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*

al., 2006; Délye *et al.*, 2010). 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

87 growth and/or fitness differences between R and S genotypes compared to those that
88 focus solely on measures of fecundity (Vila-Aiub *et al.*, 2009b).
89 Despite recommendations to control/increase the number of genetic backgrounds
90 (Bergelson & Purrington, 1996), and to examine multiple life history stages when
91 determining if resistance incurs a cost (Primack & Kang, 1989; Vila-Aiub *et al.*, 2009b),
92 a recent review shows that only 25% of herbicide resistance studies control for
93 background effects and only 7-10% of cost studies examine multiple stages of the life
94 cycle (Vila-Aiub *et al.*, 2009b). Fewer still examine the potential for fitness costs using a
95 large number of populations sampled from a species' range, an approach suggested
96 almost 20 years ago (Cousens *et al.*, 1997; Strauss *et al.*, 2002). Just as the mechanism of
97 resistance can vary among species, populations of the same weed have been shown to
98 harbor different mechanisms of resistance to the same herbicide (Christopher *et al.*, 1991;
99 Christopher *et al.*, 1992; Christopher *et al.*, 1994; Preston & Powles, 1998; Yu *et al.*,
100 2008; Délye *et al.*, 2010), thus increasing the likelihood that costs may likewise vary
101 among populations. It is also possible, though rarely tested, that fitness costs have been
102 ameliorated in some herbicide resistant populations relative to other populations due to
103 the evolution of modifier loci (Darmency *et al.*, 2015). The above hypotheses for the lack
104 of costs are all interrelated: because resistance could be due to a variety of mechanisms
105 (Délye *et al.*, 2013a), costs may be apparent at only certain life history stages, expressed
106 in particular environments (Vila-Aiub *et al.*, 2009b), or apparent in some populations but
107 not others. Thus, there remain crucial gaps in our understanding of where in the life
108 history of a plant costs might be apparent, and further, how ubiquitous costs may be
109 across a species' range (Vila-Aiub *et al.*, 2011; Neve *et al.*, 2014).
110 The common morning glory, *Ipomoea purpurea*, a noxious weed of US
111 agriculture (Webster & MacDonald, 2001), provides an excellent system to examine the
112 strength and type of costs that may be present in natural populations. This species
113 exhibits variability in resistance to glyphosate (Baucom & Mauricio, 2008; Kuester *et al.*,
114 *2015*), which is the main ingredient in the herbicide RoundUp®. RoundUp® is currently
115 the most widely used herbicide in agriculture (Fernandez-Cornejo *et al.*, 2014), and of the
116 approximately 30 resistant weeds that have been examined (Heap, 2015), only a third are
117 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

149 (hereafter field-collected seeds) were used in several experiments to determine resistance,
150 germination and early growth characteristics. To homogenize the effects of maternal
151 environment on seed quality, we chose a subset of the populations (N=18), grew them in
152 a common greenhouse for one generation and collected the autonomously self-pollinated
153 seeds from a similar growing and mating system environment (hereafter once-selfed
154 seeds).
155
156 *Estimate of herbicide resistance*
157 To determine glyphosate resistance across populations, a dose-response experiment was
158 conducted by planting a single field-collected seed from 10 randomly chosen maternal
159 lines from each population in six glyphosate treatments (including a non-herbicide
160 control treatment) in each of two greenhouse rooms. Full details of the dose-response
161 experiment are presented in Kuester *et al.* (*2015*) - for simplicity, we present resistance as
162 the percent survival per population at 1.70 kg a.i./ha of glyphosate, a rate which is
163 slightly higher than the suggested field rate of 1.54 kg a.i./ha. Individual seeds were
164 scarified, planted, allowed to grow until approximately 13 cm in height, and then treated
165 with the herbicide using a hand-held CO_2 pressurized sprayer (Spraying Systems Co.,
166 Wheaton, IL). Survival was scored three weeks after treatment application, and the
167 population estimate of resistance was determined as the proportion of individuals that
168 survived glyphosate.
169
170 *Germination*
171 To determine the effects of resistance on seed traits, we measured seed weight and
172 germination characteristics using field-collected seeds from each population (N=43). Up
173 to five (ave 4.6) seeds from 8-79 maternal lines per population (ave 38, total 1621, see
174 Table S1 for exact sample sizes per population) were haphazardly chosen for the
175 germination test. From this pool of seeds we randomly chose a subset of families per
176 population (8-49 maternal lines per population; Table S1) for which the selected seeds
177 were weighed (as a group) to determine the average seed weight. All of the selected seeds
178 were placed in a small petri dish (one dish per family) with water and allowed to
179 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.

303

304 *Visualization of cost-related traits*

305 We next examined germination and early growth traits from the original field-collected
306 seeds using a principle components analysis (PCA) to determine if there was variation
307 among populations in the expression of cost-related traits (full results Table S3). The first
308 3 factors of the PCA explained 77% of the variance, with the first factor loading with the
309 early growth traits while the second loaded with seed traits and the third with the
310 proportion of seeds that required scarification to successfully germinate. Populations with
311 higher resistance scored lower on factor 1 ($b = -2.22$, $r^2 = 0.28$, $t_{41} = -4.03$, $P = 0.0002$)
312 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
313 $= 0.06$, $t_{41} = -1.61$, $P = 0.12$). Using the first two factors to plot the results, resistant
314 populations occur mostly in the lower left quadrant (smaller plants, lighter seeds, lower
315 germination and more abnormally germinating seeds) and have a wider spread than less
316 resistant populations (Fig. 3). Furthermore, while R populations scored lower on factors 1
317 and 2 in comparison to S populations, some R populations exhibited early growth traits
318 that were similar to S populations and yet scored very low on germination traits (e.g., pop
319 num 5) whereas other R populations exhibited similar germination traits compared to the
320 S populations, but were smaller in stature than S populations (e.g., pop num 51). Thus, it
321 appears that the type of cost may vary among populations sampled from North America.

322

323 **Discussion**

324 Here we show that glyphosate resistant populations of the common morning glory
325 exhibit costs of herbicide resistance, and, that the type of cost may vary among
326 populations. Our series of experiments uncovered three notable findings: First, we found
327 a strong negative linear correlation between germination and resistance indicating that
328 resistant populations have a lower germination rate than susceptible populations. We
329 uncovered similar trends using seeds generated from a common greenhouse environment
330 showing that this result is not due simply to field environmental and/or maternal effects.
331 Second, we found that individuals from resistant populations were smaller than
332 individuals from susceptible populations, indicating that resistance may incur a cost in
333 terms of plant growth. Interestingly, we also found evidence that the two types of cost

334 may differ among populations—using PCA, we show that some R populations produce
335 normally sized plants, but score low on germination rate, and *vice versa*—some R
336 populations germinate at a rate similar to S populations, but tend to have smaller
337 individuals than S populations. Below, we detail how these results add further strength to
338 the suggestion that a variety of life stages and populations sampled across the species'
339 range should be assessed when testing the hypothesis that resistance incurs a fitness cost
340 (Délye *et al.*, 2013a). We then place our results into a broader context using a simple
341 model to examine how the cost identified in this work may influence the evolutionary
342 trajectory of resistance in this species.
343
344 *Fitness costs: seed germination and early plant size*
345 It is difficult to ascertain the frequency of germination costs associated with
346 herbicide resistance in nature since many studies of resistant weeds focus on seed
347 quantity rather than seed quality. There is some indication that germination may be
348 affected in other glyphosate resistant species–Dinelli *et al.* (2013) found reduced
349 germination of glyphosate resistant *Ambrosia trifida* populations, while Ismail *et al.*
350 (2002) found greater germination of resistant biotypes of goosegrass (*Eleusine indica)*.
351 Costs may be specific to the species or the type of mutation conferring resistance
352 (O'Donovan *et al.*, 1999; Vila-Aiub *et al.*, 2005; Délye *et al.*, 2013b). For example, only
353 one of two different resistance mutations in ACCase resistant *Lolium rigidum* had more
354 stringent germination requirements (germinated poorly in the dark and required
355 fluctuating temperatures to break seed dormancy) than the susceptible genotype (Vila-
356 Aiub *et al.*, 2005). Similarly, Délye *et al.* (2013b) found differential effects on
357 germination among resistance mutations to ACCase in *Alopecurus myosuroides*. Both of
358 these studies report that the resistance mutation led to delayed germination; such a delay
359 in germination may affect fitness, especially in agricultural settings where germinating
360 too early can lead to removal by pre-sowing practices and germinating too late can lead
361 to intensified competition with already established plants (Weaver & Cavers, 1979;
362 Barrett, 1983; Mortimer, 1997; Forcella *et al.*, 2000; Owen *et al.*, 2014).
363 While delays in germination may explain some of the results we present, in
364 general, the low germination we uncovered in the R populations was due to non-viable

365 embryos (53% of R seeds vs 18% of S seeds) rather than later germination. In the normal
366 progression of seed germination in this species, the radicle first emerges from the seed
367 coat and the cotyledons rapidly follow. In comparison, the non-viable seeds swell, split in
368 half, and eject the entire embryo from the seed coat. Many of these seeds appear fully
369 provisioned, and, although they weigh less than seeds from S populations, the differences
370 in weight are not drastic, nor are there visible indications that would suggest that seeds
371 from R populations would be of such low quality.

372 The reduced growth of R compared to S populations that we uncovered could lead
373 to decreased competitive ability and subsequent lower fitness in the presence of
374 competition, as has been found in other herbicide resistant species (Weaver & Warwick,
375 1982; Ahrens & Stoller, 1983; Holt, 1988; Alcocer-Ruthling *et al.*, 1992; Williams *et al.*,
376 1995; Vila-Aiub *et al.*, 2005; Tardif *et al.*, 2006; Vila-Aiub *et al.*, 2009a). However,
377 similar to studies of germination differences, results from studies comparing growth of
378 resistant and susceptible genotypes suggest this cost is species, mutation and environment
379 specific. For example, *Lolium rigidum* has evolved herbicide resistance via a variety of
380 mutations ranging from target site (Christopher *et al.*, 1992; Yu *et al.*, 2008) to non-target
381 site (Christopher *et al.*, 1991; Christopher *et al.*, 1994; Preston & Powles, 1998). Target
382 site mutations in the acetohydroxyacid synthase gene result in little cost in growth (Yu *et*
383 *al.*, 2010). On the other hand, herbicide resistance mediated by the cytochrome P450
384 complex resulted in reduced biomass and decreased competitive ability (Vila-Aiub *et al.*,
385 2009a).

386 An alternative explanation for the decline in germination and growth we identify
387 using the field-collected seeds is that some other co-varying population characteristic
388 such as soil fertility, spraying regime, herbivore levels, or the many other biotic and
389 abiotic factors that can influence seed development differed among R and S populations
390 (Roach & Wulff, 1987; Fenner, 1991; Schmitt *et al.*, 1992; Platenkamp & Shaw, 1993;
391 Galloway, 2001). However, several lines of evidence suggest the relationship between
392 resistance and germination that we uncovered represents a true fitness cost rather than
393 simply an effect of the environment (e.g., driven by maternal effects, latitude, spray
394 environment). First, we identified a very strong linear correlation between resistance and
395 germination. If the fitness cost was due simply to glyphosate exposure, with resistant

396 populations exhibiting abnormal seed development after surviving glyphosate
397 application, we would expect to see a binary distribution of seed quality of populations
398 that had been sprayed and those that had not, rather than a linear trend with resistance.
399 Second, the negative correlation between germination and resistance is present only
400 among resistant populations—strongly suggesting that as populations become more
401 resistant, they exhibit a correspondingly higher fitness penalty when in the absence of the
402 herbicide. Third, the negative relationship is maintained after a generation in a common
403 growing/mating system environment. Finally, our results parallel those from the recent
404 experiment that specifically controlled for genetic background and environmental effects
405 using *I. purpurea* plants from a single population (Debban *et al.*, *In press*). Individuals
406 from this population were artificially selected for increased or decreased resistance for
407 three generations under controlled greenhouse conditions, and, similar to results
408 presented here, the increased resistance lines had a larger percentage of "bad" seeds that
409 ejected the embryo. That the results from one population utilizing a controlled genetic
410 background are mirrored across many populations collected from the landscape provides
411 strong evidence that a fitness cost of glyphosate resistance is present in this weed species.
412 Interestingly, by visually examining the germination and growth traits in a PCA,
413 we find variation in the type of cost trait among R populations. While some R
414 populations fell into the "poor germination" axis, other R populations fell into the "poor
415 growth" axis compared to S populations. There are at least three possible reasons for this
416 difference: different resistance genes, different compensatory mutations or different
417 genetic backgrounds. First, the gene(s) involved in resistance may vary among
418 populations leading to different costs. Independent origins of resistance to herbicide have
419 been found in other species (Délye *et al.*, 2010) and these different mutations often incur
420 different fitness costs (Vila-Aiub *et al.*, 2005; Délye *et al.*, 2013b). Second, the resistance
421 gene(s) may be the same amongst populations but each population may have different
422 compensatory mutations that lead to different costs (Darmency *et al.*, 2015). Third, the
423 resistance gene(s) may behave differently in different genetic backgrounds (Paris *et al.*,
424 2008). These distinctions are important because they would differentially affect the
425 evolutionary trajectory of herbicide resistance. For example, if populations differ in the

426 gene(s) involved, each population may have a very different set of costs, benefits and
427 evolutionary trajectories, which would need to be incorporated in models.
428 It is currently unknown if the costs identified here are pleiotropic or due to
429 linkage to the resistance gene. The most restrictive definition of a cost requires that the
430 decrease in fitness is due to the resistance allele itself - either the actual allele or through
431 it acting pleiotropically (Bergelson & Purrington, 1996). Given that we do not know the
432 identity of the loci involved in either resistance or the abnormal germination and reduced
433 growth, we cannot entirely rule out physical linkage between resistance genes and cost
434 genes, in which case the "cost" could quickly become unlinked over generations
435 (Lewontin, 1974; Hartl & Clark, 1989). For some species, easily identifiable mutations in
436 the enzyme targeted by the herbicide can be linked to resistance, i.e. target site resistance
437 (TSR). However, preliminary work suggests that glyphosate resistance in *I. purpurea* is
438 due to non-target site mechanism (NTSR; Leslie and Baucom, *unpublished data*), and as
439 such elucidating the genetic basis of both resistance and the cost will be a non-trivial
440 endeavor. Furthermore, it is rare that genes underlying costs are identified; most
441 documented cases of the genes involved in the cost of resistance is when TSR mutations
442 lead to poor performance of the enzyme on its natural substrate (Vila-Aiub *et al.*, 2009b).
443 As far as we are aware, no study has identified the genes involved in the cost of
444 resistance when the mechanism of resistance is NTSR. One intriguing possibility for this
445 species stems from a previous study that compared transcript expression levels of
446 artificially selected lines of resistant and susceptible *I. purpurea* plants following
447 herbicide application (Leslie & Baucom, 2014). One of the differences between the
448 replicated R and S lines was a lower expression of pectin methylesterase (PME) in the
449 resistant plants. This enzyme has been shown to play a role in breaking seed dormancy
450 (Ren & Kermode, 2000) and stem elongation (Pilling *et al.*, 2000). Thus, the decreased
451 expression of PME in resistant plants may explain both the reduced germination and
452 growth in populations with higher resistance.
453
454 *How might costs influence the evolutionary trajectory of resistance in this species?*
455 Recent work in this system has shown that populations of the common morning
456 glory sampled from 2012 exhibit higher levels of resistance compared to the same

457 populations sampled in 2003 (Kuester et al, *In* Review). Interestingly, however, the
458 difference in resistance between sampling years was only slight, i.e., 62% survival at 1.7
459 kg ai/ha in 2012 vs 57% survival in 2003 samples. It is very likely that the fitness cost we
460 identified here is responsible for maintaining resistance between sampling years. In light
461 of this finding, we elected to model the influence of the germination cost on the evolution
462 of resistance in this species, making simplifying assumptions about the genetic basis of
463 resistance and pattern of mating in this species. We used a single locus Hardy-Weinberg
464 model of a panmictic population with the resistance allele dominant to the susceptible
465 allele. We examined the frequency of a resistance allele as a function of the fitness cost
466 and the proportion of the population not under selection (*i.e.* not sprayed). We ran the
467 model using one of three costs – no cost, a moderate cost, or a high cost to demonstrate
468 the differing evolutionary trajectories given different costs. On one extreme, we show
469 that the frequency of a resistance allele without a cost increases relative to the proportion
470 of the population under selection (Fig. 4a). On the other extreme, if a resistance mutation
471 leads to a strong cost—similar to that of the germination cost identified here—the
472 frequency of the resistance allele can decrease when enough of the population is free
473 from selection, i.e., not sprayed with herbicide (Fig. 4b). Populations or species with high
474 costs will have a slower rate of increase of the resistant allele compared to those that
475 exhibit a moderate cost (Fig. 4c) (such as found when examining the negative
476 repercussions of slower growth on competitiveness of resistant plants (Vila-Aiub *et al.*,
477 2009a)).
478 This simple model can also demonstrate the importance of accurate estimations of
479 costs when making management decisions. For example, we can use a "back of the
480 envelope" calculation to examine the threshold proportion of selection-free land that will
481 result in a decrease in resistance for a given cost of resistance. We estimated the threshold
482 proportion of non-treated land as $(1-C_R/(C_R+C_S))$, or one minus the cost of resistance
483 divided by the sum of the cost of susceptibility (i.e. herbicide efficiency) and the cost of
484 resistance. From the cost obtained in this study using the field-collected seeds (and
485 assuming complete control of susceptible individuals when spraying), this proportion is
486 0.54, *i.e*. more than 54% of acreage would need to be non-treated to decrease the
487 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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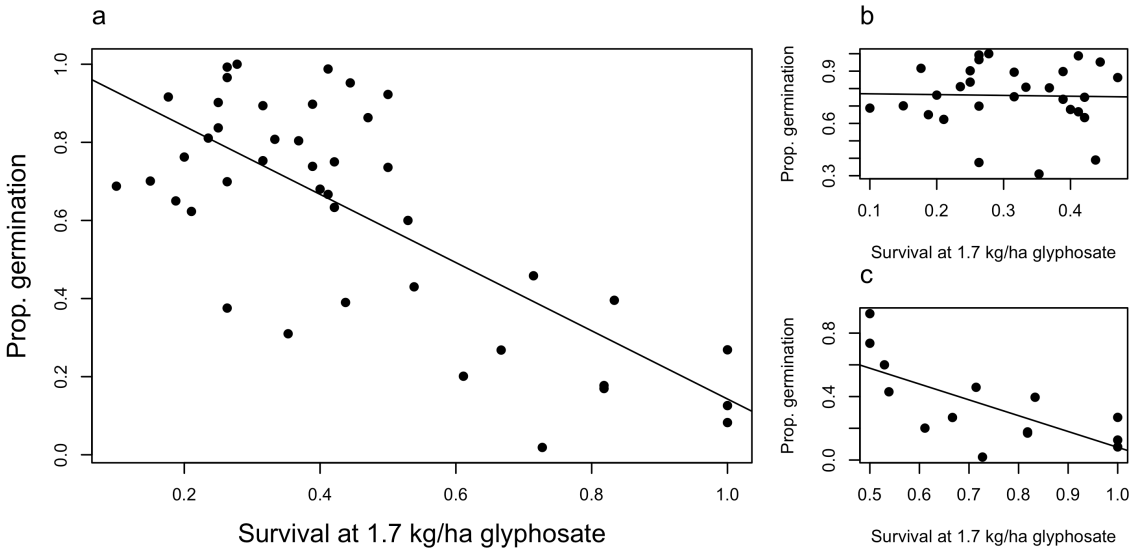
513 **Figure Legends**

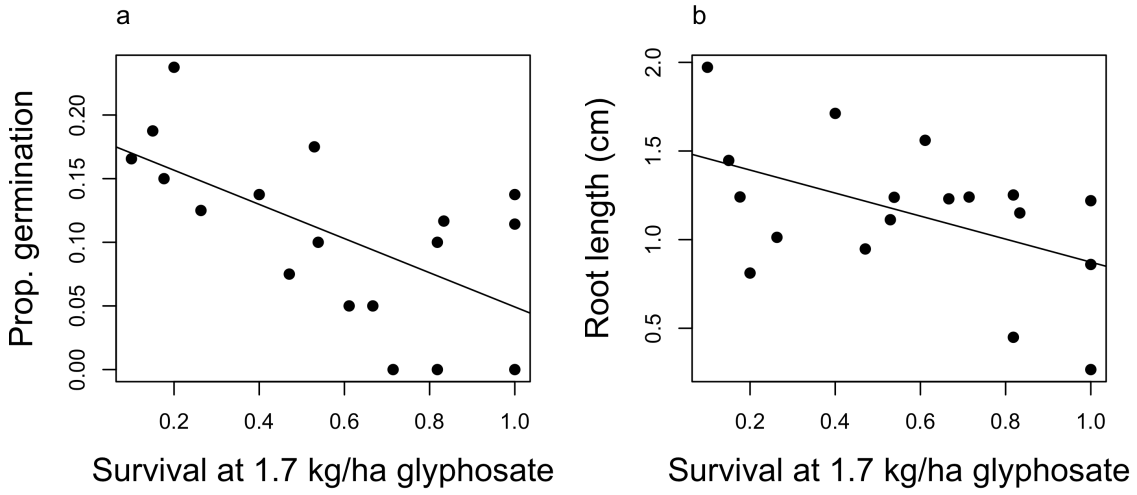
514

515 Fig 1. Regression between survival at 1.7 kg a.i./ha glyphosate and germination rate of
516 field-collected seeds across all populations (a), in susceptible populations (b) and
517 resistant populations (c).

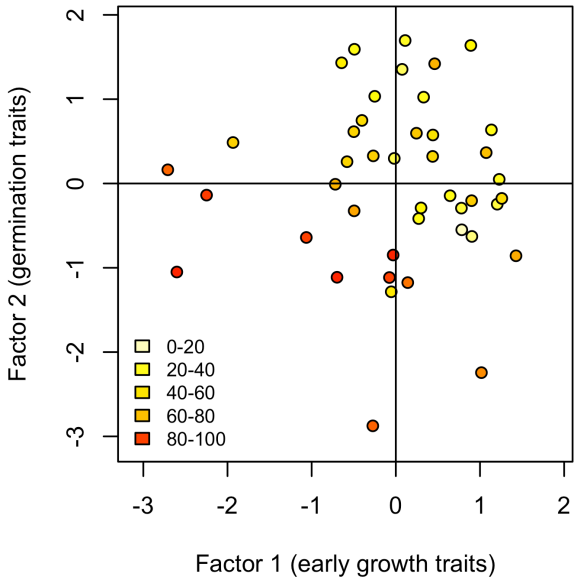


518

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.

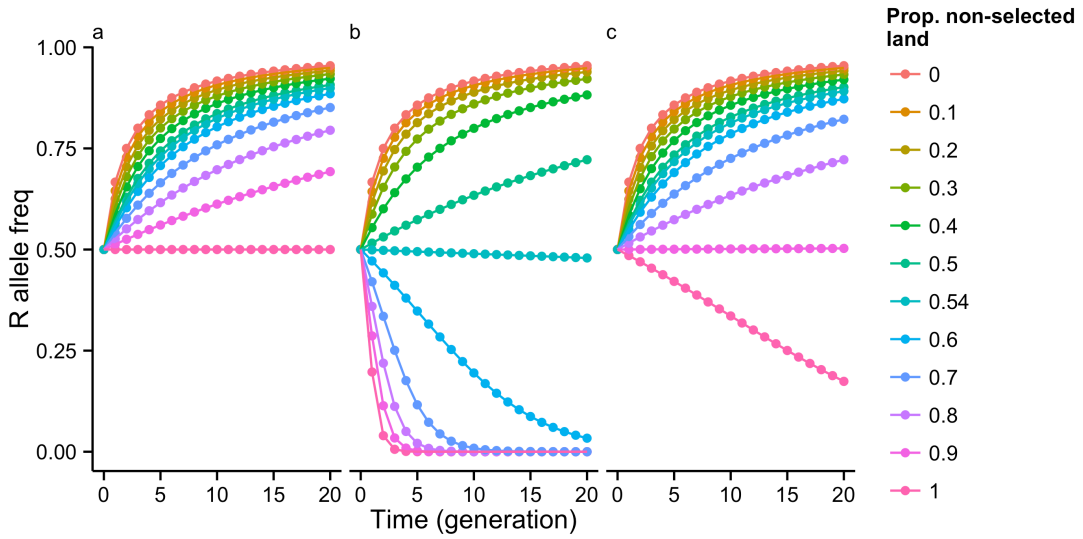


522 Fig 3. Scatter plot of PCA results showing average factor 1 (higher values indicate larger
523 plants) and factor 2 (higher values indicate heavier seeds, higher germination, and fewer
524 abnormally germinating seeds) values for field-collected seeds with circle color
525 indicating survival at 1.7 kg/ha glyphosate.



526

527 Fig 4. A simulation of resistance allele frequencies over time given different selection
528 regimes. Without a cost to resistance (a) the frequency of resistance increases if under
529 selection. When costs are high (b; C_R=0.86, from field-collected germination rate) some
530 selection regimes lead to a decrease in resistance frequency. At moderate levels of costs
531 (c), such as reproductive output under competitive environments found in Vila-Auib *et al.*
532 (2009a, C_R=0.11) resistance can decrease under a small subset of conditions. We used a
533 simple single-locus, 2 allele Hardy-Weinberg population genetics model, with resistance
534 being completely dominant to susceptibility. The net strength of selection for resistance
535 was the weighted average of the strength of selection in sprayed (0) and non-sprayed
536 (cost of resistance = C_R) areas weighted by the proportion of land in each. Similarly the
537 net strength of selection for susceptibility was the weighted average of the strength of
538 selection in sprayed (efficacy of spraying = C_S = 1) and non-sprayed areas (0) weighted
539 by the proportion of land in each.



540

541 **Supporting information**

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

545

Population number	State	Crop	Latitude	Longitude	N_{fam} germ	N_{seeds} germ	N early growth
1	TN	Corn	35.775237	-85.903419	8	31	10
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
9	NC	Soy	34.924044	-77.796171	40	189	46
10	NC	Soy	34.983161	-78.039309	28	123	24
11	NC	Corn	34.527135	-78.756704	38	178	81
12	SC	Cotton	34.145812	-79.865313	42	202	79
14	NC	Soy	35.424763	-77.917121	40	192	76
15	SC	Soy	34.104209	-79.073735	41	193	81
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
20	TN	Corn	35.830692	-85.777871	38	169	13
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
30	TN	Corn	35.31105	-85.945003	42	206	82
31	TN	Corn	35.608482	-85.846379	31	141	69
32	TN	Corn	35.099356	-86.225509	15	56	33
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
43	VA	Soy	36.886448	-78.553156	24	89	28
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
47	SC	Soy	34.282132	-79.746597	41	201	83
48	TN	Corn	35.31653	-87.35373	31	151	74
51	TN	Soy	35.533413	-85.951902	24	118	56
52	OH	Corn	41.284684	-83.847252	62	310	88

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

		Survival at 1.7 kg a.i. /ha		Latitude		Longitude	
Trait	d.f.	*b*	t	*b*	t	*b*	t
Seed weight	39	-0.00506	-2.24*	0.00068	2.75**	-0.00033	-1.95
Percent germination	39	-0.8484	-7.65***	0.03227	2.64**	-0.01885	-2.3*
Percent abnormally germinating seeds	39	0.67661	5.88***	-0.02331	-1.84	0.024	2.82**
Percent needing scarification	39	0.09051	1.53	-0.01955	-3.0**	-0.01346	-3.07**
Percent of scarified that germinated	36	-0.49897	-2.44*	0.01504	0.66	-0.0022	-0.15
Height	39	-3.23167	-3.49**	-0.07628	-0.75	0.14928	2.18*
Leaf number	39	-0.71189	-3.36**	0.0067	0.29	0.03242	2.07*
Largest leaf size	39	-1.28809	-4.21**	0.0274	0.81	0.03655	1.61

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

554 **References**

555 **Abramoff MD, Magelhaes PJ, Ram SJ. 2004.** Image Processing with ImageJ.
556 *Biophotonics International* **11**(7): 36-42.
557 **Ahrens WH, Stoller E. 1983.** Competition, growth rate, and CO_2 fixation in triazine-
558 susceptible and-resistant smooth pigweed (*Amaranthus hybridus*). *Weed*
559 *Science* **31**(4): 438-444.
560 **Alcocer-Ruthling M, Thill DC, Shafii B. 1992.** Differential competitiveness of
561 sulfonylurea resistant and susceptible prickly lettuce (*Lactuca serriola*).
562 *Weed Technology* **6**(2): 303-309.
563 **Andreev D, Kreitman M, Phillips TW, Beeman RW. 1999.** Multiple origins of
564 cyclodiene insecticide resistance in *Tribolium castaneum* (Coleoptera:
565 Tenebrionidae). *Journal of Molecular Evolution* **48**(5): 615-624.
566 **Barrett SH. 1983.** Crop mimicry in weeds. *Economic Botany* **37**(3): 255-282.
567 **Baucom RS, Mauricio R. 2004.** Fitness costs and benefits of novel herbicide
568 tolerance in a noxious weed. *Proceedings of the National Academy of Sciences*
569 *of the United States of America* **101**(36): 13386-13390.
570 **Baucom RS, Mauricio R. 2008.** Constraints on the evolution of tolerance to
571 herbicide in the common morning glory: resistance and tolerance are
572 mutually exclusive. *Evolution* **62**(11): 2842-2854.
573 **Bergelson J, Purrington CB. 1996.** Surveying patterns in the cost of resistance in
574 plants. *American Naturalist* **148**(3): 536-558.
575 **Brabham CB, Gerber CK, Johnson WG. 2011.** Fate of glyphosate-resistant giant
576 ragweed (*Ambrosia trifida)* in the presence and absence of glyphosate. *Weed*
577 *Science* **59**(4): 506-511.
578 **Christopher JT, Powles SB, Holtum JA. 1992.** Resistance to acetolactate synthase-
579 inhibiting herbicides in annual ryegrass (*Lolium rigidum*) involves at least
580 two mechanisms. *Plant Physiology* **100**(4): 1909-1913.
581 **Christopher JT, Powles SB, Liljegren DR, Holtum JA. 1991.** Cross-resistance to
582 herbicides in annual ryegrass (*Lolium rigidum*) II. chlorsulfuron resistance
583 involves a wheat-like detoxification system. *Plant Physiology* **95**(4): 1036-
584 1043.
585 **Christopher JT, Preston C, Powles SB. 1994.** Malathion antagonizes metabolism-
586 based chlorsulfuron resistance in *Lolium rigidum*. *Pesticide Biochemistry and*
587 *Physiology* **49**(3): 172-182.
588 **Coley PD, Bryant JP, Chapin III FS. 1985.** Resource availability and plant
589 antiherbivore defense. *Science* **230**: 895-899.
590 **Cousens R, Gill G, Speijers EJ. 1997.** Comment: number of sample populations
591 required to determine the effects of herbicide resistance on plant growth and
592 fitness. *Weed Research* **37**(1): 1-4.
593 **Culpepper AS. 2006.** Glyphosate-induced weed shifts. *Weed Technology* **20**(2):
594 277-281.
595 **Daborn P, Yen J, Bogwitz M, Le Goff G, Feil E, Jeffers S, Tijet N, Perry T, Heckel**
596 **D, Batterham P. 2002.** A single P450 allele associated with insecticide
597 resistance in *Drosophila*. *Science* **297**(5590): 2253-2256.

598 **Darmency H, Menchari Y, Le Corre V, Délye C. 2015.** Fitness cost due to herbicide
599 resistance may trigger genetic background evolution. *Evolution* **69**(1): 271-
600 278.
601 **Debban C, Matthews S, Elliot L, Pieper K, Wilson A, Baucom RS. *In press*.** An
602 examination of fitness costs of glyphosate resistance in the common morning
603 glory, *Ipomoea purpurea*. *Ecology and Evolution*.
604 **Délye C, Jasieniuk M, Le Corre V. 2013a.** Deciphering the evolution of herbicide
605 resistance in weeds. *Trends in Genetics* **29**(11): 649-658.
606 **Délye C, Menchari Y, Michel S, Cadet É, Le Corre V. 2013b.** A new insight into
607 arable weed adaptive evolution: mutations endowing herbicide resistance
608 also affect germination dynamics and seedling emergence. *Annals of Botany*
609 **111**(4): 681-691.
610 **Délye C, Michel S, Bérard A, Chauvel B, Brunel D, Guillemin JP, Dessaint F, Le**
611 **Corre V. 2010.** Geographical variation in resistance to acetyl‐coenzyme A
612 carboxylase‐inhibiting herbicides across the range of the arable weed
613 *Alopecurus myosuroides* (black‐grass). *New Phytologist* **186**(4): 1005-1017.
614 **Dinelli G, Marotti I, Catizone P, Bosi S, Tanveer A, Abbas R, Pavlovic D. 2013.**
615 Germination ecology of *Ambrosia artemisiifolia* L. and *Ambrosia trifida* L.
616 biotypes suspected of glyphosate resistance. *Open Life Sciences* **8**(3): 286-
617 296.
618 **Fenner M. 1991.** The effects of the parent environment on seed germinability. *Seed*
619 *Science Research* **1**(02): 75-84.
620 **Fernandez-Cornejo J, Nehring R, Osteen C, Wechsler S, Martin A, Vialou A.**
621 **2014.** Pesticide use in U.S. agriculture: 21 selected crops, 1960-2008, EIB-
622 124: U.S. Department of Agriculture, Economic Research Service.
623 **Fisher RA. 1958.** *The genetical theory of natural selection*. New York: Dover
624 Publishing Inc.
625 **Forcella F, Arnold RLB, Sanchez R, Ghersa CM. 2000.** Modeling seedling
626 emergence. *Field Crops Research* **67**(2): 123-139.
627 **Galloway LF. 2001.** The effect of maternal and paternal environments on seed
628 characters in the herbaceous plant *Campanula americana* (Campanulaceae).
629 *American Journal of Botany* **88**(5): 832-840.
630 **Gemmill AW, Read AF. 1998.** Counting the cost of disease resistance. *Trends in*
631 *Ecology & Evolution* **13**(1): 8-9.
632 **Giacomini D, Westra P, Ward SM. 2014.** Impact of genetic background in fitness
633 cost studies: an example from glyphosate-resistant *Palmer amaranth*. *Weed*
634 *Science* **62**(1): 29-37.
635 **Glettner CE, Stoltenberg DE. 2015.** Noncompetitive growth and fecundity of
636 Wisconsin giant ragweed resistant to glyphosate. *Weed Science* **63**(1): 273-
637 281.
638 **Goh SS, Vila‐Aiub MM, Busi R, Powles SB. 2015.** Glyphosate resistance in
639 *Echinochloa colona*: phenotypic characterisation and quantification of
640 selection intensity. *Pest management science*.
641 **Gould F. 1995.** Comparisons between resistance management strategies for insects
642 and weeds. *Weed Technology*: 830-839.

Hartl DL, Clark AG. 1989. *Principles of population genetics*. Sunderland, Mass.: Sinauer associates.

Hartley C, Newcomb R, Russell R, Yong C, Stevens J, Yeates D, La Salle J, Oakeshott J. 2006. Amplification of DNA from preserved specimens shows blowflies were preadapted for the rapid evolution of insecticide resistance. *Proceedings of the National Academy of Sciences* **103**(23): 8757-8762.

Heap I 2015. The International survey of herbicide resistant weeds.

Herms DA, Mattson WJ. 1992. The dilemma of plants: to grow or defend. *Quarterly Review of Biology* **67**(3): 283-335.

Holt J. 1988. Reduced growth, competitiveness, and photosynthetic efficiency of triazine-resistant *Senecio vulgaris* from California. *Journal of Applied Ecology* **25**(1): 307-318.

Ismail B, Chuah T, Salmijah S, Teng Y, Schumacher R. 2002. Germination and seedling emergence of glyphosate‐resistant and susceptible biotypes of goosegrass (*Eleusine indica* [L.] Gaertn.). *Weed Biology and Management* **2**(4): 177-185.

Jasieniuk M, Maxwell BD. 1994. Populations genetics and the evolution of herbicide resistance in weeds. *Phytoprotection* **75**(4): 25-35.

Kuester A, Chang SM, Baucom RS. *2015*. The geographic mosaic of herbicide resistance evolution in the common morning glory, *Ipomoea purpurea*: Evidence for resistance hotspots and low genetic differentiation across the landscape. *Evolutionary Applications*.

Lande R. 1983. The response to selection on major and minor mutations affecting a metrical trait. *Heredity* **50**(1): 47-65.

Leslie T, Baucom RS. 2014. De novo assembly and annotation of the transcriptome of the agricultural weed *Ipomoea purpurea* uncovers gene expression changes associated with herbicide resistance. *G3: Genes| Genomes| Genetics* **4**(10): 2035-2047.

Lewontin RC. 1974. *The genetic basis of evolutionary change*. New York: Columbia University Press.

Macnair M. 1991. Why the evolution of resistance to anthropogenic toxins normally involves major gene changes: the limits to natural selection. *Genetica* **84**(3): 213-219.

Menchari Y, Camilleri C, Michel S, Brunel D, Dessaint F, Le Corre V, Délye C. 2006. Weed response to herbicides: regional‐scale distribution of herbicide resistance alleles in the grass weed *Alopecurus myosuroides*. *New Phytologist* **171**(4): 861-874.

Menchari Y, Chauvel B, Darmency H, Délye C. 2008. Fitness costs associated with three mutant acetyl‐coenzyme A carboxylase alleles endowing herbicide resistance in black‐grass *Alopecurus myosuroides*. *Journal of Applied Ecology* **45**(3): 939-947.

Mortimer AM. 1997. Phenological adaptation in weeds—an evolutionary response to the use of herbicides? *Pesticide science* **51**(3): 299-304.

686 **Neve P, Busi R, Renton M, Vila‐Aiub MM. 2014.** Expanding the eco‐
687 evolutionary context of herbicide resistance research. *Pest management*
688 *science* **70**(9): 1385-1393.
689 **O'Donovan J, Newman J, Blackshaw R, Harker K, Derksen D, Thomas A. 1999.**
690 Growth, competitiveness, and seed germination of triallate/difenzoquat-
691 susceptible and-resistant wild oat populations. *Canadian Journal of Plant*
692 *Science* **79**(2): 303-312.
693 **Owen MJ, Goggin DE, Powles SB. 2014.** Intensive cropping systems select for
694 greater seed dormancy and increased herbicide resistance levels in *Lolium*
695 *rigidum* (annual ryegrass). *Pest management science*.
696 **Paris M, Roux F, Berard A, Reboud X. 2008.** The effects of the genetic background
697 on herbicide resistance fitness cost and its associated dominance in
698 *Arabidopsis thaliana*. *Heredity* **101**(6): 499-506.
699 **Pedersen BP, Neve P, Andreasen C, Powles SB. 2007.** Ecological fitness of a
700 glyphosate-resistant *Lolium rigidum* population: Growth and seed production
701 along a competition gradient. *Basic and Applied Ecology* **8**(3): 258-268.
702 **Pilling J, Willmitzer L, Fisahn J. 2000.** Expression of a *Petunia inflata* pectin
703 methyl esterase in *Solanum tuberosum* L. enhances stem elongation and
704 modifies cation distribution. *Planta* **210**(3): 391-399.
705 **Platenkamp GA, Shaw RG. 1993.** Environmental and genetic maternal effects on
706 seed characters in *Nemophila menziesii*. *Evolution* **47**(2): 540-555.
707 **Powles SB, Yu Q. 2010.** Evolution in action: plants resistant to herbicides. *Annual*
708 *review of plant biology* **61**: 317-347.
709 **Preston C, Powles S. 2002.** Evolution of herbicide resistance in weeds: initial
710 frequency of target site-based resistance to acetolactate synthase-inhibiting
711 herbicides in *Lolium rigidum*. *Heredity* **88**(1): 8-13.
712 **Preston C, Powles SB. 1998.** Amitrole inhibits diclofop metabolism and synergises
713 diclofop-methyl in a diclofop-methyl-resistant diotype of *Lolium rigidum*.
714 *Pesticide Biochemistry and Physiology* **62**(3): 179-189.
715 **Primack RB, Kang H. 1989.** Measuring fitness and natural selection in wild plant
716 populations. *Annual Review of Ecology and Systematics* **20**: 367-396.
717 **Rausher MD, Simms EL. 1989.** The evolution of resistance to herbivory in *Ipomoea*
718 *purpurea.* I. Attempts to detect selection. *Evolution* **43**(4): 563-572.
719 **Ren C, Kermode AR. 2000.** An increase in pectin methyl esterase activity
720 accompanies dormancy breakage and germination of yellow cedar seeds.
721 *Plant Physiology* **124**(1): 231-242.
722 **Roach DA, Wulff RD. 1987.** Maternal effects in plants. *Annual Review of Ecology and*
723 *Systematics* **18**: 209-235.
724 **Schmitt J, Niles J, Wulff RD. 1992.** Norms of reaction of seed traits to maternal
725 environments in *Plantago lanceolata*. *American Naturalist* **139**(3): 451-466.
726 **Shrestha A, Steinhauer KM, Moretti ML, Hanson BD, Jasieniuk M, Hembree KJ,**
727 **Wright SD. 2014.** Distribution of glyphosate-resistant and glyphosate-
728 susceptible hairy fleabane (*Conyza bonariensis*) in central California and their
729 phenological development. *Journal of pest science* **87**(1): 201-209.

730 **Strauss SY, Rudgers JA, Lau JA, Irwin RE. 2002.** Direct and ecological costs of
731 resistance to herbivory. *Trends in Ecology & Evolution* **17**(6): 278-285.
732 **Tardif FJ, Rajcan I, Costea M. 2006.** A mutation in the herbicide target site
733 acetohydroxyacid synthase produces morphological and structural
734 alterations and reduces fitness in *Amaranthus powellii*. *New Phytologist*
735 **169**(2): 251-264.
736 **Vila-Aiub MM, Goh SS, Gaines TA, Han H, Busi R, Yu Q, Powles SB. 2014.** No
737 fitness cost of glyphosate resistance endowed by massive EPSPS gene
738 amplification in *Amaranthus palmeri*. *Planta* **239**(4): 793-801.
739 **Vila-Aiub MM, Neve P, Powles SB. 2009a.** Evidence for an ecological cost of
740 enhanced herbicide metabolism in *Lolium rigidum*. *Journal of Ecology* **97**(4):
741 772-780.
742 **Vila-Aiub MM, Neve P, Powles SB. 2009b.** Fitness costs associated with evolved
743 herbicide resistance alleles in plants. *New Phytologist* **184**(4): 751-767.
744 **Vila-Aiub MM, Neve P, Roux F. 2011.** A unified approach to the estimation and
745 interpretation of resistance costs in plants. *Heredity* **107**(5): 386-394.
746 **Vila-Aiub MM, Neve P, Steadman K, Powles S. 2005.** Ecological fitness of a
747 multiple herbicide‐resistant *Lolium rigidum* population: dynamics of seed
748 germination and seedling emergence of resistant and susceptible
749 phenotypes. *Journal of Applied Ecology* **42**(2): 288-298.
750 **Vogwill T, Lagator M, Colegrave N, Neve P. 2012.** The experimental evolution of
751 herbicide resistance in *Chlamydomonas reinhardtii* results in a positive
752 correlation between fitness in the presence and absence of herbicides.
753 *Journal of Evolutionary Biology* **25**(10): 1955-1964.
754 **Weaver S, Warwick S. 1982.** Competitive relationships between atrazine resistant
755 and susceptible populations of *Amaranthus retroflexus* and *A. powellii* from
756 southern Ontario. *New Phytologist* **92**(1): 131-139.
757 **Weaver SE, Cavers PB. 1979.** The effects of date of emergence and emergence
758 order on seedling survival rates in *Rumex crispus* and *R. obtusifolius*.
759 *Canadian Journal of Botany* **57**(7): 730-738.
760 **Webster TM, MacDonald GE. 2001.** A survey of weeds in various crops in Georgia
761 1. *Weed Technology* **15**(4): 771-790.
762 **Williams MM, Jordan N, Yerkes C. 1995.** The fitness cost of triazine resistance in
763 jimsonweed (*Datura stramonium* L.). *American Midland Naturalist*: 131-137.
764 **Yu Q, Collavo A, Zheng M-Q, Owen M, Sattin M, Powles SB. 2007.** Diversity of
765 acetyl-coenzyme A carboxylase mutations in resistant *Lolium* populations:
766 evaluation using clethodim. *Plant Physiology* **145**(2): 547-558.
767 **Yu Q, Han H, Powles SB. 2008.** Mutations of the ALS gene endowing resistance to
768 ALS‐inhibiting herbicides in *Lolium rigidum* populations. *Pest management*
769 *science* **64**(12): 1229-1236.
770 **Yu Q, Han H, Vila-Aiub MM, Powles SB. 2010.** AHAS herbicide resistance
771 endowing mutations: effect on AHAS functionality and plant growth. *Journal*
772 *of Experimental Botany* **61**(14): 3925-3934.
773
774