Evidence for fine-scale habitat specialisation in an invasive weed

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Abstract

Aims
As an exotic species colonises a new continent, it must overcome enormous environmental variation in its introduced range. Local adaptation of introduced species has frequently been observed at the continent scale, particularly in response to latitudinal climatic variation. However, significant environmental heterogeneity can also exist at the landscape scale. A small number of studies have provided evidence that introduced species may also be capable of phenotypic and genetic differentiation at much smaller spatial scales. For example, previously we found US agricultural and non-agricultural populations of *Sorghum halepense* (Johnsongrass) to be phenotypically and genetically distinct. In this study, we investigated whether this phenotypic differentiation of agricultural and non-agricultural populations of *S. halepense* is the result of fine-scale local specialisation.

Methods
We surveyed a nationally collected *S. halepense* germplasm panel and also collected neighbouring agricultural and non-agricultural sub-populations of *S. halepense* at four sites throughout Western Virginia, USA, raising seedlings in common conditions mimicking both agricultural and non-agricultural habitats.

Important Findings
At the national scale, we found evidence of habitat differentiation but not specialisation. However, at the local scale, we found evidence of specialisation in two of the four local populations to non-agricultural habitat, but no evidence of specialisation to agricultural habitat. These results show that local specialisation is a possible, but not guaranteed consequence of kilometre-scale habitat heterogeneity in invasive species. This finding contributes to a growing awareness of the importance of fine-scale local adaptation in the ecology and management of introduced and weedy species.

Keywords: Johnsongrass, local adaptation, rapid evolution, *Sorghum halepense* (L.)

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INTRODUCTION

As an exotic species colonises a new continent it must overcome significant, and often novel, environmental variation. How exotic species do this has been a focus of scientific interest since the inception of invasion ecology (Baker 1974; Baker and Stebbins 1965; Marshall and Jain 1968). While initial studies posited plasticity, particularly in disturbance-adapted species, as a major factor allowing colonising species to tolerate environmental variation, it is now recognised that rapid evolution and local specialisation can play a major role in this process (Lee 2002). Introduced species have been seen to differentiate from native-range populations (Blair and Wolfe 2004), to evolve rapidly along abiotic clines (Maron *et al.* 2004; Huey *et al.* 2000), and to exhibit high neutral genetic variation and population structure despite founder effects (Dlugosch and Parker 2008; Lee 2002).

Among introduced plants, adaptation to continent-scale abiotic clines has also been frequently observed. For example, Scandinavian populations of *Capsella bursa-pastoris*, which was introduced in the Middle Ages, vary in their flowering phenology along latitudinal clines (Neuffer 1990), as do recently-introduced North American populations (Neuffer and Hurka 1999). In a pair of studies, Maron *et al.* (2004, 2007) found that *Hypericum perforatum* had evolved rapidly along a latitudinal gradient in the introduced range of the United States, displaying variation in size and fecundity consistent with latitudinal variation in its native range. Moreover, *H. perforatum* demonstrated higher than expected diversity in neutral AFLP markers in its introduced range even as compared to its native

range (Maron *et al.* 2004). Similar results have been observed in *Impatiens gandulifera* (Kollmann and Bañuelos 2004), *Eschscholzia californica* (Leger and Rice 2003, 2007), *Lythrum salicaria* (Montague *et al.* 2008), *Tamarix* spp. (Friedman *et al.* 2008) and *Sorghum halepense* (Atwater *et al.* 2016; McWhorter 1971). As a result of this research, we know that introduced species commonly evolve along latitudinal clines, suggesting that the specialisation of introduced subpopulations to their local environment is an expected consequence of colonisation (Colautti *et al.* 2009).

While introduced species are known to evolve in response to continent-scale environmental variation, less is known about whether they also evolve in response to environmental variation at or below the landscape scale, despite the fact that local adaptation is common in plants (Leimu and Fischer 2008; Hereford 2009). For example, Leger *et al.* (2009) observed that low- and high-elevation populations of invasive *Bromus tectorum* varied phenotypically and genetically despite being separated by only 4.2 kilometres. Low-elevation populations outperformed the high-elevation populations in a low-, but not high-, elevation common garden, suggesting that local specialisation had occurred. *Bromus tectorum* has also been found to vary genetically and phenotypically among habitat types in a Utah landscape (Scott *et al.* 2010), as well as throughout its United States distribution, in a manner consistent with local adaptation by some genotypes but not others (Merrill *et al.* 2012). In *Prunella vulgaris*, sun and shade sub-populations were found to have locally adapted in a National Park in Chile (Godoy *et al.* 2011). However, in a study in Germany, *Impatiens glandulifera* was found to vary phenotypically across sites and habitats, but not in a manner consistent with local adaptation (Pahl *et al.* 2013).

Sorghum halepense (Johnsongrass), is a globally invasive weed (Holm *et al.* 1977) that expresses ecotypic variation throughout its North American range (Atwater *et al.* 2016; McWhorter 1971; Taylorson and McWhorter 1969; Warwick *et al.* 1986). Ecotypic variation in *S. halepense* has been linked to variation in cold tolerance (Warwick *et al.* 1986) and growth in common gardens in different climates (Atwater *et al.* 2016). *Sorghum halepense* invades both managed agricultural fields and unmanaged habitats such as ditches, rights-of-way, abandoned fields and field margins (Holm *et al.* 1977; Rout *et al.* 2012; Warwick and Black 1983), with agricultural and non-agricultural populations differing both phenotypically and genetically, even among neighbouring populations (Atwater *et al.* 2016). Genetic evidence suggests that differentiation of agricultural and non-agricultural populations may have played an important role in the North American invasion of *S. halepense*, as more derived lineages of *S. halepense* are found exclusively in non-agricultural habitat (Sezen *et al.* 2016). Thus, there appears to have been a 'two-pronged' invasion of *S. halepense*, which progressed *via* partially independent invasion fronts in both agricultural and non-agricultural habitat (Atwater *et al.* 2016; Sezen *et al.* 2016). However, it is not known whether agricultural and non-agricultural populations

are locally adapted. The question of whether introduced species experience fine-scale local specialisation has profound implications for the evolution, expansion and management of introduced species (Espeland 2013; Sax *et al.* 2007).

In this study, we investigated whether *S. halepense* shows evidence of habitat specialisation at the landscape scale. We collected seeds of agricultural and non-agricultural populations of *S. halepense* in two ways: we used a subset of the germplasm collected nationally for a previous study (Atwater *et al.* 2016), and we collected from paired agricultural and non-agricultural sub-populations at four sites in Western Virginia, where our common garden was located. We investigated the following questions: (i) whether *S. halepense* exhibits significant phenotypic variation among populations; (ii) whether *S. halepense* from agricultural and non-agricultural habitats have distinct phenotypes and (iii) whether *S. halepense* performs best when grown in its home habitat. In order to determine that phenotypic differentiation is due to local adaptation, it is best to perform reciprocal transplant studies (Leimu and Fischer 2008; Hereford 2009). However, in some systems, this is not viable due to logistical, ethical or landowner concerns. In such cases, common gardens provide an excellent opportunity to explore both local adaptation and traits that may be experiencing selection (e.g. Godoy *et al.* 2011).

MATERIALS AND METHODS

Study species

Sorghum halepense is a perennial C4 grass that is native to North Africa and Anatolia, having spontaneously evolved as a hybrid between annual *S. bicolor* and wild, rhizome-producing perennial *S. propinquum* (Morden *et al.* 1990; Paterson *et al.* 1995, 2009). Currently, it is a globally invasive species (Holm *et al.* 1977; Monaghan 1979; Warwick and Black 1983) that threatens both natural (Rout *et al.* 2012) and agricultural systems worldwide (Holm *et al.* 1977; Warwick and Black 1983). Like other members of the *Sorghum* genus, *S. halepense* is capable of both self- and cross-pollination, although self-pollination occurs with much greater frequency (Warwick and Black 1983). Seeds have pronounced dormancy (Taylorson and McWhorter 1969), allowing *S. halepense* to form a seed bank. Individual seeds can live up to six years in soil, depending on planting depth, although a majority of seeds germinate 1 to 3 years after planting (Leguizamón 1986). *Sorghum halepense* can also reproduce vegetatively *via* a fast-growing rhizome network (McWhorter 1961). *Sorghum halepense* maintains high genetic diversity despite being primarily self-pollinated, which is partially explained by frequent introgression with *S. bicolor* (Arriola and Ellstrand 1996; Morrell *et al.* 2005). Perhaps as a result, *S. halepense* has differentiated into distinct ecotypes throughout its United States introduced range (Atwater *et al.* 2016; McWhorter 1971; Taylorson and McWhorter 1969; Warwick *et al.* 1986). Recent evidence suggests that *S. halepense* phenotypes vary according to both their home climate and home habitat, with agricultural and

non-agricultural accessions having distinct phenotypes and genotypes, suggesting that *S. halepense* has evolved in response to both continent-scale and landscape-scale environmental variation (Atwater *et al.* 2016). However, it is not known whether such variation influences growth in agricultural versus non-agricultural habitats, nor whether the differentiation that exists between agricultural and non-agricultural populations separated by tens or hundreds of kilometers also exists at finer spatial scales. Therefore, we wanted to test for local specialisation at two geographic scales—continental and local—using the same system.

Seed collection

We used two seed sources for this experiment: nationally and locally collected seeds. Our rationale for doing this was to determine whether there was evidence for habitat specialisation throughout the United States range of *S. halepense* (using nationally collected seeds, representing a subset of Atwater *et al.* 2016), as well as to determine whether there was evidence for specialisation at extremely fine spatial scales (<1 km, using locally collected seeds). We restricted our local-scale sampling to Western Virginia to avoid confounding effects of home climate, and to ensure that the collections were close to the common garden location.

The nationally collected seeds were harvested in June to August of 2011 from 24 sites. Each population was identified as being either agricultural (i.e. collected from an actively managed agricultural field; principally corn, soy or rice) or non-agricultural (i.e. collected from a ditch, right-of-way or long-abandoned field). The mean annual temperature (MAT) and mean annual precipitation (MAP) for each site were estimated using historic (1955–2000) averages from the BIOCLIM database (Hijmans *et al.* 2005). The 24 populations were a subset of 70 populations collected for a previous study (Atwater *et al.* 2016) and were not collected from intentionally paired agricultural and non-agricultural sites. However, for this study, we carefully selected populations that represented a full range of climates for both agricultural- and non-agricultural sites across the US distribution (Table 1). Multiple individuals (1–10) were collected from each site, for a total of 80 accessions in the experiment. The nationally collected seeds were planted and the germplasm was maintained *via* cuttings in a greenhouse. In May 2014, we planted an increase garden and harvested mature seeds from that garden in September 2014 to be used in this experiment. This procedure was designed to both increase the number of available seeds and remove maternal effects associated with the maternal growing environment.

Locally collected *S. halepense* seeds were harvested in July 2014 from four locations located in the Virginia Ridge and Valley physiographic province near Blacksburg, VA, USA (Table 2). Each of these populations occurred in a floodplain site with a mix of residential dwellings, unmanaged forest and active farmland intersected by roads and railways. The nearest populations were separated by 4.5 km and the most distant by 13.5 km and 28 km. At each location, we identified a population of *S. halepense* individuals occurring in active farmland, with field corn (*Zea mays*) planted at all sites except the Shawsville site, which was planted with pumpkins. We also identified a nearby (<1.5 km distant) population of *S. halepense* occurring on non-agricultural land (a roadside at Glenvar, a railroad right-of-way at Kentland and abandoned lots at Shawsville and Allegheny Springs). We harvested ripe seeds from 6 to 10 individuals (i.e. accessions) from each population, only harvesting individuals that were >5 m distant from one another to increase the probability of sampling multiple genotypes. Seeds were overwintered in a climate-controlled shade house (~4°C). In March 2015, we removed the seeds from cold storage, soaked them in full-strength commercial bleach solution for 4 h, washed them in tap water for 1 h and then planted them in flats containing a 1:2 mix of local topsoil and sand, by volume. After germination, individual seedlings were transplanted into randomised 120 ml pots. Seedlings were grown for an additional 6 weeks and then transplanted into the field in the last week of May, 2015.

Common garden experiment

Plots were located at the Kentland Research Farm, Virginia Tech, near McCoy, VA (37.19391 N, 80.57300 W), 100–200 m from the Kentland seed collection sites. Common garden conditions consisted of three treatments: a weed-competition treatment, a corn-competition treatment and a bare-ground control treatment. These treatments represent the habitat types *S. halepense* was collected from and most commonly occurs on, while the weed-free treatment served as a competition-free control. In the bare-ground treatment, all weeds were removed by application of glyphosate, dicamba and 2,4-D before and after transplantation. Care was taken to avoid spraying transplanted *S. halepense* with this mixture. In the weed-competition treatment, weeds were allowed to recruit naturally after *S. halepense* was planted and no further management was applied. In the corn-competition treatment, two weeks prior to transplantation of *S. halepense*, commercially available glyphosate-resistant field corn was planted at a conventional row width and seeding rate. This corn was fertilised twice with urea and chemically managed according to conventional practice, except that herbicide was selectively applied rather than broadcast in order to avoid injuring *S. halepense* transplants. We did not spray water on the control plots to account for water added during herbicide use for several reasons: (i) the amount of herbicide sprayed was inconsistent among plots because it was selectively applied, (ii) we did not want to risk dosing plants with residual herbicide still in the backpack sprayer, (iii) the amount of water added during treatment was miniscule (about 10 ml of water per m^2 of area, or about 0.1 mm of precipitation), most of which never encountered the soil and evaporated quickly.

The planting design differed slightly between experiments. Nationally collected populations were planted into five blocks, each of which contained six plots with randomised treatments.

Table 1: locations of Johnsongrass populations collected in different states in the USA (Ag.: agricultural habitats, non-Ag.: non-agricultural habitats)

Population	MAT (°C)	MAP (mm)	Habitat	Lat. (DD)	Lon. (DD)
Alabama 1	15.0	1474	Non-Ag.	34.2778	−86.8898
Alabama 2	15.5	1419	Non-Ag.	34.6483	−87.1055
Alabama 3	15.3	1430	Non-Ag.	34.7522	−86.9590
Arizona 1	20.6	305	Non-Ag.	32.2734	−110.9031
Arizona 2	21.1	201	Non-Ag.	33.1033	−111.9743
California 1	17.2	253	Ag.	36.1990	−119.2519
California 2	17.1	248	Non-Ag.	36.2337	−119.3121
California 3	17.0	250	Non-Ag.	36.3088	−119.3806
Florida 1	19.2	1475	Non-Ag.	30.5520	−84.6770
Florida 2	19.4	1321	Ag.	30.8367	−82.4560
Georgia 1	17.1	1284	Non-Ag.	32.8917	−84.3272
Georgia 2	18.5	1170	Non-Ag.	32.2050	−82.3635
Georgia 3	17.6	1205	Non-Ag.	33.2907	−81.3373
Georgia 4	16.7	1219	Ag.	33.5250	−83.4442
Georgia 5	15.1	1387	Non-Ag.	34.4655	−84.6995
Kansas 1	12.8	736	Non-Ag.	38.8833	−97.7344
Kansas 2	13.0	795	Ag.	38.7061	−97.4278
South Carolina 1	16.5	1211	Non-Ag.	34.0307	−81.5883
Texas 1	19.0	874	Ag.	31.0600	−97.3422
Texas 2	17.9	975	Ag.	33.2797	−96.8927
Texas 3	13.9	422	Non-Ag.	34.3309	−102.9762
Texas 4	13.9	434	Non-Ag.	34.3156	−102.7736
Virginia 1	15.3	1127	Ag.	37.2114	−80.4877
Virginia 2	15.3	1127	Non-Ag.	37.1940	−80.5739

Mean annual temperature (MAT) and mean annual precipitation (MAP) also shown.

Table 2: locations of locally collected source populations and the distances between agricultural and non-agricultural populations at each locality

Population	Agricultural		Non-Agricultural		Distance (m)
	Latitude	Longitude	Latitude	Longitude	
Glenvar	37.26952 N	80.13705 W	37.27053 N	80.13393 W	298
Kentland	37.19391 N	80.57526 W	37.19360 N	80.57433 W	89
Shawsville	37.18306 N	80.24093 W	37.17772 N	80.24666 W	782
Allegheny Springs	37.14962 N	80.26544 W	37.13477 N	80.26588 W	1654

Each plot consisted of five rows of eight plants (40 total), with a 1 m planting interval within rows and a 1.52 m separation between rows. This arrangement was used to accommodate the 30 inch (0.76 m) row width of corn, which was planted in some of the plots. A single seedling of each accession was planted exactly once per plot. Seedlings were at the 3-leaf stage (10–15 cm) when planted. Locally collected populations were planted similarly, except that they were sown into six blocks of three randomised plots. After three weeks of growth survival was assessed in all treatments. Planting locations were randomised. Individual plants were marked with 50 cm flags and pot tags. Johnsongrass individuals that emerged naturally were killed. Any plants that did not survive were assumed to have died due to transplant shock and were removed from further analysis. In October 2015, the height of surviving plants was measured, their flower-producing culms were counted, and their aboveground biomass was collected, dried and weighed.

Statistical analysis

We estimated treatment effects on biomass, height and number of flowering culms using linear mixed models. For the nationally collected populations, we modelled fixed effects of treatment (bare-ground, weed-competition and

corn-competition) and home habitat (agricultural or non-agricultural). We also included linear and quadratic effects of home MAT and MAP, and all possible interactions involving treatment, habitat and one, but not both, of the climate variables. We declined to include transplantation height as a covariate to avoid removing variance caused by seedling growth rate, which might represent an ecologically relevant trait associated with local specialisation. It was necessary to specify a random effect of plot, rather than block, to ensure independence of the residuals. To avoid over-fitting, we compared AIC_C values of models containing each combination of predictors and selected the models with the most terms that had an $\Delta AIC_C \leq 2$. We excluded models that did not involve main effects of habitat, treatment and plot, and we did not allow quadratic effects of MAT and MAP to exist unless linear effects were also present. Model comparison was done using package "MuMIn" (Barton 2016) in R version 3.3.0 (R Core Team 2016).

For locally collected accessions, we modelled home habitat type, population (Glenvar, Kentland, Shawsville or Allegheny Springs) and experimental treatment (bare ground, weed competition or corn competition) as fixed factors with all possible interactions included. Plot and accession were modelled as random effects. Population was modelled as a fixed, rather than random effect, because it is difficult to accurately measure random effects distributions in factors with few levels (Bolker *et al.* 2009). These models were run with natural-log-transformed biomass, height and number of flowering culms as response variables. We also modelled effects of treatment on natural-log-transformed stature (height/biomass) for the locally collected seeds. This was not done for nationally collected seeds due to strong correlations between ln-biomass and ln-stature.

Biomass, height and stature models were fitted using general linear mixed models, although it was necessary to log-transform biomass and stature to ensure normality of the residuals. To model treatment effects on the number of flowering culms, we specified a generalised mixed model with a negative binomial error distribution with the canonical link. This was used, rather than a Poisson error distribution, to account for zero-inflation and over-distribution of the response variable. General models were run using package 'lme4' (Bates *et al.* 2015), and the negative binomial model was run using 'glmmADMB' (Fournier *et al.* 2012).

To evaluate models of log-transformed biomass, height and log-transformed stature, we calculated *F* tests, with degrees of freedom determined using Kenward–Roger approximation (Kenward and Roger 1997) using 'pbkrtest' and 'lmerTest' (Halekoh and Hojsgaard 2014; Kuznetsova *et al.* 2016). For the negative binomial model, we estimated parameter significance using log-likelihood ratios with package 'car' (Fox and Weisberg 2002). In all cases, 'Type III' tests were used. To determine whether home habitat (agricultural vs. non-agricultural) influenced performance for plants from each population and in each treatment, we performed planned contrasts, with Tukey adjustment, using package 'lsmeans' (Lenth 2016).

RESULTS

Nationally collected populations

Experimental treatment had major effects on *S. halepense* growth (Table 3), with plants grown in bare ground achieving a median 278 g of aboveground biomass (with a maximum of 1.8 kg), compared to 4 g for *S. halepense* experiencing competition with weeds and 23 g for those competing with corn (Fig. 1). Competition with resident weeds caused severe stunting of most *S. halepense*, with few individuals growing tall enough (mean height: 73.4 cm) to escape the weed canopy, which primarily comprised *Amaranthus* spp., *Arctium minus*, *Conyza canadensis*, *Cyperus esculentus*, *Datura stramonium*, *Ipomoea* spp., *Oxalis* spp., *Setaria* spp. and *Trifolium* spp., all of which spontaneously emerged from the seed bank. The locally collected and nationally collected experiments were planted on opposite sides of a farm road, with slightly different land use histories and slightly different weed communities. Thus, effects of weed competition may not be directly comparable between experiments. In the corn-competition treatment, *S. halepense* produced few culms but grew nearly as tall as the competition-free plants (mean height in corn-competition, local accessions: 129.5 cm, bare ground: 138.2 cm).

Home habitat had significant effects in models of log-transformed biomass, height and flowering culm number ($P \leq 0.050$), although these effects were complex and contingent on home climate (Table 3; Fig. 1). Non-agricultural accessions grew larger on average (median biomass, bare-ground treatment, ag.: 257 g, non-ag: 339 g), although agricultural accessions from warm climates were much larger than non-agricultural accessions from similar climates. However, there was no evidence that effects of home habitat depended on experimental treatment – habitat × treatment interactions were absent from top models of biomass and culm number (see online supplementary Table S1), and did not have statistically significant effects on height (Table 3). Thus, for the nationally collected accessions, there is no evidence that differentiation between agricultural and non-agricultural accessions has influenced their responses to the experimental treatments imposed in this study.

Locally collected populations

As with the nationally collected populations, treatment had strong effects on biomass (Table 4; Fig. 2). Plants grown in bare ground achieved a median 277 g of aboveground biomass (with a maximum of 1.4 kg), compared to 1.4 g for *S. halepense* experiencing competition with weeds and 23 g for those competing with corn. Treatment had major effects on the stature of *S. halepense*, with competition causing plants to grow tall and spindly, which was especially pronounced in the corn-competition treatment (Table 4).

Population was the most important predictor of growth after treatment (Table 4); *Sorghum halepense* plants from different populations varied in their log-transformed mass, height and number of flowering culms, with population having

Table 3: omnibus tests of each treatment on biomass (log-transformed), height and the number of flowering culms for nationally-collected samples

	ln-Biomass			Height			Fl. Culms	
	SS	F	P	SS	F	P	X^2	P
Habitat	8734	$5.00_{1,644.4}$	0.026	26 151	$14.90_{1,644.4}$	<0.001	2.56_1	0.110
Treatment	208 326	$59.62_{2,25.9}$	<0.001	75 769	$21.59_{2,209.5}$	<0.001	120.76_2	< 0.001
MAP	9403	$5.38_{1,641.0}$	0.021	12 238	$6.97_{1,618.7}$	0.008	-	
MAP^2	10 002	$5.73_{1,648.6}$	0.017	668	$0.38_{1,621.6}$	0.537	-	
MAT	-			13 271	$7.56_{1,622.5}$	0.006	0.01_1	0.918
MAT^2	-			13	$0.01_{1,622.1}$	0.931	4.40_1	0.036
Habitat × Treat.	-			2521	$0.72_{2,620.3}$	0.488	-	
Habitat × MAP	6405	$3.67_{1,640.7}$	0.056	1874	$1.07_{1,621.2}$	0.302	-	
Habitat × MAP^2	12688	$7.26_{1,646.6}$	0.007	26 732	$15.24_{1,621.1}$	<0.001	-	
Habitat × MAT	-			8462	$4.82_{1,618.7}$	0.028	0.53_1	0.465
Habitat × MAT^2	-			7240	$4.13_{1,629.5}$	0.043	5.58_1	0.018
Treat. × MAP	-			721	$0.21_{2,618.7}$	0.814	-	
Treat. × MAP^2	-			1649	$0.47_{2,622.4}$	0.625	-	
Treat. × MAT	-			134	$0.04_{2,621.7}$	0.963	-	
Treat. × MAT^2	-			1609	$0.46_{2,621.9}$	0.632	-	
Habitat × Treat. × MAP	-			4703	$1.34_{2,621.2}$	0.263	-	
Habitat × Treat. × MAP^2	-			3261	$0.93_{2,618.7}$	0.395	-	
Habitat × Treat. × MAT	-			1938	$0.55_{2,620.9}$	0.576	-	
Habitat × Treat. × MAT^2	-			670	$0.19_{2,629.2}$	0.826	-	

F-statistics are shown for general linear models and X^2-statistic calculated using log-likelihood ratios is shown for the negative binomial model of flowering culms. Terms not in the top model were excluded (-).

significant main effects in models of log-transformed above-ground biomass and height ($P \leq 0.007$). Population participated in significant second- and third-order interactions with habitat and treatment to affect the number of flowering culms ($P \leq 0.024$). These effects were also marginally significant in the biomass ($P \leq 0.057$) and height models ($P = 0.051$). Home habitat did not have significant main effects in any models, although it participated in a marginally significant interaction with population in the model of log-transformed biomass ($P = 0.057$), with population and treatment in the model of height ($P = 0.051$), and with population ($P = 0.019$) and both population and treatment ($P = 0.001$) in the model of flowering culms. *Sorghum halepense* stature varied among populations but not between habitats.

We specified contrasts in order to determine whether agricultural and non-agricultural accessions from different populations and in different treatments grew differently, as indicated by the significant three-way interaction between population, treatment and habitat in the flowering culm model ($P = 0.001$) and the trend towards significant interactions in models of log-transformed biomass ($P = 0.096$) and height ($P = 0.051$; Table 5). These contrasts were not performed for stature, for which there was no indication of significant effects involving habitat. Contrasts were significant only for the weed-competition treatment. If habitat specialisation caused

non-agricultural accessions to grow better under weed competition than agricultural accessions, we should have observed positive contrasts in the weed-competition treatment. This was true for *S. halepense* from Allegheny Springs, which had a positive contrast for log-transformed biomass ($P = 0.033$) and a trend for height ($P = 0.091$), and also for *S. halepense* from Shawsville, which had a positive contrast for culm number ($P = 0.002$). However, *S. halepense* from Kentland showed the opposite pattern, with agricultural accessions having higher log-transformed biomass ($P = 0.035$), possibly greater height ($P = 0.053$) and more flowering culms ($P = 0.017$) than non-agricultural accessions (Table 5, Figs. 2 and 3).

DISCUSSION

Ecotypic variation along temperature clines has been well documented in a range of invasive species, including *S. halepense* (Atwater *et al.* 2016; McWhorter 1971; Taylorson and McWhorter 1969; Warwick *et al.* 1986). In a study of almost 500 accessions of *S. halepense* collected from 70 sites, Atwater *et al.* (2016) found evidence that *S. halepense* has differentiated genetically and phenotypically in response to habitat variation, leaving open the possibility that such differentiation was due to local specialisation. Using a subset of the accessions grown by Atwater *et al.*, here we investigated whether agricultural and non-agricultural accessions
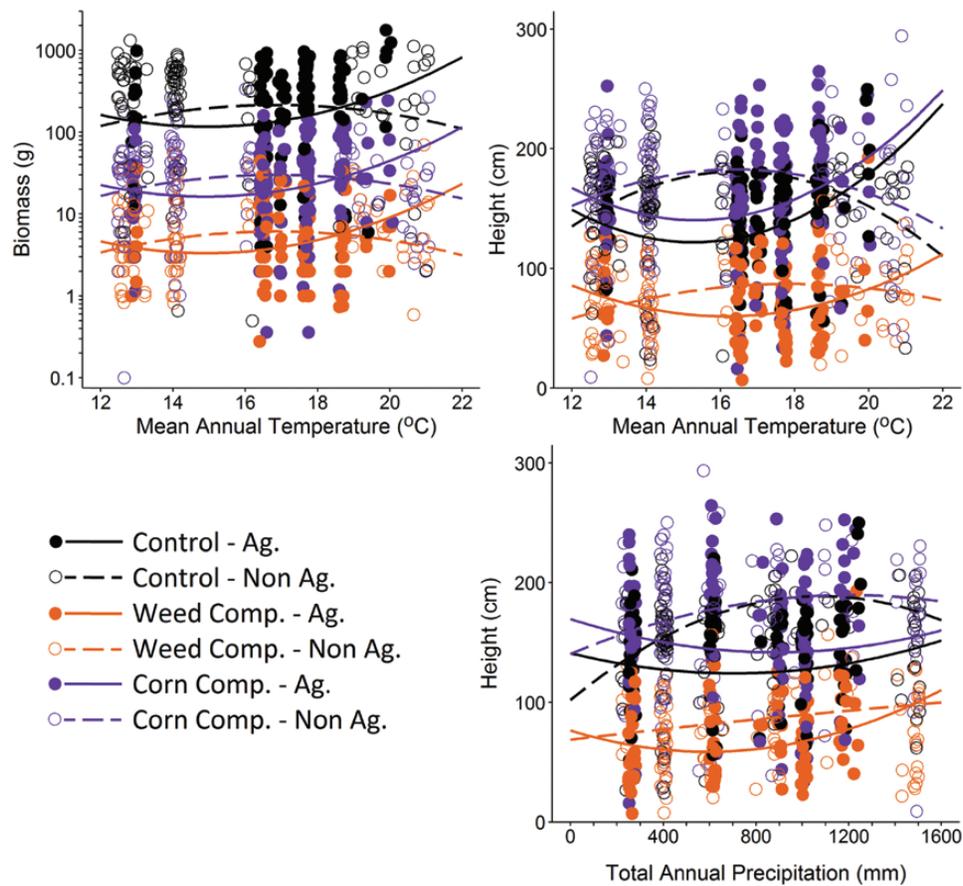
Figure 1: effects of home climate, home habitat (agricultural: solid lines, closed circles; non-agricultural: dashed lines, open circles) and treatment (bare ground; weed-competition; corn-competition) on the biomass and height of nationally collected populations. Effects of precipitation on biomass are not shown as these were not present in the top model of ln-biomass.

Table 4: omnibus tests of each treatment on biomass (log-transformed), height, stature (log-transformed) and number of flowering culms for locally collected populations

	ln-Biomass			Height			ln-Stature			Fl. Culms	
	SS	F	P	SS	F	P	SS	F	P	X^2	P
Habitat	0.39	$0.43_{1,52.6}$	0.514	641	$0.65_{1,51.7}$	0.423	0.08	$0.14_{1,52.6}$	0.71	2.47_1	0.116
Population	19.27	$7.17_{3,51.7}$	<0.001	13441	$4.56_{3,51.2}$	0.007	11.14	$6.36_{3,51.2}$	<0.001	4.22_3	0.239
Treatment	511.50	$285.39_{2,16.4}$	<0.001	29 6170	$150.80_{2,16.8}$	<0.001	354.15	$303.40_{2,16.6}$	<0.001	189.27_2	<0.001
Habitat × Pop.	7.19	$2.67_{3,51.9}$	0.057	5424	$1.84_{3,51.2}$	0.151	2.46	$1.40_{3,51.8}$	0.253	9.98_3	0.019
Habitat × Treat.	2.07	$1.16_{2,651.0}$	0.315	3636	$1.85_{2,655.8}$	0.158	0.67	$0.57_{2,651.3}$	0.565	2.17_2	0.338
Pop. × Treat.	5.1	$0.95_{6,647.8}$	0.460	6998	$1.19_{6,652.6}$	0.311	2.13	$0.61_{6,648.0}$	0.725	14.53_6	0.024
Habitat × Pop. × Treat.	9.69	$1.80_{6,648.2}$	0.096	12 394	$2.10_{6,652.6}$	0.051	4.84	$1.38_{6,648.4}$	0.220	22.26_6	0.001

F-statistics are shown for general linear models and X^2-statistic calculated using log-likelihood ratios is shown for the negative binomial model of flowering culms.

differed in their growth in conditions similar to a managed crop field and to unmanaged, non-agricultural habitat. While we found patterns of phenotypic differentiation very similar to those reported previously (Atwater *et al.* 2016), there was no evidence that growth of nationally collected *S. halepense* accessions different treatments depended upon whether accessions were collected from agricultural or non-agricultural habitats. Thus, although agricultural and non-agricultural accessions differentiated phenotypically, there was no evidence for local specialisation among the nationally collected samples. This may have been partly influenced by the confounding effects of home climate on phenotypic expression in the Virginia common garden.
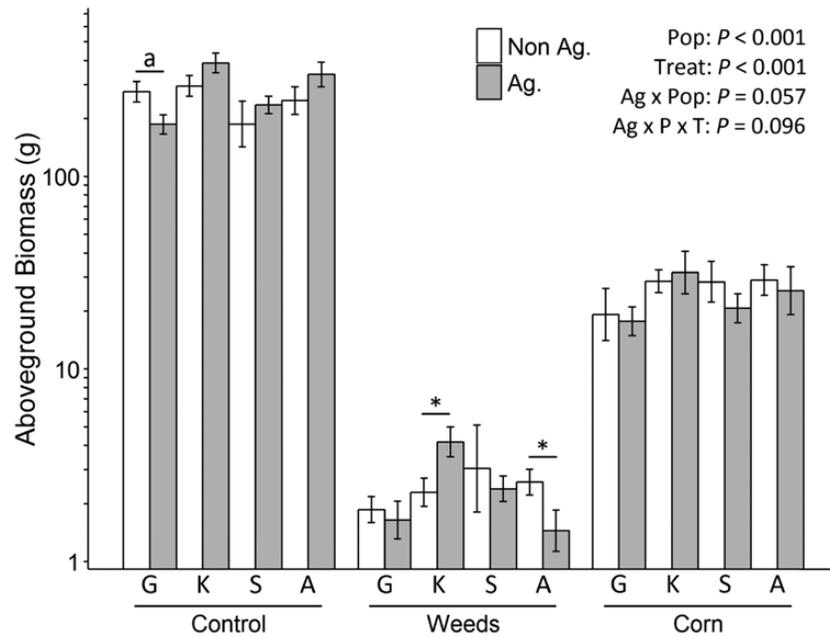
Figure 2: bar plot showing aboveground dry biomass of non-agricultural (white) and agricultural (grey) populations of *Sorghum halepense* from each locally collected source (G: Glade, K: Kentland, S: Shawsville, A: Allegheny Springs) in each treatment. Means are shown ± s.e. Omnibus tests of effects on log-transformed biomass are shown in the box. Asterisks indicate significant effects of habitat (ag. vs. non-ag) at for each population in each treatment (*P ≤ 0.050; [a]P = 0.070).

Table 5: contrasts showing effects of home habitat (agricultural vs. non-agricultural) for accessions from each locally collected paired-population in each treatment

	Biomass			Height			Num. flowering culms		
	$B \pm se$	t	P	$B \pm se$	t	P	$B \pm se$	z	P
Bare ground									
Glenvar	0.403 ± 0.221	$1.82_{220.5}$	0.070	0.780 ± 7.887	$0.10_{172.1}$	0.921	0.290 ± 0.185	1.571	0.116
Kentland	−0.300 ± 0.223	$-1.34_{267.5}$	0.180	2.760 ± 7.797	$0.35_{206.1}$	0.724	−0.240 ± 0.274	−0.879	0.379
Shawsville	−0.210 ± 0.311	$-0.68_{318.1}$	0.500	−5.783 ± 10.857	$-0.53_{250.4}$	0.595	−0.200 ± 0.320	−0.623	0.533
Alleghany Springs	−0.344 ± 0.252	$-1.37_{244.0}$	0.173	−12.356 ± 8.905	$-1.39_{188.4}$	0.167	−0.175 ± 0.287	−0.609	0.543
Weeds									
Glenvar	0.051 ± 0.274	$0.19_{354.4}$	0.853	−0.549 ± 9.493	$-0.06_{285.5}$	0.954	−0.309 ± 0.541	−0.571	0.568
Kentland	−0.559 ± 0.264	$-2.11_{395.8}$	0.035	−17.687 ± 9.087	$-1.95_{316.8}$	0.053	−1.138 ± 0.477	−2.383	0.017
Shawsville	0.406 ± 0.345	$1.18_{343.5}$	0.240	11.045 ± 11.667	$0.95_{227.9}$	0.345	1.570 ± 0.509	3.087	0.002
Alleghany Springs	0.667 ± 0.312	$2.14_{411.3}$	0.033	18.349 ± 10.832	$1.69_{332.1}$	0.091	0.939 ± 0.693	1.355	0.175
Corn									
Glenvar	0.080 ± 0.259	$0.31_{323.7}$	0.757	13.040 ± 9.067	$1.44_{258.4}$	0.152	0.074 ± 0.358	0.207	0.836
Kentland	−0.066 ± 0.247	$-0.27_{351.3}$	0.790	−11.905 ± 8.580	$-1.39_{274.8}$	0.166	−0.180 ± 0.357	−0.503	0.615
Shawsville	0.375 ± 0.317	$1.18_{351.9}$	0.238	16.363 ± 11.061	$1.48_{272.8}$	0.140	0.136 ± 0.399	0.342	0.733
Alleghany Springs	0.131 ± 0.284	$10.46_{337.1}$	0.646	15.701 ± 9.948	$1.58_{265.1}$	0.116	−0.152 ± 0.395	−0.394	0.694

A positive contrast (*B*) indicates that non-agricultural accessions grew larger than agricultural accessions. Test statistics (*t* or *z*) and their degrees of freedom, and significance (*P*) are also shown.

To determine whether *S. halepense* experiences habitat specialisation at very fine spatial scales, we also collected seeds from paired agricultural and non-agricultural populations at four sites in Western Virginia, with the paired agricultural and non-agricultural populations being separated by only 89–1654 m. In contrast to the nationally collected samples, we found evidence consistent with local specialisation in two of the four locally collected populations. However, this was only true for the weed-competition treatment. In the corn-competition treatment, all accessions performed uniformly. Thus, there is evidence for the existence of specialised non-agricultural populations of *S. halepense*, but there is no evidence of agricultural
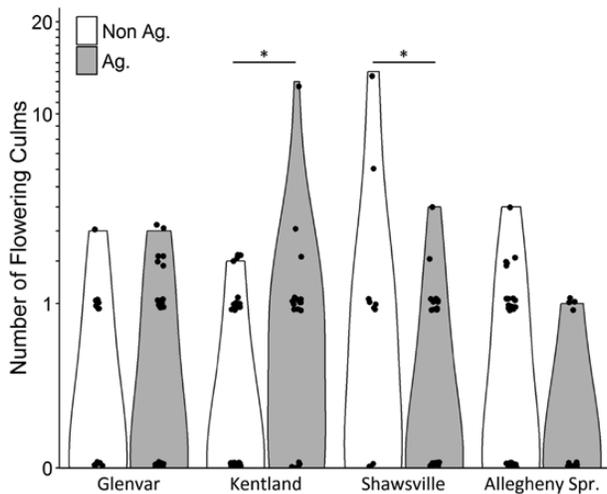
Figure 3: violin plot of number of flowering culms of non-agricultural (white) and agricultural (grey) sub-populations of *S. halepense* from each locally collected population (Glade, Kentland, Shawsville, Allegheny Springs) in the weed-competition treatment. Actual data are shown as points. Asterisks indicate significant effects of habitat (ag. vs. non-ag) at for each population in each treatment (*$P \leq 0.050$).

specialisation. We note that these results cannot be taken as definitive proof of specialisation, which requires estimation of lifetime fitness. It is also possible that maternal effects influenced our results, although it is not clear why maternal affects would influence growth in the weed competition treatment but not in the other treatments. We note that maternal effects were unlikely to have influenced the performance of nationally-collected accessions, which were grown in an increase garden for one generation.

Throughout the South-eastern US, *S. halepense* is a common weed of agricultural fields, especially corn, where light competition is high. As observed in this study, *S. halepense* expresses a unique phenotype in corn fields during the establishment year, growing tall but with very low culm production, especially relative to plants not experiencing competition. We hypothesised that, due to local specialisation, agricultural accessions of *S. halepense* would display this distinctive morphology under all experimental conditions, but did not find that to be the case. Instead, all accessions, regardless of their home habitat and whether they were nationally or locally collected, readily adopted the 'agricultural morphology' when grown with corn, but not in the other treatments. However, while we did not find evidence that agricultural accessions outperformed non-agricultural accessions when grown in agricultural conditions (i.e. in a high-resource fertilised environment with low weed pressure, but in competition with corn), we did find phenotypic differentiation in the weed-competition treatment that was consistent with local specialisation in two of four populations. Our results fit well with a study of nearly 500 accessions of *S. halepense* collected from 70 sites throughout the United States, which found that agricultural genotypes appeared to be 'general purpose' or 'jack-of-all-trades' genotypes (*sensu*

Baker and Stebbins 1965; Richards *et al.* 2006), able to grow in disparate climates (Atwater *et al.* 2016). In contrast, the non-agricultural populations showed evidence of adaptation to local climates. Taken together with the results presented here, it appears that *S. halepense* relies on plasticity to persist in agricultural environments, and on local adaptation to colonise non-agricultural habitat.

We speculate that specialisation may have occurred in some non-agricultural populations, but not in agricultural populations, because the former tend to be much more fecund. While we have not conducted a survey of *S. halepense* population sizes in different habitats in our study area, anecdotally we note that *S. halepense* obtains far greater seed densities—and likely greater propagule sizes—in unmanaged habitat than it does in managed agricultural fields (DZA, personal observation). That being the case, local adaptation of non-agricultural subpopulations would be expected (Holt 1996), with selection favouring plasticity in agricultural subpopulations (Sultan and Spencer 2002). A similar pattern was observed in *Bromus tectorum*, which showed evidence of specialisation in low-elevation core populations but not in high-elevation marginal populations (Leger *et al.* 2009). Espeland (2013) noted that this may be a common pattern in colonising species. However, Godoy *et al.* (2011) found evidence of specialisation in both sunny core populations and in nearby marginal shade populations of *Prunella vulgaris*.

In order for local specialisation to occur, species must possess sufficient additive genetic variance (Lee 2002), and enough population structure that gene flow among subpopulations does not overwhelm local selection (Slatkin 1987). It was once supposed that, due to founder effects, recently introduced species were lacking in genetic variance (Baker and Stebbins 1965). However, multiple introductions (\Novak and Mack 1993, 2005), introgression with resident species (Casa *et al.* 2005; Morrell *et al.* 2005; Paterson *et al.* 1995) and conversion of dominance and epistatic variance (Cheverud and Routman 1996; Willis and Orr 1993) have all been shown to improve additive variance in introduced populations. High phenotypic variance is now known to be common within and among introduced populations (Dlugosch and Parker 2008; Sax *et al.* 2007), even in species with reproductive systems that limit recombination (Leger *et al.* 2009).

While specialisation of plant species is apparently common at the continent scale (e.g. Atwater *et al.* 2016; Friedman *et al.* 2008; Leger and Rice 2003; Maron *et al.* 2004; Montague *et al.* 2008; Neuffer 1990), at the landscape scale, where gene flow is likely to be high, it is not clear how gene flow and selection might interact to influence specialisation of invasive species (Espeland 2013), as local adaptation of sub-populations is only a stable consequence of selection when rates of gene flow are low. Otherwise, selection is expected either to favour plasticity (Sultan and Spencer 2002) or the specialisation of the most fecund subpopulation (Holt 1996), unless specialisation also causes reproductive isolation (Carroll and Boyd 1992; Carroll *et al.* 1997; Huey *et al.* 2005). We did not perform

genetic analyses on the locally collected populations, but a genetic analysis of the nationally collected *S. halepense* accessions (Sezen *et al.*, 2016) shows that agricultural and non-agricultural accessions of *S. halepense* have differentiated genetically. Such differentiation may be facilitated by the tendency of *S. halepense* to reproduce vegetatively, and to self-fertilise when reproducing sexually (Warwick and Black 1983).

Fine-scale local adaptation could have important consequences for the management of invasive species, as unmanaged populations (e.g. non-agricultural populations of *S. halepense*) could variously represent (i) sources of propagule pressure on managed lands; (ii) demographic sinks (Howe *et al.* 1991); (iii) constituents of a highly admixed greater population, in which phenotypic differentiation is maintained only by constant, strong selection or (iv) isolated sub-populations that do not interact with other sub-populations. The category a species falls into depends upon the degree of gene flow among sub-populations, the trade-offs required for specialisation and the effective population sizes of sub-populations occupying each habitat (Espeland 2013). Our results suggest that *S. halepense* probably falls into the first category, as non-agricultural populations grew readily in the corn-competition treatment. These results suggest that, for *S. halepense*, suppression of border populations may be important for successful control in croplands, as these populations likely represent a significant propagule source. Overall, these and other recent findings (Atwater *et al.* 2016; Godoy *et al.* 2011; Leger *et al.* 2009; Merrill *et al.* 2012; Scott *et al.* 2010) provide compelling evidence that sub-population differentiation at fine spatial scales is an important feature of plant invasions (Espeland 2013) with important implications for both the ecology and management of invasive species.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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