Title: Reduced seed viability and reductions in plant size provide evidence for costs of glyphosate resistance in an agricultural weed

Short title: Fitness cost of herbicide resistance

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Summary

• Although fitness costs of adaptation to herbicide are widely expected, they are not universally detected, calling into question their generality. This lack of identified costs could be due to experimental constraints such as a limited focus on measures of plant fecundity and low replication.

• Here we examine the potential for a cost of herbicide resistance on seed germination, root growth, and above-ground growth using 43 naturally occurring populations of *Ipomoea purpurea* that vary in their resistance to RoundUp®, the most commonly used herbicide worldwide.

• We show substantial costs in all three traits. Highly resistant populations had lower germination rates, slower root growth and slower above-ground growth. A visual exploration of the data indicated that the type of cost may differ and even trade off among populations.

• We place our findings into a broad context using a simple model to show that the strength of the germination cost could act to slow the rate of resistance evolution in this species. Our results demonstrate that costs of adaptation may be present at stages other than the production of progeny. Additionally, the cumulative effect of costs at multiple life cycle stages can result in severe consequences to fitness when adapting to novel environments.

Keywords: cost of herbicide resistance, fitness cost, glyphosate, *Ipomoea purpurea*, trade-offs

Introduction

The study of adaptation to xenobiotics (chemicals such as pesticides and herbicides) can provide unique opportunities to examine the evolutionary process: we know when selection began, the strength of selection, and often its frequency. Researchers have uncovered broad insights about the process of adaptation from such studies—for example, information on the nature of genetic variation underlying resistance traits (Preston & Powles, 2002; Hartley *et al.*, 2006), the effect size of alleles (Macnair, 1991; Daborn *et al.*, 2002), and the repeatability of evolution (Andreev *et al.*, 1999; Menchari *et
Many investigations of herbicide and pesticide resistance have also examined the central idea that novel traits incur costs—e.g., the expectation of lower fitness of resistant (R) compared to susceptible (S) genotypes in the absence of the xenobiotic (reviewed in Vila-Aiub et al., 2011). Fitness costs of resistance are hypothesized to arise from increased investment to defense/resistance and a subsequent reduction in allocation to other traits (Coley et al., 1985; Herms & Mattson, 1992), and/or from mutations that decrease the efficiency of proteins on their native substrate (Fisher, 1958; Lande, 1983). Costs are universally expected since genetic variation is often found to underlie resistance traits in nature (Baucom & Mauricio, 2004; Menchari et al., 2006; Délye et al., 2010; Kuester et al., 2015); if there were no costs, resistance would increase to fixation and every individual would be highly defended (Rausher & Simms, 1989).

Despite our expectations, however, reviews of the literature consistently show that costs are not ubiquitous across herbicide resistant weeds (Bergelson & Purrington, 1996; Gemmill & Read, 1998; Vila-Aiub et al., 2009b). Three main ideas have been proposed to explain their absence. First, there are a diverse number of potential mechanisms responsible for resistance, and such mechanisms vary according to the particular herbicide and weed species in question (Powles & Yu, 2010; Vogwill et al., 2012). It is possible that some mechanisms—such as those that incur resistance to a range of different herbicides—are more likely to impose costs compared to others—i.e., a single gene nucleotide substitution that does not alter the efficiency of translated proteins (e.g. Yu et al., 2007; Yu et al., 2010). Second, costs may not be detected if the genetic background is not properly controlled (Bergelson & Purrington, 1996; Vila-Aiub et al., 2009b; Vila-Aiub et al., 2011). Control of the genetic background, either by performing crosses between R and S plants to homogenize the genetic background (Baucom & Mauricio, 2004; Menchari et al., 2008; Giacomini et al., 2014) or ensuring replication of multiple genetic backgrounds (Cousens et al., 1997; Strauss et al., 2002) increases the likelihood that a cost will be detected (Bergelson & Purrington, 1996). Third, researchers often examine only a portion of the life cycle (i.e., seed production or fecundity) and do so in artificial and/or non-competitive conditions (Vila-Aiub et al., 2009b; Vila-Aiub et al., 2011). Studies that examine a range of traits are more likely to identify potential
growth and/or fitness differences between R and S genotypes compared to those that focus solely on measures of fecundity (Vila-Aiub et al., 2009b).

Despite recommendations to control/increase the number of genetic backgrounds (Bergelson & Purrington, 1996), and to examine multiple life history stages when determining if resistance incurs a cost (Primack & Kang, 1989; Vila-Aiub et al., 2009b), a recent review shows that only 25% of herbicide resistance studies control for background effects and 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 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; Christopher et al., 1992; Christopher et al., 1994; Preston & 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 (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 in the life history of a plant costs might be apparent, and further, how ubiquitous costs 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 US agriculture (Webster & MacDonald, 2001), provides an excellent system to examine the strength and type of costs that may be present in natural populations. This species exhibits variability in resistance to glyphosate (Baucom & 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 &
Stoltenberg, 2015; Goh et al., 2015). *I. purpurea* has long been considered to exhibit low-
level resistance to glyphosate (considered ‘tolerance’ in Culpepper (2006)), and
previously we have shown that this low-level resistance has an additive genetic basis and
is under positive selection in the presence of the herbicide (Baucom & Mauricio, 2008).
More recently, a replicated dose-response experiment 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).
Strikingly, the fecundity of individuals from a single population artificially selected for
increased resistance was not significantly lower than susceptible individuals in the
absence of the herbicide, suggesting there may not be a fitness 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., *In press*). These results suggest
that reductions in progeny quality, rather than seed quantity, might be the mechanism of
fitness costs associated with glyphosate resistance in this species.

Here we determine if there is a cost to resistance in progeny quality measured at
multiple stages of the life cycle and the prevalence of this cost across populations by
examining germination, early root growth and above-ground growth across 43
populations of *I. purpurea*. We specifically ask the following: (i) is germination
correlated to resistance across this species’ range in the US?, (ii) do resistant populations
exhibit smaller size at early life history stages (*i.e.,* early germinant, young plant)?, and
(iii) do resistant populations exhibit the same type of fitness cost? Finally, we place our
findings of a strong fitness cost associated with germination (see Results) into a broad
context by using a simple model to examine the potential evolutionary trajectory of
resistance in this species.

**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 US (Table 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 non-herbicide 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 until approximately 13 cm in height, and then treated with the herbicide using a hand-held CO₂ 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

To determine the effects of resistance on seed traits, we measured seed weight and germination characteristics using field-collected seeds from each population (N=43). Up to five (ave 4.6) seeds from 8-79 maternal lines per population (ave 38, total 1621, see Table S1 for exact sample sizes per population) were haphazardly chosen for the germination test. From this pool of seeds we randomly chose a subset of families per population (8-49 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) with water and allowed to germinate in the lab under ambient light. Petri dishes were completely randomized across
lab benches. Germination was scored after 16 days, with successful germination considered the emergence of a normal radicle. At this time, seeds that had not imbibed water were scarified and germination was again scored after 1 week. We calculated the percentage of seeds exhibiting normal germination, the percentage of seeds needing scarification, the percentage of scarified seeds that germinated, and the percentage that had abnormal germination. In addition, to compare results between this petri dish assay and germination in soil, we examined germination data from one replicate of the dose-response experiment mentioned earlier in which seeds were scarified and planted in conetainers. Germination was scored after three weeks and used to calculate the percentage of seeds that germinated.

We next repeated the germination assay experiment using seeds from maternal lines that were selfed once in the greenhouse to examine the potential that germination rates were influenced by the environment. Two sets of five seeds for up to 8 maternal lines (randomly selected) for each of 18 populations were placed in petri dishes with water. Germination was scored after 11 days. If seeds had not imbibed water they were scarified and scored again in one week. We calculated the percentage of seeds with normal germination prior to scarification, the percentage of seeds that germinated after scarification, and the percentage that had abnormal germination.

Early root and above-ground growth

We carried out another assay to measure early root growth by placing scarified once-selfed seeds in water and measured root growth after four days. We chose to first scarify the seeds in this assay to standardize water absorption among individuals. Two sets of five seeds for up to 8 maternal lines (randomly selected) for each of 18 populations were scarified and placed in petri dishes with water. Germination was scored after 4 days and petri dishes were scanned. For each germinated seed the root length was measured using Image J (Abramoff et al., 2004).

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. Approximately three weeks after planting (and prior to spraying) we measured the height of the stem, the number of leaves and length of the largest leaf on each individual planted per treatment per population (total N=2908, Table S1 for exact sample sizes per population). Means for each trait per population were obtained by calculating the LSMeans from models with replicate, population, and planting tray within replicate as predictors using PROC GENMOD (SAS v9.1), using the distribution resulting in normal residuals and the lowest AIC value (normal for leaf number and leaf length; negative binomial for height).

Statistical analysis
To determine if there was a relationship between resistance and progeny quality, the population mean of each trait averaged across maternal lines was used as the dependent variable in individual regressions with the population level of resistance using PROC REG in SAS (v9.1); residuals were examined to confirm normality. For the field-collected seed germination assay the traits analyzed were average seed weight, percent germination, percent abnormal germination, percent needing scarification and the percent germination for scarified seeds. Visual examination of the regression between percent germination of field-collected seeds and resistance suggested a different pattern between resistant and susceptible populations. Therefore we applied separate regressions for susceptible (defined as <50% survival) and resistant populations (>50% survival).

Additionally, previous studies had indicated there was 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 included resistance as well as latitude and longitude of the population in the above regressions. Similar to the field-collected seeds, the population means for the once-selfed seeds were used in separate regressions between population-level resistance and percent germination prior to scarification or percent germination after scarification.

To examine early plant size traits (root length of once-selfed seeds and height, leaf number and leaf size of field-collected seeds), we used population means in a regression with population-level resistance.
Finally, we performed a Principle Components Analysis (PCA) using the population averages of several traits from the field-collected seeds, which included seed weight, germination percentage, percentage of abnormally germinating seeds, percentage of successfully germinating scarified seeds, early plant height, leaf number and leaf size to determine if different populations had different suites of costs—i.e., a difference in germination costs or differences in early plant size. This analysis was performed using PROC FACTOR in SAS 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 correlation between resistance and the percentage of field-collected seeds that germinated \(b = -0.87, r^2 = 0.54, t_{41} = -6.94, P < 0.0001; \) Fig. 1a); increasing resistance by 1% decreased germination by 0.87%. When we grouped populations into resistant (R) and susceptible (S) populations based on greater or less than 50% survival after glyphosate application, respectively, we found that, on average, R populations exhibit ~35% germination compared to ~76% germination among S populations. Interestingly, the negative trend between germination and resistance is present among R populations but not among S populations (Fig. 1b,c). Thus, the fitness cost in this species is apparent only above a certain resistance threshold. We also uncovered a negative relationship between germination and resistance when seeds from these populations were planted in soil in the greenhouse \(b = -0.18, r^2 = 0.31, t_{41} = -4.24, P = 0.0001\), showing that decreased germination associated with increased resistance is consistent across experimental conditions. 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 \(b = 0.88, r^2 = 0.59, t_{41} = 7.59, P < 0.0001\) in that, instead of exhibiting normal germination, a non-viable embryo would be 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.
Populations with greater resistance had more seeds that needed scarification ($b = 0.16$, $r^2 = 0.14$, $t_{41} = 2.58$, $P = 0.01$), of which fewer seeds that subsequently germinated ($b = -0.52$, $r^2 = 0.17$, $t_{38} = -2.76$, $P = 0.009$). We also found that populations with higher resistance produced lighter seeds ($b = -0.006$, $r^2 = 0.11$, $t_{41} = -2.26$, $P = 0.03$), indicating that resistance influenced multiple measures of seed quality.

Using multiple regression analysis, we confirmed a geographic pattern in resistance, namely a decline in resistance with increasing latitude ($b = -0.033$, $t_{40} = -1.99$, $P = 0.05$) but not longitude ($b = -0.02$, $t_{40} = -1.72$, $P = 0.09$). However, resistance remained significant for all traits after accounting for the effect of geography except for the proportion of seeds needing scarification (Table S2). This suggests that the patterns we find between seed quality and resistance are not entirely due to geography.

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. Very few of the greenhouse grown seeds germinated prior to scarification (2.0%) and there was no effect of resistance ($b = -0.006$, $r^2 = 0.01$, $t_{17} = -0.324$, $P = 0.75$). However, after scarification there was a negative relationship between germination and resistance ($b = -0.13$, $r^2 = 0.37$, $t_{17} = -3.08$, $P = 0.007$, Fig. 3a) as was found in the field-collected seeds. As opposed to the field-collected seeds, there was a very low rate of abnormal germination (~10%) and the level of abnormal germination showed no relationship with resistance ($b = 0.02$, $r^2 = 0.01$, $t_{16} = 0.318$, $P = 0.75$).

**Early root and aboveground growth**

There was a significant negative correlation between root length and resistance ($b = -0.02$, $r^2 = 0.01$, $t_{17} = -2.63$, $P = 0.009$; Fig. 3b), indicating that the more resistant populations have either smaller or slower growing roots. Furthermore, plants from more resistant populations had smaller above-ground structures on average than plants from less resistant populations (height: $b = -3.41$, $r^2 = 0.25$, $t_{41} = -3.66$, $P = 0.0007$; leaf number: $b = -0.80$, $r^2 = 0.27$, $t_{41} = -3.89$, $P = 0.0004$; largest leaf width: $b = -1.43$, $r^2 = 0.45$, $t_{41} = -4.92$, $P < 0.0001$). Specifically, plants from resistant populations (i.e., populations that exhibited >50% survival post-spray) were ~10% shorter, and had ~7% fewer and ~13% smaller leaves.
Visualization of cost-related traits

We next examined germination and early growth traits from the original field-collected seeds using a principle components analysis (PCA) to determine if there was variation among populations in the expression of cost-related traits (full results Table S3). The first 3 factors of the PCA explained 77% of the variance, with the first factor 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 factor 1 \( (b = -2.22, r^2 = 0.28, t_{41} = -4.03, P = 0.0002) \) and factor 2 \( (b = -2.12, r^2 = 0.26, t_{41} = -3.79, P = 0.0005) \), but not on factor 3 \( (b = -1.02, r^2 = 0.06, t_{41} = -1.61, P = 0.12) \). Using the first two factors to plot the results, resistant populations occur mostly in the lower left quadrant (smaller plants, lighter seeds, lower germination and more abnormally germinating seeds) and have a wider spread than less resistant populations (Fig. 3). Furthermore, while R populations scored lower on factors 1 and 2 in comparison to S populations, some R populations exhibited early growth traits that were similar to S populations and yet scored very low on germination traits (e.g., pop num 5) whereas other R populations exhibited similar germination traits compared to the S populations, but were smaller in stature than S 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 costs of herbicide resistance, and, that the type of cost may vary among populations. Our series of experiments uncovered three notable findings: First, we found a strong negative linear correlation between germination and resistance indicating that resistant populations have a lower germination rate than susceptible populations. We uncovered similar trends using seeds generated from a common greenhouse environment showing that this result is not due simply to field environmental and/or maternal effects. Second, we found that individuals from resistant populations were smaller than individuals from susceptible populations, indicating that resistance may incur a cost in terms of plant growth. Interestingly, we also found evidence that the two types of cost
may differ among populations—using PCA, we show that some R populations produce normally sized plants, but score low on germination rate, and vice versa—some R populations germinate at a rate similar to S populations, but tend to have smaller individuals than S populations. 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). We then place our results into a broader context using a simple model to examine how the cost identified in this work may influence the evolutionary trajectory of resistance in this species.

Fitness costs: seed germination and early plant size

It is difficult to ascertain the frequency of germination costs associated with herbicide resistance in nature since many studies of resistant weeds 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*). Costs may be specific to the species 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 (germinated poorly in the dark and required fluctuating temperatures to break seed 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 intensified competition with already established plants (Weaver & Cavers, 1979; Barrett, 1983; Mortimer, 1997; Forcella et al., 2000; Owen et al., 2014).

While delays in germination may explain some of the results we present, in general, the low germination we uncovered in the R populations was due to non-viable
embryos (53% of R seeds vs 18% of S seeds) rather than later germination. In the normal progression of seed germination in this species, the radicle first emerges from the seed coat and the cotyledons rapidly follow. In comparison, the non-viable seeds swell, split in half, and eject the entire embryo from the seed coat. Many of these seeds appear fully provisioned, and, although they weigh less than seeds from S populations, the differences in weight are not drastic, nor are there visible indications that would suggest that seeds from R populations would be of such low quality.

The reduced growth of R compared to S populations that we uncovered could lead to decreased competitive ability and subsequent lower fitness in the presence of competition, as has been found in other herbicide resistant species (Weaver & Warwick, 1982; Ahrens & Stoller, 1983; Holt, 1988; Alcocer-Ruthling et al., 1992; Williams et al., 1995; Vila-Aiub et al., 2005; Tardif et al., 2006; Vila-Aiub et al., 2009a). However, similar to studies of germination differences, results from studies comparing growth of resistant and susceptible genotypes suggest this cost is species, mutation and environment specific. For example, Lolium rigidum has evolved herbicide resistance via a variety of mutations ranging from target site (Christopher et al., 1992; Yu et al., 2008) to non-target site (Christopher et al., 1991; Christopher et al., 1994; Preston & 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 co-varying 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 R and S populations (Roach & Wulff, 1987; Fenner, 1991; Schmitt et al., 1992; Platenkamp & Shaw, 1993; Galloway, 2001). However, several lines of evidence 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, we identified a very strong linear correlation between resistance and germination. If the fitness cost was 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 correlation between germination and resistance is present only among resistant populations—strongly suggesting that as populations become more resistant, they exhibit a correspondingly higher fitness penalty when in the absence of the herbicide. Third, the negative relationship is maintained after a generation in a common growing/mating system environment. Finally, our results parallel those from the recent experiment that specifically controlled for genetic background and environmental effects using *I. purpurea* plants from a single population (Debban *et al.*, In press). 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 provides strong evidence that a fitness cost of glyphosate resistance is present in this weed species.

Interestingly, by visually examining the germination and growth traits in a PCA, we find variation in the type of cost trait among R populations. While some R populations fell into the “poor germination” axis, other R populations fell into the “poor growth” axis compared to S populations. 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 amongst 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 costs 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 - either the actual allele or through it acting pleiotropically (Bergelson & 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 & Clark, 1989). For some species, easily identifiable mutations in the enzyme targeted by the herbicide can be linked to resistance, i.e. target site resistance (TSR). However, preliminary work suggests that glyphosate resistance in *I. purpurea* is due to non-target site mechanism (NTSR; Leslie and Baucom, *unpublished data*), and as such elucidating the genetic basis of both resistance and the cost will be a non-trivial 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 & Baucom, 2014). One of the differences between the replicated R and S 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 & 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 costs influence the evolutionary trajectory of resistance in this species?*

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, *In Review*). Interestingly, however, the difference in resistance between sampling years was only slight, i.e., 62% survival at 1.7 kg ai/ha in 2012 vs 57% survival in 2003 samples. It is very likely that the fitness cost we identified here is responsible for maintaining resistance between sampling years. In light of this finding, we elected to model the influence of the germination cost on the evolution of resistance in this species, making simplifying assumptions about the genetic basis of resistance and pattern of mating in this species. We used a single locus Hardy-Weinberg model of a panmictic population with the resistance allele dominant to the susceptible allele. We examined the frequency of a resistance allele as a function of the fitness cost and the proportion of the population not under selection (*i.e.* not sprayed). We ran the model using one of three costs – no cost, a moderate cost, or a high cost to demonstrate the differing evolutionary trajectories given different costs. On one extreme, we show that the frequency of a resistance allele without a cost increases relative to the proportion of the population under selection (Fig. 4a). On the other extreme, if a resistance mutation leads to a strong cost—similar to that of the germination cost identified here—the frequency of the resistance allele can decrease when enough of the population is free from selection, *i.e.*, not sprayed with herbicide (Fig. 4b). Populations or species with high costs will have a slower rate of increase of the resistant allele compared to those that exhibit a moderate cost (Fig. 4c) (*such as found when examining the negative repercussions of slower growth on competitiveness of resistant plants (Vila-Aiub et al., 2009a)*).

This simple model can also demonstrate the importance of accurate estimations of costs when making management decisions. For example, we can use a “back of the envelope” calculation to examine the threshold proportion of selection-free land that will result in a decrease in resistance for a given cost of resistance. We estimated the threshold proportion of non-treated land as \((1-C_R/(C_R+C_S))\), or one minus the cost of resistance divided by the sum of the cost of susceptibility (*i.e.* herbicide efficiency) and the cost of resistance. From the cost obtained in this study using the field-collected seeds (and assuming complete control of susceptible individuals when spraying), this proportion is 0.54, *i.e.* more than 54% of acreage would need to be non-treated to decrease the frequency of the resistant allele. With no fitness cost, 100% of acreage would need to be
non-treated. Clearly, estimates of fitness costs are necessary to decide which management
decisions will be both feasible and effective. Additionally, if populations differ in their
costs, these management decisions will likely differ by population. Although the
calculation we present here is consistent with expectations of a simple model of allele
frequency change (Jasieniuk & Maxwell, 1994), we caution that our estimates do not
include important dynamics such as the influence of population subdivision, selfing rates,
and the contribution of migrants from the seed bank (among other biologically relevant
forces).

In summary, we found fitness costs in seed quality across replicated populations
of the common morning glory and demonstrate that these costs can play a substantial role
in herbicide resistance evolution. 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. 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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07191 to RSB and SMC.
Figure Legends

Fig 1. Regression between survival at 1.7 kg a.i./ha glyphosate and germination rate of field-collected seeds across all populations (a), in susceptible populations (b) and resistant populations (c).
Fig 2. Regression between survival at 1.7 kg a.i./ha glyphosate and germination rate (a) and root growth (b) of once-selfed seeds generated in the greenhouse.
Fig 3. Scatter plot of PCA results showing average factor 1 (higher values indicate larger plants) and factor 2 (higher values indicate heavier seeds, higher germination, and fewer abnormally germinating seeds) values for field-collected seeds with circle color indicating survival at 1.7 kg/ha glyphosate.
Fig 4. A simulation of resistance allele frequencies over time given different selection regimes. Without a cost to resistance (a) the frequency of resistance increases if under selection. When costs are high (b; $C_R=0.86$, from field-collected germination rate) some selection regimes lead to a decrease in resistance frequency. At moderate levels of costs (c), such as reproductive output under competitive environments found in Vila-Auib et al. (2009a, $C_R=0.11$) resistance can decrease under a small subset of conditions. We used a simple single-locus, 2 allele Hardy-Weinberg population genetics model, with resistance being completely dominant to susceptibility. The net strength of selection for resistance was the weighted average of the strength of selection in sprayed (0) and non-sprayed (cost of resistance = $C_R$) areas weighted by the proportion of land in each. Similarly the net strength of selection for susceptibility was the weighted average of the strength of selection in sprayed (efficacy of spraying = $C_S=1$) and non-sprayed areas (0) weighted by the proportion of land in each.
Supporting information

Table S1. Population characteristics including population number, long name, state, crop type, latitude and longitude and sample sizes for experiments with field-collected seeds.

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<th>Longitude</th>
<th>N\textsubscript{fam} germ</th>
<th>N\textsubscript{seeds} germ</th>
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Table S2. Regression results between resistance and seed quality traits, accounting for geography (latitude and longitude) for field-collected seeds.

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Table S3. PCA loadings and variance explained.

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References


