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

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Abstract

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 rates, 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.

Introduction

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, traits 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; Yu et al. 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; Vila-Aiub et al. 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 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 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, since 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; Christopher et al. 1992; Christopher et al. 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 in the life history of a plant tradeoffs 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 US 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). *I. 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 US 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 above-ground 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 US, 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 the same type of potential trade-off, 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

133 Multiple fruits were collected from up to 79 individuals separated by at least 2 m from 43
134 populations located across the Midwest and Southeastern US (Table S1; Fig S1). These
135 seeds (hereafter field-collected seeds) were used in several experiments to determine
136 resistance, germination and early growth characteristics. To homogenize the effects of
137 maternal environment on seed quality, we chose a subset of the populations (N=18), grew
138 them in a common greenhouse for one generation and collected the autonomously self-
139 pollinated seeds from a similar growing and mating system environment (hereafter once-
140 selfed seeds).
141
142 *Estimate of herbicide resistance*
143 To determine glyphosate resistance across populations, a dose-response experiment was
144 conducted by planting a single field-collected seed from 10 randomly chosen maternal
145 lines from each population in six glyphosate treatments (including a non-herbicide
146 control treatment) in each of two greenhouse rooms. Full details of the dose-response
147 experiment are presented in Kuester *et al.* (2015) - for simplicity, we present resistance as
148 the percent survival per population at 1.70 kg a.i./ha of glyphosate, a rate which is
149 slightly higher than the suggested field rate of 1.54 kg a.i./ha. Individual seeds were
150 scarified, planted, allowed to grow for three weeks, and then treated with the herbicide
151 (PowerMax Roundup; Monsanto, St. Louis, Missouri) using a hand-held CO_2 pressurized
152 sprayer (Spraying Systems Co., Wheaton, IL). Survival was scored three weeks after
153 treatment application, and the population estimate of resistance was determined as the
154 proportion of individuals that survived glyphosate.
155

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 lab 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 8-79 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 (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), submerged in filtered water and allowed to germinate in the lab under ambient light and temperature. Water was added as necessary every three days to prevent drying out. Petri dishes were completely randomized across lab benches. Germination was scored periodically until no further germination was recorded. Final pre-scarification 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 (by visual determination) were scarified and germination was again scored after 1 week. We recorded the final number of seeds exhibiting normal germination, the number of seeds needing scarification, the number of scarified seeds that germinated, and the number that

179 had abnormal germination. For the second germination assay, we examined germination
180 data from one replicate (housed in a single greenhouse room) of the dose-response
181 experiment mentioned earlier in which seeds were scarified and planted in conetainers
182 with 1 seed per pot (10 maternal families per population). Germination was scored after
183 three weeks and used to calculate the percentage of seeds that germinated.
184 For the third and final germination assay, we used seeds from maternal lines that
185 were selfed once in the greenhouse. Two sets of five seeds for up to 8 maternal lines
186 (randomly selected) for each of 18 populations were placed in petri dishes with water.
187 Germination was scored after 11 days. If seeds had not imbibed water they were scarified
188 and scored again in one week. We calculated the percentage of seeds with normal
189 germination prior to scarification, the percentage of seeds that germinated after
190 scarification, and the percentage that had abnormal germination.
191
192 *Early root and above-ground growth*
193 To examine early root growth, we again used the once-selfed seeds and measured root
194 length four days after the germination assay began. We chose to first scarify the seeds in
195 this assay to standardize water absorption among individuals. Two sets of five seeds for
196 up to 8 maternal lines (randomly selected) for each of 18 populations were scarified and
197 placed in petri dishes with water. Germination was scored after 1, 4 and 7 days. On day 4
198 petri dishes were scanned and the root length was measured using Image J (Abramoff et
199 al. 2004) for each germinated seed.
200 We next examined early growth traits of greenhouse-grown individuals to
201 determine if there was a relationship between resistance and plant size (i.e., are plants

202 from resistant populations smaller?). To do so we used measurements from plants from
203 the dose-response experiment prior to herbicide application. Three weeks after planting
204 (and prior to spraying) we measured the height of the stem, the number of leaves and
205 length of the largest leaf on each individual planted per treatment per population (total
206 N=2908, Table S1 for exact sample sizes per population).
207
208 *Statistical analysis*
209 *Field-collected seeds*—We assessed the relationship between resistance and progeny
210 quality using mixed model analyses of variance. We used a generalized linear mixed-
211 effect model to examine final germination, germination before scarification, abnormal
212 germination, seeds needing scarification, and germination after scarification with
213 resistance and population (random) as predictors using the glmer function in the R
214 package lme4 with a binomial distribution. All of the binary measures were coded as 1 or
215 0. Seed weight (g) was modeled using a mixed model with resistance and population
216 (random) as predictors using the lmer function in the R package lme4. Additionally,
217 previous studies have indicated a geographic pattern of resistance in this species (Kuester
218 et al. 2015). To ensure that the above results were not an artifact of geography, we added
219 latitude and longitude (scaled) of the population in the above models. For the experiment
220 examining germination in soil, we modeled germination with resistance level and
221 population (random) as predictors using a binomial distribution.
222 *Once-selfed* seeds—Similar to the field-collected seeds, we used mixed-model binomial
223 regressions to assess the effect of resistance on germination characteristics of the once-
224 selfed, greenhouse generated seeds. We modeled germination before scarification and

225 germination after scarification with resistance and population (random) as predictors
226 using a binomial model. To determine if the maternal environment in which the seeds
227 developed influenced germination, in a separate model we compared germination
228 between maternal environments (i.e., field collected seeds versus seeds propagated in the
229 greenhouse) by including maternal environment as a treatment effect in the model. To do
230 so, we modeled final germination using treatment, resistance, population (random) and
231 treatment*resistance as predictors using a binomial distribution. An interaction between
232 treatment and resistance would indicate that the maternal environment differently
233 influenced germination.
234 *Early growth and size*—We next used mixed model analyses of variance to determine if
235 more resistant populations exhibited early growth life-history trade-offs. We separately
236 considered root length of the early germinant and plant size. We examined root length
237 using the once-selfed seeds in two different models. The first and more basic model
238 examined the influence of resistance and population (random) on log-transformed root
239 length (cm) 4 days post germination. A difference in root length, however, could be due
240 to differences in either growth rate of the radicle or differences due to the timing of
241 germination, *i.e.*, when growth began following germination. To distinguish between
242 these two potential explanations, we calculated an estimate of germination speed, the
243 time to 50% germination – a shorter time would suggest that seeds began growing sooner
244 after water was added. We used the germination data from days 1, 4, and 7 to obtain a
245 population level estimate of the time to 50% germination using a germination Hill
246 function (El-Kassaby et al. 2008). This function decomposes germination into 4
247 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 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 (cm), leaf number and leaf size (cm) of plants grown from field-collected seeds (~3 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 ($\lambda = 2.0$) was used to achieve better fit.

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 visually examine the data and determine how populations differed along the two axes retained. 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 relationship between resistance and the percentage of field-collected seeds that germinated (Fig. 1a). This is true both of seeds that germinated before scarification ($\beta = -4.93$, $\chi^2_1 = 24.66$ $P < 0.0001$) and the total number that germinated (including those that germinated after being scarified; $\beta = -5.20$, $\chi^2_1 = 24.80$, $P < 0.0001$; 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 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 ($\beta = 1.52$, $\chi^2_1 = 5.00$, $P = 0.03$), of which fewer seeds that subsequently germinated ($\beta = -5.50$, $\chi^2_1 = 8.81$, $P = 0.003$). We also found that populations with higher resistance produced lighter seeds ($\beta = -0.005$, $\chi^2_1 = 4.69$, $P = 0.03$), 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

293 and germinated (2.0%) and there was no effect of resistance ($\beta = 184.2$, $\chi^2_1 = 0.81$, P =
294 0.37). After scarification, however, there was a significant negative relationship between
295 germination and resistance ($\beta = -1.18$, $\chi^2_1 = 6.42$, P = 0.01, Fig. 1c). This effect remained
296 significant after accounting for latitude and longitude ($\beta = -1.13$, $\chi^2_1 = 5.49$, P = 0.02).
297 Interestingly, the decrease in germination of once-selfed, greenhouse-generated seeds
298 was significantly less than the field collected seeds (treatment * resistance: $\beta = 2.14$, $\chi^2_1 =$
299 27.8, P < 0.0001) suggesting that maternal environmental conditions influence the quality
300 of seeds produced. In addition, we found a much lower rate of abnormal germination in
301 the once-selfed seeds (~10%) compared to the field collected seeds, and the level of
302 abnormal germination showed no relationship with resistance ($\beta = 0.31$, $\chi^2_1 = 0.23$, P =
303 0.63). These results suggest that, while germination costs are consistently detected
304 between experiments in which the maternal environment differed, field environmental
305 conditions exacerbate the strength of the germination cost.
306
307 *Early root and aboveground growth*
308 To test whether growth differed according to resistance status, we scarified and
309 germinated the once-selfed seeds then measured root growth after 4 days. There was a
310 much higher germination rate of these seeds (86%) compared to the previous experiment
311 (2% pre-scarification) and the majority occurred before day 4. While there was a nearly
312 significant negative relationship between root length and resistance ($\beta = -0.38$, $\chi^2_1 = 3.19$,
313 P = 0.07; Fig. 2a), including the time to 50% germination in the model removed this
314 effect (resistance: $\beta = -0.07$, $\chi^2_1 = 0.09$, P = 0.76; time to 50% germination: $\beta = -0.37$, χ^2_1
315 = 4.85, P = 0.03), suggesting that the difference in root length was due to the timing of

316 germination rather than a difference in growth rate. A difference in plant size was also
317 found in the 3-4 week old plants grown in soil from the field-collected seeds - plants from
318 more resistant populations had smaller above-ground structures on average than plants
319 from less resistant populations (height: $\beta = -7.42$, $\chi^2_1 = 7.20$, $P = 0.007$; leaf number: $\beta =$
320 -1.11, $\chi^2_1 = 15.86$, $P < 0.0001$; largest leaf width: $\beta = -4.97$, $\chi^2_1 = 17.32$, $P < 0.0001$; Fig.
321 2b-d).
322
323 *Visualization of cost-related traits*
324 We next examined germination and early growth traits from the original field-collected
325 seeds using a principle components analysis (PCA) to determine if there was variation
326 among populations in the expression of cost-related traits (full results Table S3). The first
327 3 principle components of the PCA explained 77% of the variance, with the first principle
328 component (PC) loading with the early growth traits while the second loaded with seed
329 traits and the third with the proportion of seeds that required scarification to successfully
330 germinate. Populations with higher resistance scored lower on PC1 ($b = -2.22$, $r^2 = 0.28$,
331 $t_{41} = -4.03$, $P = 0.0002$) and PC2 ($b = -2.12$, $r^2 = 0.26$, $t_{41} = -3.79$, $P = 0.0005$), but not on
332 PC3 ($b = -1.02$, $r^2 = 0.06$, $t_{41} = -1.61$, $P = 0.12$). Using the first two PCs to plot the results,
333 resistant populations occur mostly in the lower left quadrant (smaller plants, lighter seeds,
334 lower germination and more abnormally germinating seeds) and have a wider spread than
335 less resistant populations (Fig. 3). Furthermore, while resistant populations scored lower
336 on PC1 and PC2 in comparison to susceptible populations, some resistant populations
337 exhibited early growth traits that were similar to susceptible populations and yet scored
338 very low on germination traits (e.g., pop num 5) whereas other resistant populations

339 exhibited similar germination traits compared to the susceptible populations, but were
340 smaller in stature than susceptible populations (e.g., pop num 51). Thus, it appears that
341 the type of cost may vary among populations sampled from North America.
342
343 **Discussion**
344 Here we show that glyphosate resistant populations of the common morning glory
345 exhibit life-history trade-offs associated with resistance, and, that these trade-offs may
346 vary among populations. Our series of experiments uncovered three notable findings:
347 First, we found a negative linear relationship between germination and resistance
348 indicating that resistant populations have a lower germination rate than susceptible
349 populations. This negative relationship persisted when using seeds generated from a
350 common greenhouse environment showing that this result is not due solely to field
351 environmental and/or maternal effects. Second, we found that individuals from resistant
352 populations were smaller than individuals from susceptible populations, indicating that
353 resistance influences early plant growth. Interestingly, we also found evidence that the
354 two types of trade-off may differ among populations—using PCA, we show that some
355 resistant populations produce normally sized plants, but score low on germination traits,
356 and *vice versa*. Below, we detail how these results add further strength to the suggestion
357 that a variety of life stages and populations sampled across the species' range should be
358 assessed when testing the hypothesis that resistance incurs a fitness cost (Délye et al.
359 2013a).
360
361 *Fitness costs: seed germination and early plant size*

362 It is difficult to determine how common germination differences associated with
363 resistance may be among weeds since many studies focus on seed quantity rather than
364 seed quality. There is some indication that germination may be affected in other
365 glyphosate resistant species. Dinelli et al. (2013) found reduced germination of
366 glyphosate resistant *Ambrosia trifida* populations, while Ismail et al. (2002) found greater
367 germination of resistant biotypes of goosegrass (*Eleusine indica)*. More broadly, life
368 history trade-offs may be specific to the herbicide and/or species in question or the type
369 of mutation conferring resistance (O'Donovan et al. 1999; Vila-Aiub et al. 2005; Délye et
370 al. 2013b). For example, only one of two different resistance mutations in ACCase
371 resistant *Lolium rigidum* had more stringent germination requirements (seeds germinated
372 poorly in the dark and required fluctuating temperatures to break dormancy) than the
373 susceptible genotype (Vila-Aiub et al. 2005). Similarly, Délye *et al.* (2013b) found
374 differential effects on germination among resistance mutations to ACCase in *Alopecurus*
375 *myosuroides*. Both of these studies report that the resistance mutation led to delayed
376 germination. Such a delay in germination may affect fitness, especially in agricultural
377 settings where germinating too early can lead to removal by pre-sowing practices and
378 germinating too late can lead to intensified competition with already established plants
379 (Weaver and Cavers 1979; Barrett 1983; Mortimer 1997; Forcella et al. 2000; Owen et al.
380 2014). Our analysis of root growth suggests that differences in plant size in *I. purpurea*
381 may be due to a similar delay in germination in resistant populations.
382 The reduced growth of resistant compared to susceptible populations that we
383 uncovered could lead to decreased competitive ability and subsequent lower fitness in the
384 presence of competition if, as has been found in other herbicide resistant weeds, the

385 difference in growth persists to adult plants (Weaver and Warwick 1982; Ahrens and
386 Stoller 1983; Holt 1988; Alcocer-Ruthling et al. 1992; Williams et al. 1995; Vila-Aiub et
387 al. 2005; Tardif et al. 2006; Vila-Aiub et al. 2009a). This type of life-history trade-off,
388 which ultimately may manifest as a fitness cost, is also likely to be species, mutation and
389 environment specific. For example, *Lolium rigidum* has evolved herbicide resistance via
390 a variety of mutations ranging from target site (Christopher et al. 1992; Yu et al. 2008) to
391 non-target site (Christopher et al. 1991; Christopher et al. 1994; Preston and Powles
392 1998). Target site mutations in the acetohydroxyacid synthase gene result in little cost in
393 growth (Yu et al. 2010). On the other hand, herbicide resistance mediated by the
394 cytochrome P450 complex resulted in reduced biomass and decreased competitive ability
395 (Vila-Aiub et al. 2009a).

396 An alternative explanation for the decline in germination and growth we identify
397 using the field-collected seeds is that some other co-varying population characteristic
398 such as soil fertility, spraying regime, herbivore levels, or the many other biotic and
399 abiotic factors that can influence seed development differed among resistant and
400 susceptible populations (Roach and Wulff 1987; Fenner 1991; Schmitt et al. 1992;
401 Platenkamp and Shaw 1993; Galloway 2001). These differences may explain the stronger
402 decline in germination in the field-collected seeds compared to the once-selfed seeds.
403 Several lines of evidence, however, suggest the relationship between resistance and
404 germination that we uncovered represents a true fitness cost rather than simply an effect
405 of the environment (e.g., driven by maternal effects, latitude, spray environment). First,
406 the relationship between resistance and germination appears approximately linear, which
407 is expected as the frequency of resistant individuals increases. If the trade-offs identified

408 herein were due simply to glyphosate exposure, with resistant populations exhibiting
409 abnormal seed development after surviving glyphosate application, we would expect to
410 see a binary distribution of seed quality of populations that had been sprayed and those
411 that had not, rather than a linear trend with resistance. Second, the negative relationship
412 between resistance and germination is maintained after a generation in a common
413 greenhouse environment—an effect should disappear if the decrease in fitness was due to
414 glyphosate exposure in the field. Finally, our results parallel those from a recent
415 experiment that specifically controlled for genetic background and environmental effects
416 using *I. purpurea* plants from a single population (Debban et al. 2015). Individuals from
417 this population were artificially selected for increased or decreased resistance for three
418 generations under controlled greenhouse conditions, and, similar to results presented
419 here, the increased resistance lines had a larger percentage of "bad" seeds that ejected the
420 embryo. That the results from one population utilizing a controlled genetic background
421 are mirrored across many populations collected from the landscape provides strong
422 evidence that lower germination represents a fitness cost of glyphosate resistance in this
423 weed species.

424 While we detected lower germination in herbicide resistant populations across
425 multiple experiments suggesting a true trade-off, we also found differences between
426 experiments in the strength of the relationship. This suggests both an underlying genetic
427 basis as well as an environmental component influence the expression of the trade-off.
428 Compared to the field-collected, unscarified seeds, the once-selfed, unscarified seeds
429 (Fig. 1) had a low germination rate suggesting that the seed coat was perhaps more
430 pristine in seeds generated in the greenhouse. However, if scarified, we found that the

431 once-selfed seeds exhibited high germination and the relationship between resistance and
432 germination remained negative indicating a fitness decline associated with resistance.
433 The physical seed coat is the primary mechanism of dormancy in this species (Brechu-
434 Franco et al. 2000); thus, environmentally-induced physical differences in the seed coat
435 (e.g. thickness or waxiness) or its degree of degradation (e.g. mechanical disruption or
436 seed storage differences) likely influences germination timing. Although it is clear that
437 the environment influences seed germination in this species, that we consistently
438 observed a decline in germination with resistance across multiple experiments suggests
439 that there is an underlying genetic basis to the cost of resistance.

440 Another striking difference between experiments was in the frequency of
441 abnormal seeds produced. The once-selfed seeds had almost no abnormal germination
442 (i.e., no dead embryos that were ejected from the seed coat) while some of the field-
443 collected populations had a high level of abnormal germination. In fact, the strong
444 decline in germination for field-collected seeds was due primarily to this abnormal
445 germination. Abnormal germination could be due to a variety of environmental causes
446 (e.g. herbicide application, nutrient availability, competition, etc) or be a cost of
447 resistance that is only induced under field conditions. Our results suggest that in a benign
448 environment, such as the greenhouse, the seeds in general are of high quality (high
449 germination, fewer abnormal germinants) but there is a cost of resistance that increases
450 the time it takes to germinate (based on root growth experiment), possibly leading to
451 smaller plants at any given point. On the other hand, under field conditions, populations
452 with higher resistance produce more abnormal seeds (due to either environmental
453 differences or an environmentally induced cost of resistance) and the normal seeds may

454 still have an increase in the time it takes to germinate leading to smaller plants at any
455 given point.

456 Interestingly, by visually examining the germination and growth traits in a PCA,
457 we find variation in the type of potential cost among resistant populations. While some
458 resistant populations fell into the "poor germination" axis, other resistant populations fell
459 into the "poor growth" axis compared to susceptible populations. There are at least three
460 possible reasons for this difference: different resistance genes, different compensatory
461 mutations or different genetic backgrounds. First, the gene(s) involved in resistance may
462 vary among populations leading to different costs. Independent origins of resistance to
463 herbicide have been found in other species (Délye et al. 2010) and these different
464 mutations often incur different fitness costs (Vila-Aiub et al. 2005; Délye et al. 2013b).
465 Second, the resistance gene(s) may be the same amongst populations but each population
466 may have different compensatory mutations that lead to different costs (Darmency et al.
467 2015). Third, the resistance gene(s) may behave differently in different genetic
468 backgrounds (Paris et al. 2008). These distinctions are important because they would
469 differentially affect the evolutionary trajectory of herbicide resistance. For example, if
470 populations differ in the gene(s) involved, each population may have a very different set
471 of costs, benefits and evolutionary trajectories, which would need to be incorporated in
472 models.

473 It is currently unknown if the trait trade-offs identified here are pleiotropic or due
474 to linkage to the resistance gene. The most restrictive definition of a cost requires that the
475 decrease in fitness is due to the resistance allele itself - either the actual allele or through
476 it acting pleiotropically (Bergelson and Purrington 1996). Given that we do not know the

477 identity of the loci involved in either resistance or the abnormal germination and reduced
478 growth, we cannot entirely rule out physical linkage between resistance genes and cost
479 genes, in which case the "cost" could quickly become unlinked over generations
480 (Lewontin 1974; Hartl and Clark 1989). For some species, easily identifiable mutations in
481 the enzyme targeted by the herbicide can be linked to resistance, i.e. target site resistance
482 (TSR). However, preliminary work suggests that glyphosate resistance in *I. purpurea* is
483 due to non-target site mechanism (NTSR; Leslie and Baucom, *unpublished data*), and as
484 such elucidating the genetic basis of both resistance and the cost will be a non-trivial
485 endeavor. Furthermore, it is rare that genes underlying costs are identified; most
486 documented cases of the genes involved in the cost of resistance is when TSR mutations
487 lead to poor performance of the enzyme on its natural substrate (Vila-Aiub et al. 2009b).
488 As far as we are aware, no study has identified the genes involved in the cost of
489 resistance when the mechanism of resistance is NTSR. One intriguing possibility for this
490 species stems from a previous study that compared transcript expression levels of
491 artificially selected lines of resistant and susceptible *I. purpurea* plants following
492 herbicide application (Leslie and Baucom 2014). One of the differences between the
493 replicated resistant and susceptible lines was a lower expression of pectin methylesterase
494 (PME) in the resistant plants. This enzyme has been shown to play a role in breaking seed
495 dormancy (Ren and Kermode 2000) and stem elongation (Pilling et al. 2000). Thus, the
496 decreased expression of PME in resistant plants may explain both the reduced
497 germination and growth in populations with higher resistance.
498

How might life history trade-offs 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 possible that the life-history differences that we identified here are responsible, at least in part, for maintaining resistance between sampling years. For example, the lower germination of resistant types would manifest as a fitness cost if resistant and susceptible types 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 types would not be at a relative disadvantage. While we have not examined seed production across all 43 populations examined herein, a common garden study of glyphosate susceptible and resistant families 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 a single population 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 if in competition, an effect which remains to be tested in this system.

522 In summary, we found reductions in seed quality across replicated herbicide
523 resistant populations of the common morning glory. Although most studies use seed
524 quantity as a proxy for fitness, our results highlight that reductions in progeny quality are
525 an equally, if not more, important cost of adaptation in *I. purpurea*. Given that fitness
526 costs are thought to arise from a variety of mechanisms (allocation of resources,
527 ecological costs, etc.), our results suggest that a high priority should be placed on the
528 examination of multiple stages of the life cycle when assessing potential costs and not
529 just seed quantity. Furthermore, because the strength of this cost could maintain the
530 efficacy of a globally important herbicide, this work illustrates the utility and importance
531 of integrating evolutionary principles into management scenarios (Gould 1995).
532
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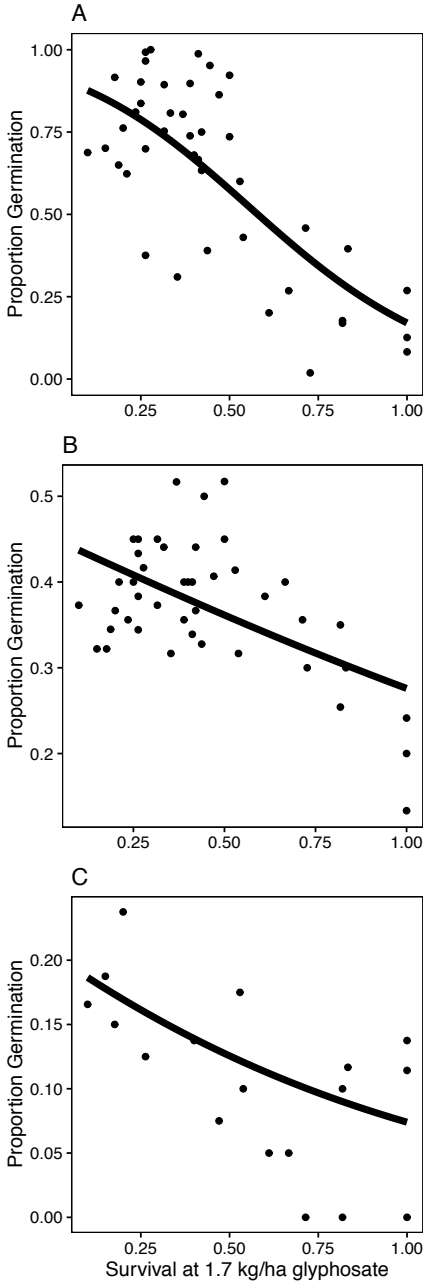
537

538 **Figure Legends**

539 Fig 1. Relationship between herbicide resistance and proportion germination for (a) field-
540 collected seeds in petri dishes, (b) field-collected seeds in soil, and (c) once-selfed seeds
541 (note the differences in the y-axis scale). Points are the mean per population, lines are the
542 average marginal predicted probabilities from the appropriate model.



543

544

Fig 2. Relationship between herbicide resistance and (a) root growth after 4 days, (b) shoot height (c) leaf number and (d) width of the largest leaf after 3 weeks. Points are the mean per population and lines are the average marginal predicted probabilities from the appropriate model.

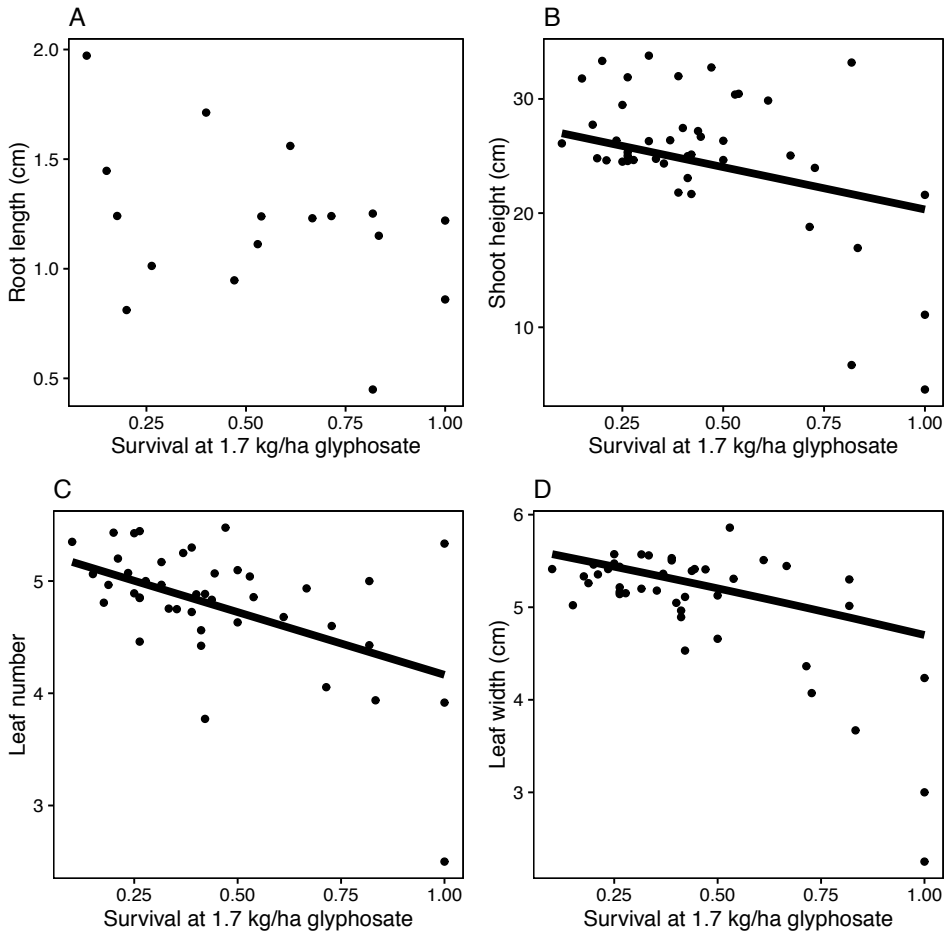
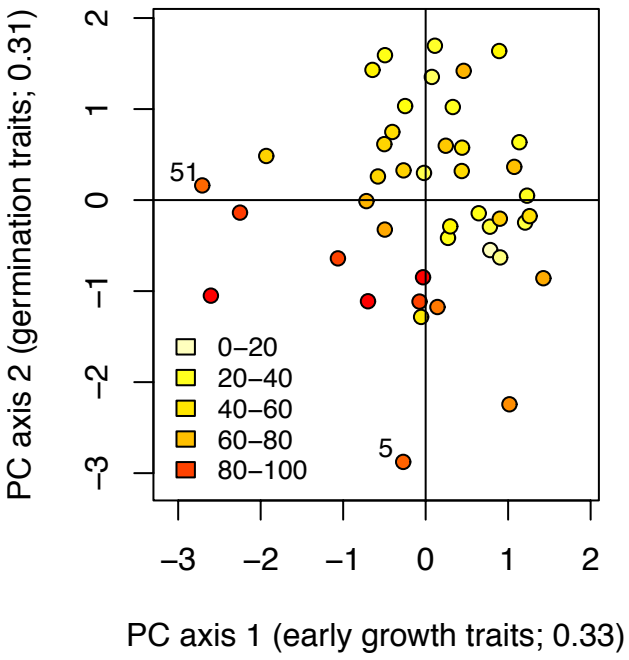


Fig 3. Scatter plot of PCA results showing average principle components axis 1 (higher values indicate larger plants) and principle components axis 2 (higher values indicate heavier seeds, higher germination, and fewer abnormally germinating seeds) values for field-collected seeds with circle color indicating survival at 1.7 kg/ha glyphosate. The proportion variation explained by each axis is noted in axis labels.



556 **Supporting information**

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

563

Population number	State	Crop	Latitude	Longitude	N_{fam} germ	N_{seeds} germ	N early growth	N_{fam} once selfed
1	TN	Corn	35.775237	-85.903419	8	31	10	4
2	NC	Corn	34.595714	-77.927484	79	387	60	
4	NC	Corn	34.556672	-79.125602	39	178	79	
5	SC	Soy	33.859875	-79.909072	34	146	27	
8	SC	Corn	34.297195	-79.991259	40	180	73	8
9	NC	Soy	34.924044	-77.796171	40	189	46	8
10	NC	Soy	34.983161	-78.039309	28	123	24	8
11	NC	Corn	34.527135	-78.756704	38	178	81	8
12	SC	Cotton	34.145812	-79.865313	42	202	79	8
14	NC	Soy	35.424763	-77.917121	40	192	76	8
15	SC	Soy	34.104209	-79.073735	41	193	81	8
16	SC	Alfalfa	34.10535	-79.183234	41	203	78	
17	SC	Soy	34.159155	-79.272908	27	135	82	
18	SC	Corn	34.156593	-79.27027	39	193	79	
19	NC	Corn	34.508193	-78.70899	35	138	70	8
20	TN	Corn	35.830692	-85.777871	38	169	13	2

21	TN	Soy	35.369816	-77.877314	40	200	70	
22	NC	Corn	36.1436	-78.053422	17	78	69	
23	TN	Corn	35.067905	-86.62955	40	195	77	
26	TN	Soy	35.533413	-85.951902	50	250	86	
28	SC	Corn	34.097917	-80.377715	35	172	80	
29	NC	Corn	34.705135	-78.738897	30	122	75	7
30	TN	Corn	35.31105	-85.945003	42	206	82	
31	TN	Corn	35.608482	-85.846379	31	141	69	8
32	TN	Corn	35.099356	-86.225509	15	56	33	5
33	OH	Corn	39.858763	-83.669821	52	260	79	
34	OH	Corn	39.44316	-83.910189	48	238	82	
35	IN	Corn	39.853945	-85.770156	28	120	80	
36	IN	Corn	40.565608	-85.503826	33	146	85	
37	OH	Corn	39.583755	-83.758264	49	243	79	
38	OH	Soy	39.515447	-83.407431	46	230	88	
39	IN	Corn	39.988984	-85.742262	38	188	75	
40	OH	Soy	41.284684	-83.847252	50	237	84	
41	VA	Corn	38.636343	-78.472921	41	200	78	
42	VA	Corn	38.373523	-78.662516	49	230	82	8
43	VA	Soy	36.886448	-78.553156	24	89	28	2
44	VA	Soy	38.285415	-78.797088	20	98	75	
45	VA	Soy	36.847945	-78.595042	42	209	76	
46	TN	Soy	35.536019	-86.17985	34	100	17	2
47	SC	Soy	34.282132	-79.746597	41	201	83	
48	TN	Corn	35.31653	-87.35373	31	151	74	8
51	TN	Soy	35.533413	-85.951902	24	118	56	8
52	OH	Corn	41.284684	-83.847252	62	310	88	

564 Table S2. Regression results between resistance and seed quality traits, accounting for geography (latitude and longitude) for field-

565 collected seeds.

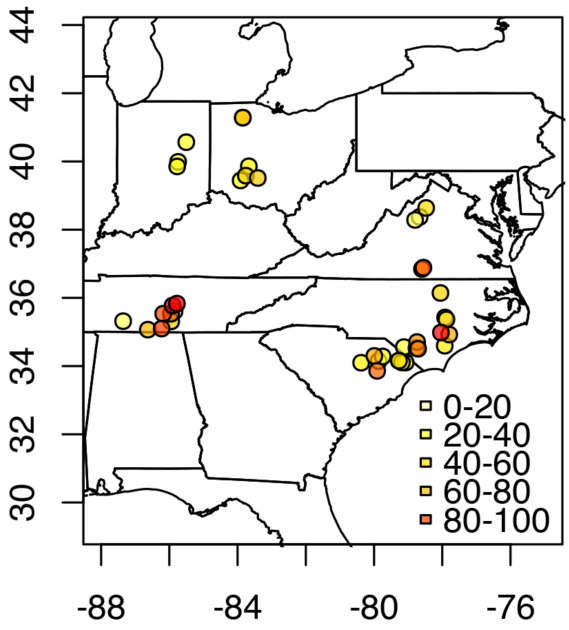
Trait	Population number	Survival at 1.7 kg a.i. /ha		Latitude (scaled)		Longitude (scaled)	
		b	χ^2_1	*b*	χ^2_1	*b*	χ^2_1
Seed weight (g)	43	-0.0047	4.63*	0.0016	7.33**	-0.0010	3.91*
Final germination	43	-4.89	28.62***	0.6298	8.80**	-0.3784	4.13*
Abnormally germinating seeds	42	4.24	32.52***	-0.1774	1.24	0.1826	1.58
Seeds needing scarification	43	0.83	1.77	-0.4636	7.19**	-0.3488	5.22*
Scarified seeds that germinated	40	-5.87	8.85**	0.0061	0.0002	-0.3342	0.65
Height (cm)	43	-7.39	7.26**	-0.3336	0.38	1.2210	4.76*
Leaf number	43	-1.08	17.57***	-0.0070	0.02	0.1589	9.17**
Largest leaf size (Box cox transformed cm)	43	-4.40	14.04***	0.4080	2.96	0.4642	3.81

566

Table S3. PCA loadings and variance explained.

Trait	Factor1	Factor2	Factor3
Seed weight	0.09506	0.8414	-0.16627
Percent germination	0.29276	0.82272	0.37541
Percent viable dormant seeds	0.0339	0.12801	0.92794
Percent abnormally germinating seeds	-0.15651	-0.81953	-0.42013
Height	0.94253	0.04143	0.05526
Leaf number	0.86728	0.13535	0.06511
Largest leaf size	0.74853	0.28353	0.00642
Prop. Variance explained	0.3316	0.3105	0.1734

Fig S1. Map of the Eastern US showing the population locations and their survival at 1.7 kg a.i./ha (color of circle).



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