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Fitness costs of herbicide resistance across natural populations of the common morning glory, *Ipomoea purpurea*

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Although fitness costs associated with plant defensive traits are widely expected, they are not universally detected, calling into question their generality. Here, we examine the potential for life-history trade-offs associated with herbicide resistance by examining seed germination, root growth, and above-ground growth across 43 naturally occurring populations of *Ipomoea purpurea* that vary in their resistance to RoundUp®, the most commonly used herbicide worldwide. We find evidence for life-history trade-offs associated with all three traits; highly resistant populations had lower germination, shorter roots, and smaller above-ground size. A visual exploration of the data indicated that the type of trade-off may differ among populations. Our results demonstrate that costs of adaptation may be present at stages other than simply the production of progeny in this agricultural weed. Additionally, the cumulative effect of costs at multiple life cycle stages can result in severe consequences to fitness when adapting to novel environments.

KEY WORDS: **Cost of herbicide resistance, early life history, fitness cost, germination, glyphosate, *Ipomoea purpurea*, seed quality, trade-offs.**

Plant defense is generally hypothesized to involve a cost. This expectation stems from the surprising observation of genetic variation underlying plant defense traits in many natural systems, whether the elicitor of damage is an herbivore, a pathogen, or an herbicide (Simms and Rausher 1987, 1989; Stahl et al. 1999; Baucom and Mauricio 2004; Bakker et al. 2006; Menchari et al. 2006; Délye et al. 2010; Kuester et al. 2015). If there were no costs associated with defense, alleles conferring either resistance or tolerance to damage should increase to fixation, rendering all individuals in the population highly defended (Rausher and Simms 1989). Despite our expectations of a trade-off between fitness and defense, however, reviews of the literature consistently show that costs are not ubiquitous regardless of the elicitor of selection or the study organism at hand (Bergelson and Purrington 1996; Coustau and Chevillon 2000).

Three main ideas have been proposed to explain the absence of such costs. First, there are a diverse number of potential mechanisms responsible for adaptation to a damaging agent, only some of which may incur a cost (Powles and Yu 2010; Vogwill et al. 2012). A single-gene nucleotide substitution that leads to herbicide resistance, for example, may not alter the efficiency of translated proteins and therefore not incur a cost (e.g., Yu et al. 2007, 2010). On the other hand, a mechanism that provides resistance to a range of different herbicides through changes in growth may be more likely to impose fitness costs. Second, costs may not be detected if the genetic background is not properly controlled (Bergelson and Purrington 1996; Vila-Aiub et al. 2009b, 2011). Control of the genetic background, either by performing crosses (Baucom and Mauricio 2004; Menchari et al. 2008; Giacomini et al. 2014) or ensuring replication across multiple genetic backgrounds (Cousens et al. 1997; Strauss et al. 2002), increases the likelihood that a cost will be detected (Bergelson and Purrington 1996). Third, researchers often examine only a portion of the life cycle (i.e., seed production or fecundity) and may do so in artificial

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and/or noncompetitive conditions (Vila-Aiub et al. 2009b, 2011). Studies that examine a range of traits are more likely to identify potential growth and/or fitness differences associated with plant defense compared to those that focus solely on measures of fecundity (Vila-Aiub et al. 2009b).

The phenomenon of herbicide resistance in plant weeds provides a particularly useful system to investigate the nature and types of costs associated with plant defense, because we know when selection by the herbicide began, the strength of selection, and often the frequency of herbicide use. However, as in other systems examining the evolution of plant defense, fitness costs of herbicide resistance are often not detected (Bergelson and Purrington 1996; Gemmill and Read 1998; Vila-Aiub et al. 2009b). Despite recommendations to control/increase the number of genetic backgrounds (Bergelson and Purrington 1996), and to examine multiple life-history stages when determining if resistance incurs a cost (Primack and Kang 1989; Vila-Aiub et al. 2009b), only 25% of herbicide resistance studies control for background effects; further, only 7–10% of cost studies examine multiple stages of the life cycle (Vila-Aiub et al. 2009b). Fewer still examine the potential for fitness costs using a large number of naturally occurring populations sampled from a species' range, an approach suggested almost 20 years ago (Cousens et al. 1997; Strauss et al. 2002). Just as the mechanism of resistance can vary among species, populations of the same weed have been shown to harbor different mechanisms of resistance to the same herbicide (Christopher et al. 1991, 1992, 1994; Preston and Powles 1998; Yu et al. 2008; Délye et al. 2010), thus increasing the likelihood that costs may likewise vary among populations. It is also possible, though rarely tested, that fitness costs have been ameliorated in some herbicide resistant populations relative to other populations due to the evolution of modifier loci (i.e., compensatory evolution, Darmency et al. 2015). The above hypotheses for the lack of costs are all interrelated: because resistance could be due to a variety of mechanisms (Délye et al. 2013a), costs may be apparent at only certain life-history stages, expressed in particular environments (Vila-Aiub et al. 2009b), or apparent in some populations but not others. Thus, there remain crucial gaps in our understanding of where trade-offs between fitness-enhancing traits and resistance might be apparent, and further, how ubiquitous such trade-offs may be across a species' range (Vila-Aiub et al. 2011; Neve et al. 2014).

The common morning glory, *Ipomoea purpurea*, a noxious weed of U.S. agriculture (Webster and MacDonald 2001), provides an excellent system to examine the strength and type of potential costs that may be present in natural populations. This species exhibits variability in resistance to glyphosate (Baucom and Mauricio 2008; Kuester et al. 2015), which is the main ingredient in the herbicide RoundUp®. RoundUp® is currently the most widely used herbicide in agriculture (Fernandez-Cornejo et al. 2014), and of the approximately 30 resistant weeds that have been examined (Heap 2015), only a third are reported to express fitness costs (Ismail et al. 2002; Pedersen et al. 2007; Brabham et al. 2011; Giacomini et al. 2014; Shrestha et al. 2014; Vila-Aiub et al. 2014; Glettner and Stoltenberg 2015; Goh et al. 2015). *Ipomoea purpurea* has long been considered to exhibit low-level resistance to glyphosate (Culpepper 2006), and previously we have shown that this low-level resistance (estimated as proportion leaf damage) has an additive genetic basis and is under positive selection in the presence of the herbicide (Baucom and Mauricio 2008). Further, a recent replicated dose-response experiment of 43 populations sampled from the Southeastern and Midwest United States showed that some populations of *I. purpurea* exhibit ~100% survival after application of the field dose of RoundUp® (i.e., resistance), whereas other populations exhibit high susceptibility (Kuester et al. 2015). Although we find variability in resistance across natural populations, it is unclear if this defense trait involves a cost. We investigated this question within one population using artificial selection for increased/decreased resistance and discovered that the seed production of individuals from the increased resistance lines was not significantly lower than that of susceptible lines in the absence of the herbicide, suggesting that there may not be a fecundity cost associated with resistance in this species. However, there was some indication that progeny quality may be lower in resistant individuals—resistant lines exhibited a trend for reduced seed viability compared to susceptible lines (Debban et al. 2015). This finding suggests that trade-offs between fitness enhancing traits (e.g., germination and resistance) may be present within this species, which could manifest as a cost by reducing the overall fitness of resistant compared to susceptible lineages in the absence of herbicide.

Here, we determine if there are trade-offs associated with resistance by examining germination, early root growth, and aboveground growth across 43 populations of *I. purpurea*. We specifically ask the following: (1) are there potential trade-offs associated with resistance across this species' range in the United States manifest in the form of (i) lower germination and/or (ii) smaller size at early life-history stages (i.e., early germinant, young plant)? And (2) do resistant populations exhibit different trade-offs, which may indicate the nature and expression of fitness costs may vary across populations?

Materials and Methods

SEED COLLECTION AND CONTROL OF MATERNAL/ENVIRONMENTAL EFFECTS

Multiple fruits were collected from up to 79 individuals separated by at least 2 m from 43 populations located across the Midwest and Southeastern United States (Table S1; Fig. S1). These seeds (hereafter field-collected seeds) were used in several experiments

to determine resistance, germination, and early growth characteristics. To homogenize the effects of maternal environment on seed quality, we chose a subset of the populations ($N = 18$), grew them in a common greenhouse for one generation and collected the autonomously self-pollinated seeds from a similar growing and mating system environment (hereafter once-selfed seeds).

ESTIMATE OF HERBICIDE RESISTANCE

To determine glyphosate resistance across populations, a dose-response experiment was conducted by planting a single field-collected seed from 10 randomly chosen maternal lines from each population in six glyphosate treatments (including a nonherbicide control treatment) in each of two greenhouse rooms. Full details of the dose-response experiment are presented in Kuester et al. (2015)—for simplicity, we present resistance as the percent survival per population at 1.70 kg a.i./ha of glyphosate, a rate which is slightly higher than the suggested field rate of 1.54 kg a.i./ha. Individual seeds were scarified, planted, allowed to grow for three weeks, and then treated with the herbicide (PowerMax Roundup; Monsanto, St. Louis, MO) using a hand-held CO_2 pressurized sprayer (Spraying Systems Co., Wheaton, IL). Survival was scored three weeks after treatment application, and the population estimate of resistance was determined as the proportion of individuals that survived glyphosate.

GERMINATION

We performed three germination experiments to determine if resistance influenced seed traits. First, we examined germination using field-collected seeds in a Petri-dish assay in the laboratory; second, we examined germination of the field-collected seeds in the soil in the greenhouse; and third, we performed a Petri-dish assay in the laboratory using seeds generated via selfing in the greenhouse (once-selfed seeds) to examine the potential for maternal field environmental effects. For the first experiment using field-collected seeds, we measured seed weight and germination characteristics using field-collected seeds from each population ($N = 43$). Up to five (ave 4.6) seeds from eight to 79 maternal lines per population (ave 38, total 1621, see Table S1 for exact sample sizes per population) were randomly chosen for the germination test. From this pool of seeds, we randomly chose a subset of families per population (eight to 49 maternal lines per population; Table S1) for which the selected seeds were weighed (as a group) to determine the average seed weight. All of the selected seeds were placed in a small Petri dish (one dish per family), submerged in filtered water and allowed to germinate in the laboratory under ambient light and temperature. Water was added as necessary every three days to prevent drying out. Petri dishes were completely randomized across laboratory benches. Germination (the emergence of a normal radicle) was scored periodically until no further germination was recorded, which occurred after 16 days (hereafter referred to as prescarification germination). At this time, seeds that had not imbibed water (by visual determination) were scarified and germination was again scored after one week (hereafter referred to as postscarification germination). We recorded the final number of seeds exhibiting normal germination (including both pre- and postscarification, hereafter referred to as final germination), the number of seeds needing scarification, the number of scarified seeds that germinated, and the number that had abnormal germination. For the second germination assay, we examined germination data from one replicate (housed in a single greenhouse room) of the dose-response experiment mentioned earlier in which seeds were scarified and planted in containers (164 mL volume; Stuewe and Sons, Tangent, OR) with one seed per pot (10 maternal families per population). Germination was scored after three weeks.

For the third and final germination assay, we used seeds from maternal lines that were selfed once in the greenhouse. Two sets of five seeds for up to eight maternal lines (randomly selected) for each of 18 populations were placed in Petri dishes with water. Prescarification germination was scored after 11 days. If seeds had not imbibed water they were scarified and scored again after one week. We recorded the final number of seeds exhibiting normal germination (i.e., final germination), the number of seeds with normal germination prior to scarification (i.e., prescarification germination), the number of seeds that germinated after scarification (i.e., postscarification germination), and the number that had abnormal germination.

EARLY ROOT AND ABOVE-GROUND GROWTH

To examine early root growth, we again used the once-selfed seeds and measured root length four days after the germination assay began. We chose to first scarify the seeds in this assay to standardize water absorption among individuals. Two sets of five seeds for up to eight maternal lines (randomly selected) for each of 18 populations were scarified and placed in Petri dishes with water. Germination was scored after one, four, and seven days to estimate time to 50% germination (see details below). On day 4, Petri dishes were scanned and the root length was measured using Image J (Abramoff et al. 2004) for each germinated seed.

We next examined early growth traits of greenhouse-grown individuals to determine if there was a relationship between resistance and plant size (i.e., are plants from resistant populations smaller?). To do so, we used measurements from plants from the dose-response experiment prior to herbicide application. Three weeks after planting (and prior to spraying), we measured the height (cm) of the stem, the number of leaves, and width (cm) of the largest leaf on each individual planted per treatment per population (total $N = 2908$; Table S1 for exact sample sizes per population).

STATISTICAL ANALYSIS

Field-collected seeds

We assessed the relationship between resistance and progeny quality using mixed-model analyses of variance. We used a generalized linear mixed-effect model to examine final germination, prescarification, abnormal germination, seeds needing scarification, and postscarification germination with resistance and population (random) as predictors using the glmer function in the R package lme4 with a binomial distribution (Bates et al. 2015). All of the binary measures were coded as 1 or 0. Seed weight (g) was modeled using a mixed-model with resistance and population (random) as predictors using the lmer function in the R package lme4. Additionally, previous studies have indicated a geographic pattern of resistance in this species (Kuester et al. 2015). To ensure that the above results were not an artifact of geography, we added latitude and longitude (scaled) of the population in the above models. For the experiment examining germination in soil, we modeled germination with resistance level and population (random) as predictors using a binomial distribution.

Once-selfed seeds

Similar to the field-collected seeds, we used mixed-model binomial regressions to assess the effect of resistance on germination characteristics of the once-selfed, greenhouse-generated seeds. We modeled prescarification, postscarification, and final germination with resistance and population (random) as predictors using a binomial model. To determine if the maternal environment in which the seeds developed influenced germination, in a separate model we compared final germination between maternal environments (i.e., field-collected seeds vs. seeds propagated in the greenhouse) by including maternal environment as a treatment effect in the model on the subset of the populations used in both experiments. To do so, we modeled final germination using treatment, resistance, population (random), and treatment × resistance as predictors using a binomial distribution. An interaction between treatment and resistance would indicate that the maternal environment influences the relationship between resistance and germination.

Early growth and size

We next used mixed-model analyses of variance to determine if more resistant populations exhibited early growth life-history trade-offs. We separately considered root length of the early germinant and plant size. We examined root length using the once-selfed seeds in two different models. The first and more basic model examined the influence of resistance and population (random) on log-transformed root length (cm) four days postgermination. A difference in root length, however, could be due to differences in either growth rate of the radicle or differences due to the timing of germination, that is, when growth began following the beginning of the germination assay. To distinguish between these two potential explanations, we calculated the time to 50% germination as an estimate of germination speed—a shorter time would suggest that seeds began growing sooner after water was added. We used the germination data from days 1, 4, and 7 to obtain a population-level estimate of the time to 50% germination using a Hill function (El-Kassaby et al. 2008). This function decomposes germination into four parameters: a, the germination capacity; b, the steepness of the curve; c, the time to 50% germination; and y_0, the lag time before germination. We used the nonlinear least squares (nls) function in R (R Core Team 2016) to estimate the (b) and (c) parameters. We chose to pool the data on a population-level to increase the accuracy of the estimation. The time to 50% germination (c) was then used as a covariate in the more complex model of root length that included resistance, population (random), and time to 50% germination.

We next examined height, leaf number, and leaf width of plants grown from field-collected seeds (approximately three weeks growth in greenhouse) to determine if resistance incurs early growth life-history trade-offs. We used each trait in separate mixed-models with replicate, rack within replicate (random), resistance, and population (random) as predictors. Residuals of leaf size were not normal, so a Box–Cox transformation ($\lambda = 1.94$) was used to achieve better fit.

Finally, we performed a principal component analysis (PCA) using the population averages of several traits from the field-collected seeds to visually examine the data and determine how populations differed along the two axes retained. The traits included were seed weight, germination percentage, percentage of abnormally germinating seeds, percentage of successfully germinating scarified seeds, early plant height, leaf number, and leaf size. This analysis was performed using PROC FACTOR in SAS (SAS Institute Inc., Cary NC) with a varimax rotation to obtain more easily interpretable axes. Loadings and the proportion variance explained for each factor with an eigenvalue >1 can be found in Table S3.

Results

GERMINATION

We found a strong and significant negative relationship between resistance and the percentage of field-collected seeds that germinated (Fig. 1A). This is true for both prescarification germination ($\beta = -4.93$, $\chi^2_1 = 24.66$ $P < 0.0001$) and final germination (both pre- and postscarification germination; $\beta = -5.20$, $\chi^2_1 = 24.80$, $P < 0.0001$; Fig. 1A). In addition to a decline in germination, several other measures of seed quality also declined with increasing resistance. We found a higher percentage of abnormally germinating seeds ($\beta = 4.24$, $\chi^2_1 = 33.20$, $P < 0.0001$) in that, instead of exhibiting normal germination, a nonviable embryo would be

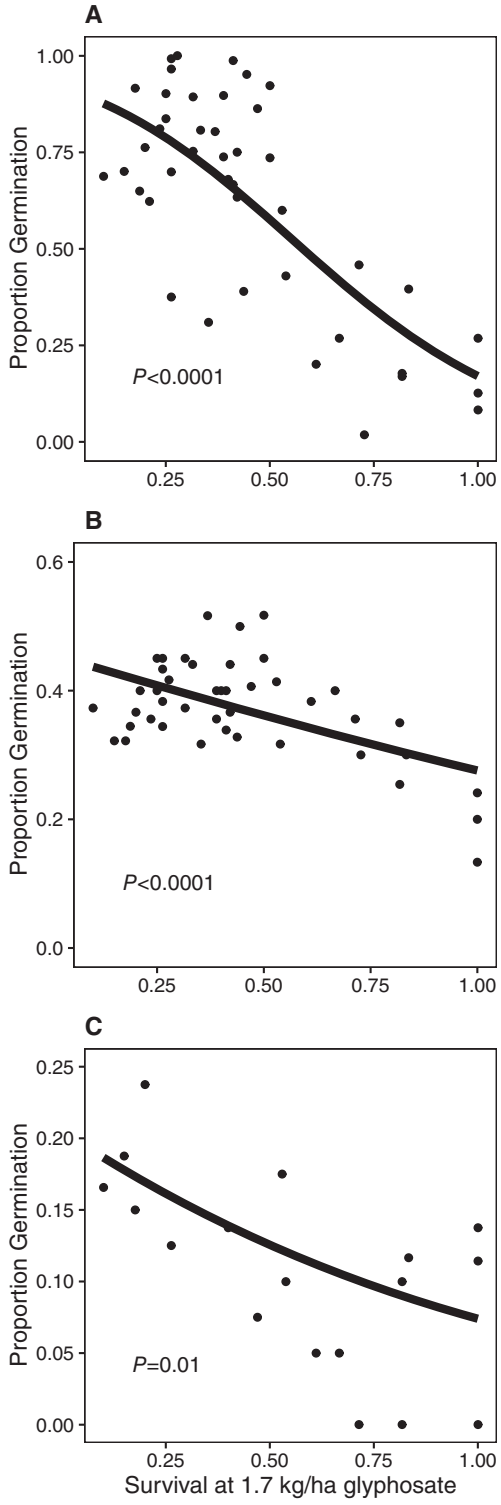

Figure 1. Final germination decreased as population-level herbicide resistance increased for (A) field-collected seeds in Petri dishes, (B) field-collected seeds in soil, and (C) once-selfed seeds (note the differences in the *y*-axis scale) in *I. purpurea*. Points are the mean per population, lines are the average marginal predicted probabilities from the appropriate model, *P* values indicate the significance of the resistance effect (see Methods for model details).

ejected from the seed coat with no further growth. Furthermore, some seeds simply did not imbibe water; we scarified these seeds to determine if they were viable but potentially dormant. As resistance increased across populations, more seeds needed scarification ($\beta = 1.52$, $\chi^2_1 = 5.00$, $P = 0.03$) and fewer of those seeds subsequently germinated ($\beta = -5.50$, $\chi^2_1 = 8.81$, $P = 0.003$). We also found that seed weight decreased as resistance increased ($\beta = -0.005$, $\chi^2_1 = 4.69$, $P = 0.03$), indicating that resistance influenced multiple measures of seed quality for seeds collected from the field. All of these relationships remain significant after accounting for longitude and latitude of the populations except for the percentage needing scarification (Table S2), suggesting that the patterns we find are not due to a simple geographic pattern. We similarly uncovered a negative relationship between germination and resistance when seeds from these populations were planted in soil in the greenhouse ($\beta = -0.79$, $\chi^2_1 = 16.09$, $P < 0.0001$; Fig. 1B).

The negative relationship between resistance and germination was supported by the results from the once-selfed seeds grown in a common environment for a generation (Fig. 1C). Prior to scarification, very few of the greenhouse-grown seeds imbibed water and germinated (2.1%) and there was no effect of resistance ($\beta = -0.70$, $\chi^2_1 = 0.80$, $P = 0.37$). After scarification, however, there was a significant negative relationship between germination and resistance (final germination: $\beta = -1.18$, $\chi^2_1 = 6.42$, $P = 0.01$, Fig. 1C). This effect remained significant after accounting for latitude and longitude (resistance: $\beta = -1.13$, $\chi^2_1 = 5.48$, $P = 0.02$). Interestingly, the decrease in final germination with resistance for once-selfed, greenhouse-generated seeds was significantly less than the field-collected seeds (treatment × resistance: $\beta = 2.14$, $\chi^2_1 = 27.8$, $P < 0.0001$), suggesting that maternal environmental conditions influence the quality of seeds produced. In addition, we found a much lower rate of abnormal germination in the once-selfed seeds (~10%) compared to the field-collected seeds (~40%), and the level of abnormal germination showed no relationship with resistance ($\beta = 0.31$, $\chi^2_1 = 0.23$, $P = 0.63$). These results suggest that, while germination costs are consistently detected between experiments in which the maternal environment differed, field environmental conditions exacerbate the strength of the germination cost.

EARLY ROOT AND ABOVE-GROUND GROWTH

To test whether growth differed with resistance, we scarified the once-selfed seeds and measured germination speed and root growth. There was a much higher germination rate of these seeds (86%) compared to the previous experiment (only 2% germination of unscarified once-selfed seeds) and the majority occurred before day 4. As in the previous experiments, there was a significant decrease in germination with increasing resistance (germination at day 4: $\beta = -1.60$, $\chi^2_1 = 4.48$, $P = 0.03$). Root growth after

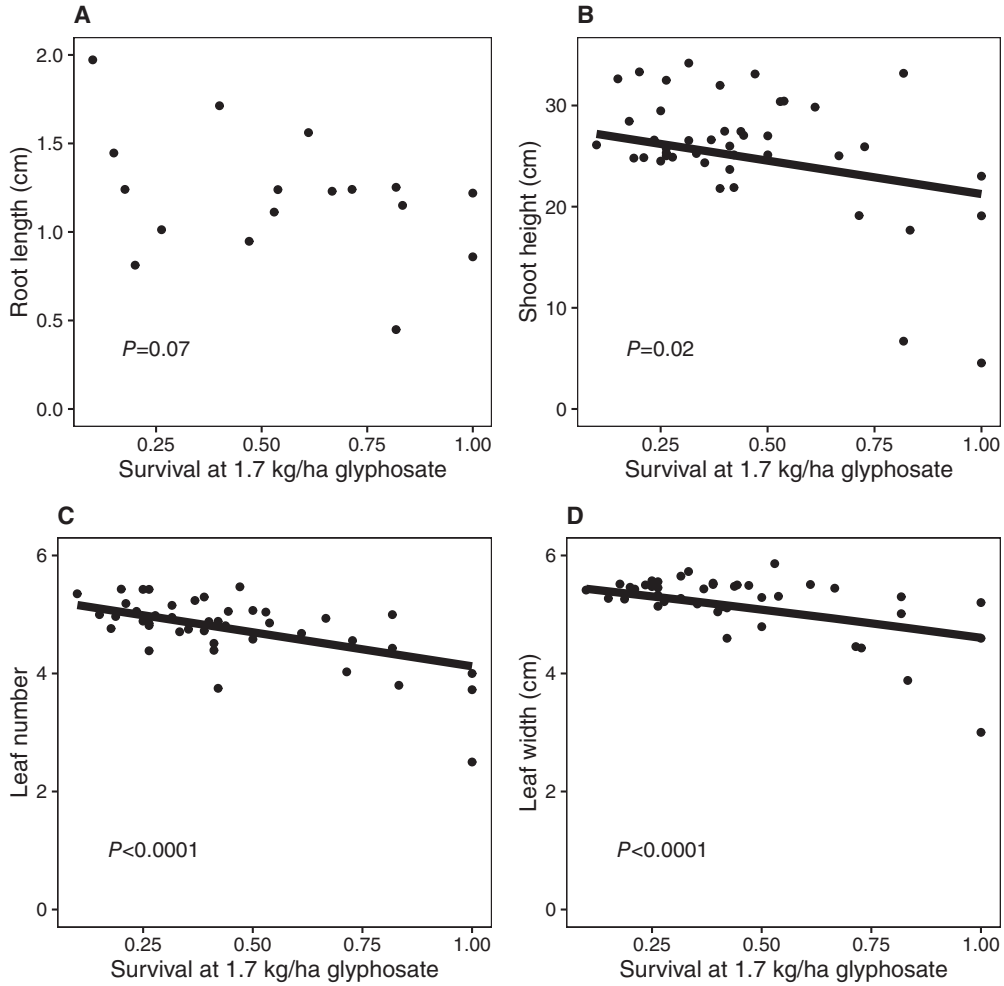

Figure 2. Plant size deceased as population-level herbicide resistance increased for (A) root growth after four days (marginally significant), (B) shoot height, (C) leaf number, and (D) width of the largest leaf after three weeks for *I. purpurea*. Points are the mean per population, lines are the average marginal predicted probabilities from the appropriate model, *P* values indicate the significance of the resistance effect (see Section Methods for model details).

four days showed a nearly significant negative relationship between root length and resistance (log-transformed root length: $\beta = -0.39$, $\chi^2_1 = 3.19$, $P = 0.07$; Fig. 2A). However, including the time to 50% germination in the model removed this effect (resistance: $\beta = -0.07$, $\chi^2_1 = 0.09$, $P = 0.76$; time to 50% germination: $\beta = -0.37$, $\chi^2_1 = 4.85$, $P = 0.03$), suggesting that the difference in root length was due to the timing of germination rather than a difference in growth rate. In fact, there was a nearly significant positive relationship between resistance and time to 50% germination ($b = 0.28$, $r^2 = 0.19$, $t_{15} = 1.90$, $P = 0.077$). A difference in plant size was also found in the three- to four-week-old plants grown in soil from the field-collected seeds—as resistance increased across populations, above-ground structures decreased in size (height: $\beta = -6.61$, $\chi^2_1 = 5.25$, $P = 0.02$; leaf number: $\beta = -1.15$, $\chi^2_1 = 15.68$, $P < 0.0001$; Box–Cox transformed largest leaf width: $\beta = -4.62$, $\chi^2_1 = 16.22$, $P < 0.0001$; Fig. 2B–D).

VISUALIZATION OF COST-RELATED TRAITS

We next examined germination and early growth traits from the original field-collected seeds using a PCA to determine if there was variation among populations in the expression of cost-related traits (full results Table S3). The first three principal components (PCs) explained 77% of the variance, with the first PC loading with the early growth traits, while the second loaded with seed traits and the third with the proportion of seeds that required scarification to successfully germinate. Populations with higher resistance scored lower on PC1 ($b = -0.13$, $r^2 = 0.28$, $t_{41} = -4.03$, $P = 0.0002$) and PC2 ($b = -0.12$, $r^2 = 0.26$, $t_{41} = -3.79$, $P = 0.0005$), but not on PC3 ($b = -0.06$, $r^2 = 0.06$, $t_{41} = -1.61$, $P = 0.12$). Using the first two PCs to plot the results, populations with higher resistance occur mostly in the lower left quadrant (smaller plants, lighter seeds, lower final germination and more abnormally germinating seeds) and have a wider spread than less resistant populations

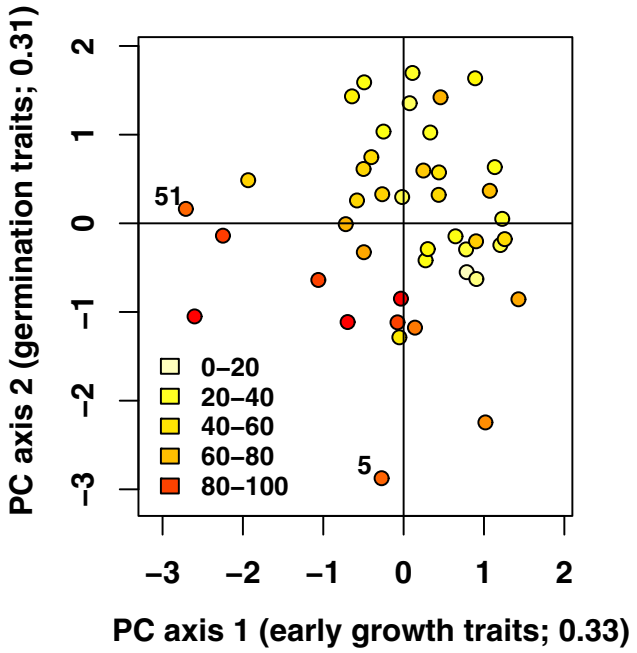

Figure 3. ***Ipomoea purpurea*** **populations with higher herbicide resistance scored lower on PCA axes 1 (growth related traits;** $P = 0.0002$**) and 2 (germination-related traits;** $P = 0.0005$**). Circle color indicates survival at 1.7 kg/ha glyphosate. The proportion variation explained by each axis is noted in axis labels.**

(Fig. 3). Furthermore, while an increase in resistance decreased scores on PC1 and PC2, there was variation among the highly resistant populations; some highly resistant populations exhibited early growth traits that were similar to highly susceptible populations and yet scored very low on germination traits (e.g., pop num 5), whereas other highly resistant populations exhibited similar germination traits compared to the highly susceptible populations, but were smaller in stature than susceptible populations (e.g., pop num 51). Thus, it appears that the type of cost may vary among populations sampled from North America.

Discussion

Here, we show that glyphosate resistant populations of the common morning glory exhibit life-history trade-offs associated with resistance, and, that these trade-offs may vary among populations. Our series of experiments uncovered three notable findings: First, we found a negative linear relationship between germination and resistance, indicating that as resistance increased, germination decreased. This negative relationship persisted when using seeds generated from a common greenhouse environment showing that this result is not due solely to field environmental and/or maternal effects. Second, we found that plant size decreased as resistance increased, indicating that resistance influences early plant growth. Third, we found evidence that the two types of trade-off may differ among populations—using a PCA, we show that some highly resistant populations produce normally sized plants, but score low on germination traits, and vice versa. Below, we detail how these results add further strength to the suggestion that a variety of life stages and populations sampled across the species' range should be assessed when testing the hypothesis that resistance incurs a fitness cost (Délye et al. 2013a).

FITNESS COSTS: SEED GERMINATION AND EARLY PLANT SIZE

It is difficult to determine how common germination differences associated with resistance may be among weeds because many studies focus on seed quantity rather than seed quality. There is some indication that germination may be affected in other glyphosate resistant species. Dinelli et al. (2013) found reduced germination of glyphosate resistant *Ambrosia trifida* populations, while Ismail et al. (2002) found greater germination of resistant biotypes of goosegrass (*Eleusine indica*). More broadly, life-history trade-offs may be specific to the herbicide and/or species in question or the type of mutation conferring resistance (O'Donovan et al. 1999; Vila-Aiub et al. 2005; Délye et al. 2013b). For example, only one of two different resistance mutations in ACCase resistant *Lolium rigidum* had more stringent germination requirements (seeds germinated poorly in the dark and required fluctuating temperatures to break dormancy) than the susceptible genotype (Vila-Aiub et al. 2005). Similarly, Délye et al. (2013b) found differential effects on germination among resistance mutations to ACCase in *Alopecurus myosuroides*. Both of these studies report that the resistance mutation led to delayed germination. Such a delay in germination may affect fitness, especially in agricultural settings where germinating too early can lead to removal by pre-sowing practices and germinating too late can lead to intensified competition with already established plants (Weaver and Cavers 1979; Barrett 1983; Mortimer 1997; Forcella et al. 2000; Owen et al. 2014). Our analysis of root growth suggests that differences in plant size in *I. purpurea* may be due to a similar delay in germination in resistant populations.

The decrease in growth with increasing resistance that we uncovered could lead to decreased competitive ability and subsequent lower fitness in the presence of competition if, as has been found in other herbicide resistant weeds, the difference in growth persists to adult plants (Weaver and Warwick 1982; Ahrens and Stoller 1983; Holt 1988; Alcocer-Ruthling et al. 1992; Williams et al. 1995; Vila-Aiub et al. 2005, 2009a; Tardif et al. 2006). This type of life-history trade-off, which ultimately may manifest as a fitness cost, is also likely to be species, mutation, and environment specific. For example, *L. rigidum* has evolved herbicide resistance via a variety of mutations ranging from target site (Christopher et al. 1992; Yu et al. 2008) to nontarget site (Christopher et al. 1991, 1994; Preston and Powles 1998). Target site

mutations in the acetohydroxyacid synthase gene result in little cost in growth (Yu et al. 2010). On the other hand, herbicide resistance mediated by the cytochrome P450 complex resulted in reduced biomass and decreased competitive ability (Vila-Aiub et al. 2009a).

An alternative explanation for the decline in germination and growth we identify using the field-collected seeds is that some other covarying population characteristic such as soil fertility, spraying regime, herbivore levels, or the many other biotic and abiotic factors that can influence seed development differed among resistant and susceptible populations (Roach and Wulff 1987; Fenner 1991; Schmitt et al. 1992; Platenkamp and Shaw 1993; Galloway 2001). These differences may explain the stronger decline in final germination in the field-collected seeds compared to the once-selfed seeds. Several lines of evidence, however, suggest the relationship between resistance and germination that we uncovered represents a true fitness cost rather than simply an effect of the environment (e.g., driven by maternal effects, latitude, spray environment). First, the relationship between resistance and germination appears approximately linear, which is expected as the frequency of resistance individuals increases. If the trade-offs identified herein were due simply to glyphosate exposure, with resistant populations exhibiting abnormal seed development after surviving glyphosate application, we would expect to see a binary distribution of seed quality of populations that had been sprayed and those that had not, rather than a linear trend with resistance. Second, the negative relationship between resistance and germination is maintained after a generation in a common greenhouse environment—an effect that should disappear if the decrease in fitness was due to glyphosate exposure in the field. Finally, our results parallel those from a recent experiment that specifically controlled for genetic background and environmental effects using *I. purpurea* plants from a single population (Debban et al. 2015). Individuals from this population were artificially selected for increased or decreased resistance for three generations under controlled greenhouse conditions, and, similar to results presented here, the increased resistance lines had a larger percentage of "bad" seeds that ejected the embryo. That the results from one population utilizing a controlled genetic background are mirrored across many populations collected from the landscape provide strong evidence that lower germination represents a fitness cost of glyphosate resistance in this weed species.

Although we detected lower final germination in populations with higher herbicide resistance across multiple experiments suggesting a true trade-off, we also found differences between experiments in the strength of the relationship. This suggests that both an underlying genetic basis and an environmental component influence the expression of the trade-off. Compared to the field-collected seeds, the once-selfed seeds had a much lower germination rate prior to scarification (44% vs. 2% for the same set of populations), suggesting that the seed coat was perhaps more pristine in seeds generated in the greenhouse. However, if scarified prior to the assay (e.g., the root growth experiment), we found that the once-selfed seeds exhibited high germination. The physical seed coat is the primary mechanism of dormancy in this species (Brechu-Franco et al. 2000); thus, environmentally induced physical differences in the seed coat (e.g., thickness or waxiness) or its degree of degradation (e.g., mechanical disruption or seed storage differences) likely influences germination timing. Although it is clear that the environment influences seed germination in this species, we consistently observed a decline in germination with resistance across multiple experiments suggesting that there is an underlying genetic basis to the cost of resistance.

Another striking difference between experiments was in the frequency of abnormal seeds produced. The once-selfed seeds had almost no abnormal germination (i.e., no dead embryos that were ejected from the seed coat), whereas some of the field-collected populations had a high level of abnormal germination. In fact, the strong decline in final germination for field-collected seeds was due primarily to this abnormal germination. Abnormal germination could be due to a variety of environmental causes (e.g., herbicide application, nutrient availability, competition, etc.) or be a cost of resistance that is only induced under field conditions. Our results suggest that in a benign environment, such as the greenhouse, the seeds in general are of high quality (high germination, fewer abnormal germinants), but there is a cost of resistance that increases the time it takes to germinate (based on the root growth experiment), possibly leading to smaller plants at any given point. On the other hand, under field conditions, populations with higher resistance produce more abnormal seeds (due to either environmental differences or an environmentally induced cost of resistance). Further experiments investigating whether field-collected seeds also have a delayed germination cost (as seen in the once-selfed seeds) and measuring the consequences of this delay under field conditions are needed to better understand the interplay between the environment, resistance, and seed quality.

Interestingly, by visually examining the germination and growth traits in the PCA, we find variation in the type of potential cost among high-resistance populations. Although some highly resistant populations fell more into the "poor germination" axis, other highly resistant populations fell more into the "poor growth" axis. There are at least three possible reasons for this difference: different resistance genes, different compensatory mutations, or different genetic backgrounds. First, the gene(s) involved in resistance may vary among populations leading to different costs. Independent origins of resistance to herbicide have been found in other species (Délye et al. 2010) and these different mutations often incur different fitness costs (Vila-Aiub et al. 2005; Délye et al. 2013b). Second, the resistance gene(s)

may be the same among populations, but each population may have different compensatory mutations that lead to different costs (Darmency et al. 2015). Third, the resistance gene(s) may behave differently in different genetic backgrounds (Paris et al. 2008). These distinctions are important because they would differentially affect the evolutionary trajectory of herbicide resistance. For example, if populations differ in the gene(s) involved, each population may have a very different set of costs, benefits, and evolutionary trajectories, which would need to be incorporated in models.

It is currently unknown if the trait trade-offs identified here are pleiotropic or due to linkage to the resistance gene. The most restrictive definition of a cost requires that the decrease in fitness is due to the resistance allele itself (Bergelson and Purrington 1996). Given that we do not know the identity of the loci involved in either resistance or the abnormal germination and reduced growth, we cannot entirely rule out physical linkage between resistance genes and cost genes in which case the "cost" could quickly become unlinked over generations (Lewontin 1974; Hartl and Clark 1989). For some species, easily identifiable mutations in the enzyme targeted by the herbicide can be linked to resistance, that is, target site resistance (TSR). However, preliminary work suggests that glyphosate resistance in *I. purpurea* is due to nontarget site mechanism (NTSR; T. Leslie and R. S. Baucom, unpubl. data), and as such elucidating the genetic basis of both resistance and the cost will be a nontrivial endeavor. Furthermore, it is rare that genes underlying costs are identified; most documented cases of the genes involved in the cost of resistance is when TSR mutations lead to poor performance of the enzyme on its natural substrate (Vila-Aiub et al. 2009b). As far as we are aware, no study has identified the genes involved in the cost of resistance when the mechanism of resistance is NTSR. One intriguing possibility for this species stems from a previous study that compared transcript expression levels of artificially selected lines of resistant and susceptible *I. purpurea* plants following herbicide application (Leslie and Baucom 2014). One of the differences between the replicated resistant and susceptible lines was a lower expression of pectin methylesterase (PME) in the resistant plants. This enzyme has been shown to play a role in breaking seed dormancy (Ren and Kermode 2000) and stem elongation (Pilling et al. 2000). Thus, the decreased expression of PME in resistant plants may explain both the reduced germination and growth in populations with higher resistance.

HOW MIGHT LIFE-HISTORY TRADE-OFFS INFLUENCE THE EVOLUTIONARY TRAJECTORY OF RESISTANCE IN THIS SPECIES?

The evolutionary trajectory of resistance should depend on the magnitude of the benefit of resistance in the presence of spraying and the magnitude of the cost of resistance in the absence of herbicide as well as the spatial/temporal frequency of spraying. Recent work in this system has shown that populations of the common morning glory sampled from 2012 exhibit higher levels of resistance compared to the same populations sampled in 2003 (Kuester et al., unpubl. ms.). Interestingly, however, the difference in resistance between sampling years was only slight (62% survival at 1.7 kg a.i./ha in 2012 vs. 57% survival in 2003 samples). It is possible that the life-history differences that we identified here are responsible, at least in part, for maintaining this intermediate level of resistance between sampling years. For example, the lower germination of resistant types would manifest as a fitness cost if resistant and susceptible individuals produce approximately the same number of total seeds (or if $R < S$); if, however, resistant types produce enough viable seed to offset the lowered germination, then overall fitness would not be impacted and resistant individuals would not be at a relative disadvantage. Although we have not examined seed production across all 43 populations examined herein, a common garden study of glyphosate susceptible and resistant families from a single population of this species found there was no difference in total seed production of resistant compared to susceptible lines (Debban et al. 2015), indicating there is no cost of resistance in terms of seed quantity. That we similarly find poor germination between these experiments and those using genetic lines developed from one population that strongly supports the finding that germination quality is a true fitness cost of glyphosate resistance in this species. Further, the differences in growth that we have detected between resistant and susceptible populations could potentially manifest as a fitness cost when seedlings are in competition, an effect which remains to be tested in this system.

In summary, we found reductions in seed quality across replicated herbicide resistant populations of the common morning glory. Although most studies use seed quantity as a proxy for fitness, our results highlight that reductions in progeny quality are an equally, if not more, important cost of adaptation in *I. purpurea*. Given that fitness costs are thought to arise from a variety of mechanisms (allocation of resources, ecological costs, etc.), our results suggest that a high priority should be placed on the examination of multiple stages of the life cycle when assessing potential costs and not just seed quantity. Furthermore, because the strength of this cost could maintain the efficacy of a globally important herbicide, this work illustrates the utility and importance of integrating evolutionary principles into management scenarios (Gould 1995).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. *Ipomoea purpurea* population characteristics, including population number, state, crop type, latitude and longitude, and sample sizes for experiments with field-collected seeds (N_{fam} germ = number of families in the germination assay for field-collected seeds; N_{seeds} germ = number of seeds in the germination assay for field-collected seeds; N early growth = number of seeds in the early growth experiment, N_{fam} once-selfed = number of families in the germination assays for the once-selfed seeds).

Table S2. Regression results between resistance and seed quality traits, accounting for geography (latitude and longitude) for field-collected seeds of *Ipomoea purpurea*.

Table S3. PCA loadings and variance explained.

Fig S1. Map of the eastern United States showing *Ipomoea purpurea* population locations and their survival at 1.7 kg a.i./ha (color of circle).