitat: An Experimental Test of Competitive Ability and Drought Tolerance in Chromolaena odorata. PLoS ONE 8.

Berger JD, Ludwig C. 2014. Contrasting adaptive strategies to terminal drought-stress gradients in Mediterranean legumes: Phenology, productivity, and water relations in wild and domesticated Lupinus luteus L. Journal of Experimental Botany 65: 6219–6229.

van Boheemen LA, Atwater DZ, Hodgins KA. 2019. Rapid and repeated local adaptation to climate in an invasive plant. New Phytologist 222: 614–627.

Burt GW. 1974. Adaptation of Johnsongrass. Weed Science 22: 59–63.

Cardoso JA, Pineda M, Jiménez J de la C, Vergara MF, Rao IM. 2015. Contrasting strategies to cope with drought conditions by two tropical forage C 4 grasses. AoB Plants 7: plv107.

Carvajal DE, Loayza AP, Rios RS, Gianoli E, Squeo FA. 2017. Population variation in drought-resistance strategies in a desert shrub along an aridity gradient: Interplay between phenotypic plasticity and ecotypic differentiation. Perspectives in Plant Ecology, Evolution and Systematics 29: 12–19.

Catford JA, Daehler CC, Murphy HT, et al. 2012. The intermediate disturbance hypothesis and plant invasions: Implications for species richness and management. Perspectives in Plant Ecology, Evolution and Systematics 14: 231–241.

Cavero J, Medina ET, Puig M, Martínez-Cob A. 2009. Sprinkler irrigation changes maize canopy
microclimate and crop water status, transpiration, and temperature. *Agronomy Journal* **101**: 854–864.

**Chapin FS. 1987.** Adaptation and physiological responses of wild plants to nutrient stress In: Gabelman WH, Loughman BC, eds. *Genetic Aspects of Plant Mineral Nutrition*. Martinus Nijhoff Publishers, 15–25.

**Cheng W, Padre AT, Sato C, et al. 2016.** Changes in the soil C and N contents, C decomposition and N mineralization potentials in a rice paddy after long-term application of inorganic fertilizers and organic matter. *Soil Science and Plant Nutrition* **62**: 212–219.

**Clark RB, Reinhard N. 1991.** Effects of soil temperature on root and shoot growth traits and iron deficiency chlorosis in sorghum genotypes grown on a low iron calcareous soil. *Plant and Soil* **130**: 97–103.

**Crooks JA, Chang AL, Ruiz GM. 2011.** Aquatic pollution increases the relative success of invasive species. *Biological Invasions* **13**: 165–176.

**Davidson AM, Jennions M, Nicotra AB. 2011.** Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters* **14**: 419–431.

**Decker KL, Allen CR, Acosta L, et al. 2012.** Land Use, Landscapes, and Biological Invasions. *Invasive Plant Science and Management* **5**: 108–116.

**Delêtre M, Soengas B, Vidaurre PJ, et al. 2017.** Ecotypic differentiation under farmers’ selection: Molecular insights into the domestication of *Pachyrhizus* Rich. ex DC. (Fabaceae) in the Peruvian Andes. *Evolutionary Applications* **10**: 498–513.

**Dimkpa CO, Andrews J, Sanabria J, et al. 2020.** Interactive effects of drought, organic fertilizer, and zinc oxide nanoscale and bulk particles on wheat performance and grain nutrient accumulation. *Science of the Total Environment* **722**: 137808.
Ehleringer JR, Cerling TE, Helliker BR. 1997. C4 photosynthesis, atmospheric CO2, and climate. *Oecologia* **32112**: 285–299.

Engel K, Tollrian R, Jeschke JM. 2011. Integrating biological invasions, climate change, and phenotypic plasticity. *Communicative and Integrative Biology* **4**: 247–250.

Espeland EK. 2013. Predicting the dynamics of local adaptation in invasive species. *Journal of Arid Land* **5**: 268–274.

Eziz A, Yan Z, Tian D, Han W, Tang Z, Fang J. 2017. Drought effect on plant biomass allocation: A meta-analysis. *Ecology and Evolution* **7**: 11002–11010.

Fahey C, Angelini C, Flory SL. 2018. Grass invasion and drought interact to alter the diversity and structure of native plant communities. *Ecology* **99**: 2692–2702.

Filippou P, Bouchagier P, Skotti E, Fotopoulos V. 2014. Proline and reactive oxygen/nitrogen species metabolism is involved in the tolerant response of the invasive plant species Ailanthus altissima to drought and salinity. *Environmental and Experimental Botany* **97**: 1–10.

Funk JL. 2008. Differences in plasticity between invasive and native plants from a low resource environment. *Journal of Ecology* **96**: 1162–1173.

Gaertner M, Wilson JRU, Cadotte MW, MacIvor JS, Zenni RD, Richardson DM. 2017. Non-native species in urban environments: patterns, processes, impacts and challenges. *Biological Invasions* **19**: 3461–3469.

Gao SB, Mo LD, Zhang LH, et al. 2018. Phenotypic plasticity vs. local adaptation in quantitative traits differences of Stipa grandis in semi-arid steppe, China. *Scientific Reports* **8**: 1–8.

Garrido E, Andraca-Gómez G, Fornoni J. 2012. Local adaptation: Simultaneously considering herbivores and their host plants. *New Phytologist* **193**: 445–453.
Gervasi DDL, Schiestl FP. 2017. Real-time divergent evolution in plants driven by pollinators. *Nature Communications* 8: 1–8.

Gitelson AA, Buschmann C, Lichtenenthaler HK. 1999. The chlorophyll fluorescence ratio F735/F700 as an accurate measure of the chlorophyll content in plants. *Remote Sensing of Environment* 69: 296–302.

Gorton AJ, Moeller DA, Tiffin P. 2018. Little plant, big city: A test of adaptation to urban environments in common ragweed (Ambrosia artemisiifolia). *Proceedings of the Royal Society B: Biological Sciences* 285.

Hengl T, De Jesus JM, MacMillan RA, et al. 2014. SoilGrids1km - Global soil information based on automated mapping. *PLoS ONE* 9.

Henn JJ, Buzzard V, Enquist BJ, et al. 2018. Intraspecific trait variation and phenotypic plasticity mediate alpine plant species response to climate change. *Frontiers in Plant Science* 871: 1–11.

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the National Academy of Sciences of the United States of America* 107: 2093–2098.

Kausar A, Gull M. 2019. Influence of salinity stress on the uptake of magnesium, phosphorus, and yield of salt susceptible and tolerant sorghum cultivars (Sorghum bicolor L.). *Journal of Applied Biology and Biotechnology* 7: 53–58.

Kawecki TJ, Ebert D. 2004. Conceptual issues in local adaptation. *Ecology Letters* 7: 1225–1241.

Kour D, Rana KL, Kaur T, et al. 2020. Microbe-mediated alleviation of drought stress and acquisition of phosphorus in great millet (Sorghum bicolor L.) by drought-adaptive and phosphorus-solubilizing microbes. *Biocatalysis and Agricultural Biotechnology* 23: 101501.

Kueffer C, Pyšek P, Richardson DM. 2013. Integrative invasion science: Model systems, multi-site
studies, focused meta-analysis and invasion syndromes. *New Phytologist* **200**: 615–633.

Lavergne S, Muenke NJ, Molofsky J. 2010. Genome size reduction can trigger rapid phenotypic evolution in invasive plants. *Annals of Botany* **105**: 109–116.

Leguizamón ES, Yanniccar ME, Guiamet JJ, Acciaresi HA. 2011. Growth, gas exchange and competitive ability of Sorghum halepense populations under different soil water availability. *Canadian Journal of Plant Science* **91**: 1011–1025.

Leimu R, Fischer M. 2008. A meta-analysis of local adaptation in plants. *PLoS ONE* **3**: 1–8.

Li Q, Chen Y, Liu M, Zhou X, Yu S, Dong B. 2008. Effects of irrigation and straw mulching on microclimate characteristics and water use efficiency of winter wheat in North China. *Plant Production Science* **11**: 161–170.

Liu YN, Xiao XY, Guo ZH. 2019. Identification of indicators of giant reed (Arundo donax L.) ecotypes for phytoremediation of metal-contaminated soil in a non-ferrous mining and smelting area in southern China. *Ecological Indicators* **101**: 249–260.

Lowry DB, Behrman KD, Grabowski P, Morris GP, Kiniry JR, Juenger TE. 2014. Adaptations between ecotypes and along environmental gradients in Panicum virgatum. *American Naturalist* **183**: 682–692.

Luo W, Jiang Y, Lü X, et al. 2013. Patterns of Plant Biomass Allocation in Temperate Grasslands across a 2500-km Transect in Northern China. *PLoS ONE* **8**: 1–8.

Lustenhouwer N, Williams JL, Levine JM. 2019. Evolution during population spread affects plant performance in stressful environments. *Journal of Ecology* **107**: 396–406.

Malíková L, Latzel V, Šmilauer P, Klimešová J. 2016. Local adaptation of annual weed populations to habitats differing in disturbance regime. *Evolutionary Ecology* **30**: 861–876.
Matesanz S, Horgan-Kobelski T, Sultan SE. 2012. Phenotypic Plasticity and Population Differentiation in an Ongoing Species Invasion. *PLoS ONE* **7**.

Mcwhorter CG. 1971. Introduction and Spread of Johnsongrass in the United States. Author (s): C. G. McWhorter Published by: Weed Science Society of America Stable URL: http://www.jstor.org/stable/4041684 Introduction and Spread of Johnsongrass in the United States ’. 19: 496–500.

Molina-Montenegro MA, Acuña-Rodríguez IS, Flores TSM, et al. 2018. Is the success of plant invasions the result of rapid adaptive evolution in seed traits? Evidence from a latitudinal rainfall gradient. *Frontiers in Plant Science* **9**: 1–15.

Oduor AMO, Leimu R, van Kleunen M. 2016. Invasive plant species are locally adapted just as frequently and at least as strongly as native plant species. *Journal of Ecology* **104**: 957–968.

Papafilippaki A, Nikolaidis NP. 2020. Comparative study of wild and cultivated populations of Cichorium spinosum: The influence of soil and organic matter addition. *Scientia Horticulturae* **261**.

Park W, Han KH, Ahn SJ. 2012. Differences in root-to-shoot cd and Zn translocation and by HMA3 and 4 could influence chlorophyll and anthocyanin content in arabidopsis ws and col-0 ecotypes under excess metals. *Soil Science and Plant Nutrition* **58**: 334–348.

Postma FM, Ågren J. 2016. Early life stages contribute strongly to local adaptation in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences of the United States of America* **113**: 7590–7595.

Qi Y, Wei W, Chen C, Chen L. 2019. Plant root-shoot biomass allocation over diverse biomes: A global synthesis. *Global Ecology and Conservation* **18**: e00606.

Rajakaruna N. 2018. Lessons on Evolution from the Study of Edaphic Specialization. *Botanical Review* **84**: 39–78.
Razgour O, Forester B, Taggart JB, et al. 2019. Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections. *Proceedings of the National Academy of Sciences of the United States of America* **116**: 10418–10423.

Reich PB, Luo Y, Bradford JB, Poorter H, Perry CH, Oleksyn J. 2014. Temperature drives global patterns in forest biomass distribution in leaves, stems, and roots. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 13721–13726.

Rout ME, Chrzanowski TH, Westlie TK, DeLuca TH, Callaway RM, Holben WE. 2013. Bacterial endophytes enhance competition by invasive plants. *American Journal of Botany* **100**: 1726–1737.

Sakaguchi S, Horie K, Ishikawa N, et al. 2019. Maintenance of soil ecotypes of Solidago virgaurea in close parapatry via divergent flowering time and selection against immigrants. *Journal of Ecology* **107**: 418–435.

Salas RA, Burgos NR, Tranel PJ, et al. 2016. Resistance to PPO-inhibiting herbicide in Palmer amaranth from Arkansas. *Pest Management Science* **72**: 864–869.

Sanchez AC, Subudhi PK, Rosenow DT, Nguyen HT. 2002. Mapping QTLs associated with drought resistance in sorghum (Sorghum bicolor L. Moench). *Plant Molecular Biology* **48**: 713–726.

Schneider LC. 2006. Invasive species and land-use: The effect of land management practices on bracken fern invasion in the region of Calakmul, Mexico. *Journal of Latin American Geography* **5**: 91–107.

Schwinning S, Meckel H, Reichmann LG, Polley HW, Fay PA. 2017. Accelerated development in Johnsongrass seedlings (Sorghum halepense) suppresses the growth of native grasses through size asymmetric competition. *PLoS ONE* **12**: 1–18.

Sezen UU, Barney JN, Atwater DZ, et al. 2016. Multi-phase US spread and habitat switching of a post-columbian invasive, Sorghum halepense. *PLoS ONE* **11**: 1–14.
Thornley JHM. 1972. A Balanced Quantitative Model for Root: Shoot Ratios in Vegetative Plants. *Annals of Botany* 36: 431–441.

Turner KG, Huffbauer RA, Rieseberg LH. 2014. Rapid evolution of an invasive weed. *New Phytologist* 202: 309–321.

Vetter VMS, Kreyling J, Dengler J, et al. 2020. Invader presence disrupts the stabilizing effect of species richness in plant community recovery after drought. *Global Change Biology* 26: 3539–3551.

Warwick & Black LD. 1983. The Biology of Canadian Weeds. 61. Sorghum halepense (L.) Pers. *Canadian Journal of Plant Science* 1014: 997–1014.

Warwick SI, Phillips D, Andrews C. 1986. Rhizome depth: the critical factor in winter survival of Sorghum halepense (L.) Pers. (Johnson grass). *Weed Research* 26: 381–388.

Washburn JD, Whitmire DK, Murray SC, et al. 2013. Estimation of Rhizome Composition and Overwintering Ability in Perennial Sorghum spp. Using Near-Infrared Spectroscopy (NIRS). *Bioenergy Research* 6: 822–829.

Wen B. 2015. Effects of high temperature and water stress on seed germination of the invasive species Mexican sunflower. *PLoS ONE* 10: 1–13.

Whitney KD, Gabler CA. 2008. Rapid evolution in introduced species, “invasive traits” and recipient communities: Challenges for predicting invasive potential. *Diversity and Distributions* 14: 569–580.

Wilsey BJ, Polley HW. 2006. Aboveground productivity and root-shoot allocation differ between native and introduced grass species. *Oecologia* 150: 300–309.

Wilson JB. 1988. A Review of Evidence on the Control of Shoot: Root Ratio, in Relation to Models. *Annals of Botany* 61: 433–449.

Xia HB, Xia H, Ellstrand NC, Yang C, Lu BR. 2011. Rapid evolutionary divergence and ecotypic
diversification of germination behavior in weedy rice populations. *New Phytologist* **191**: 1119–1127.

**Yang R, Tang J, Yang Y, Chen X. 2007.** Invasive and non-invasive plants differ in response to soil heavy metal lead contamination. *Ecology* **48**: 453–458.

**Yuan X, Wen B. 2018.** Seed germination response to high temperature and water stress in three invasive Asteraceae weeds from Xishuangbanna, SW China. *PLoS ONE* **13**: 1–16.

**Zhou H, Helliker BR, Huber M, Dicks A, Akçay E. 2018.** C4 photosynthesis and climate through the lens of optimality. *Proceedings of the National Academy of Sciences of the United States of America* **115**: 12057–12062.
Table 1. Effect tests of each linear model as reduced via backward stepwise selection for optimized AICc. Only effects that remained in at least one of the three reduced models are presented in this table. Total Biomass and Root:Shoot response variables were log-transformed to meet model assumptions. Square brackets around variable names indicate variable locking prior to stepwise selection. Alpha level of significance indicated by *** = 0.0005, ** = 0.005, * = 0.05.

|                         | Total Biomass | Root:Shoot | Chlorophyll |
|-------------------------|---------------|------------|-------------|
|                         | DF | SS     | F   | p    | DF | SS     | F   | p    | DF | SS     | F   | p    |
| [Block]                 | 4  | 0.201  | 8.655 | 0.0037** | 4  | 0.046  | 1.287 | 0.258 | 4  | 2106.2  | 0.278 | 0.5993 |
| [Initial Height]        | 1  | 1.325  | 57.11 | <0.0001***| 1  | 0.02   | 0.554 | 0.458 | 1  | 21735  | 2.856 | 0.0927 |
| [Irrigation]            | 1  | 0.662  | 28.54 | <0.0001***| 1  | 0.41   | 11.57 | 0.0008**| 1  | 204010 | 26.81 | <0.0001***|
| [Fertilization]         | 1  | 1.647  | 70.99 | <0.0001***| 1  | 0.091  | 2.574 | 0.1103 | 1  | 407796 | 53.59 | <0.0001***|
| Ecotype                 | -  | -      | -    | -      | -  | -      | -    | -      | -  | -      | -    | -      |
| MAT                     | -  | -      | -    | -      | 1  | 0.302  | 8.618 | 0.0037**| -  | -      | -    | -      |
| MAP                     | -  | -      | -    | -      | 1  | 0.062  | 1.759 | 0.1863 | -  | -      | -    | -      |
| SOC                     | -  | -      | -    | -      | -  | -      | -    | -      | 1  | 57303  | 7.5306 | 0.0067* |
| Irr. * Fert.            | 1  | 1.181  | 50.92 | <0.0001***| -  | -      | -    | -      | 1  | 121443 | 15.96 | <0.0001***|
| Irr. * MAP              | -  | -      | -    | -      | 1  | 0.215  | 6.065 | 0.0147*| -  | -      | -    | -      |
| Irr. * SOC              | -  | -      | -    | -      | -  | -      | -    | -      | 1  | 33967  | 4.464 | 0.0359* |
| Ecotype * SOC           | -  | -      | -    | -      | -  | -      | -    | -      | 1  | 32704  | 4.298 | 0.0395* |
Figure 1

Ecotype
- Agricultural
- Non-agricultural

Mean Annual Precipitation Bands
- 109 – 406mm
- 406 – 621mm
- 621 – 836mm
- 836 – 1051mm
- 1051 – 1266mm
- 1266 – 1481mm
Figure 3

A

B

Chlorophyll Content (mg m⁻²)

0 50 100 150 200 250 300 350 400 450

0 50 100 150 200 250 300 350 400

Soil Organic Carbon (g/kg fine earth fraction)

Soil Organic Carbon (g/kg fine earth fraction)
Figure 4

A

B

Root:Shoot Biomass Ratio

Mean Annual Temperature (°C)

Mean Annual Precipitation (mm)

Unirrigated
Irrigated