Adaptive and maladaptive expression plasticity underlying herbicide resistance in an agricultural weed.

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Abstract

Plastic phenotypic responses to environmental change are common, yet we lack a clear understanding of the fitness consequences of these plastic responses. Here, we use the evolution of herbicide resistance in the common morning glory (*Ipomoea purpurea*) as a model for understanding the relative importance of adaptive and maladaptive gene expression responses to herbicide. Specifically, we compare leaf gene expression changes caused by herbicide spray to the expression changes that evolve in response to artificial selection for herbicide resistance. We identify a number of genes that show plastic and evolved responses to herbicide and find that for the majority of genes with both plastic and evolved responses, plastic responses appear to be adaptive. We also find that selection for herbicide response increases gene expression plasticity. Overall, these results show the importance of adaptive plasticity for herbicide resistance in a common weed and that expression changes in response to strong environmental change can be adaptive.

Introduction

Determining the fitness consequences of plastic responses is crucial for predicting response to selection (Reed et al. 2010), understanding the maintenance of variation for phenotypes (Josephs 2018), and breeding plants for new, challenging environments (Gage et al. 2017; Kusmec et al. 2018). However, we lack a clear view of how the plastic responses affect fitness (Bradshaw 1965; Schlichting 1986; Via et al. 1995; Ghalambor et al. 2007). Despite hypotheses and evidence that gene expression changes underlie many plastic responses in traditional phenotypes, the fitness consequences of gene expression plasticity are unknown (Hodgins-Davis and Townsend 2009; Lovell et al. 2016).

A number of approaches have been used to describe the fitness consequences of plasticity. For example, some studies have measured fitness in organisms where a specific plastic response has or has not been induced to evaluate whether the plastic response is adaptive in the environments that induce it (Dudley and Schmitt 1996; Schmitt et al. 1999). However, many plastic responses, including expression level, are not amenable to these types of manipulations. An alternative approach to understanding the fitness consequences of plasticity is comparing the fitness of genetically distinct individuals that exhibit different levels of plasticity (Weis and Gorman 1990; Scheiner and Callahan 1999; Stinchcombe et al. 2004; Baythavong and Stanton 2010; Wagner and Mitchell-Olds 2018). However, these quantitative genetics based approaches require the

presence of natural genetic variation for plasticity as well as large numbers of phenotypic measurements, which are prohibitively expensive to apply to gene expression.

Here, we determine whether plastic changes in gene expression are adaptive or maladaptive by comparing plastic expression changes elicited by an environment with the expression changes that evolve during adaptation to that environment. We conduct this experiment in the context of an agricultural weed evolving herbicide resistance. If plasticity is adaptive and increases fitness in the new environment, the direction of plastic responses is expected to match the direction of evolved responses. Alternatively, if plasticity is maladaptive, we expect plastic responses to be opposite that of adaptive responses. Previous applications of this approach have found that plastic expression changes tend to be maladaptive in guppy brain expression response to predation environments (Ghalambor et al. 2015) and Drosophila gene expression responses to diet (Huang and Agrawal 2016). An analysis of the previously mentioned guppy study along with studies from *Escherichia coli* and yeast also found a preponderance of maladaptive expression plasticity (Ho and Zhang 2018; Ho and Zhang 2019). Strikingly, this approach has not been widely applied beyond these few studies.

The fitness consequences of plastic responses are especially relevant for a thorough understanding of fitness trade-offs across environments. While local adaptation has traditionally been thought of as a consequence of fitness trade-offs between different environments, there is increasing evidence that conditionally neutral loci with effects only in one environment are important for local adaptation (Kawecki and Ebert 2004; Savolainen et al. 2013; Wadgymar et al. 2017). If plastic responses tend to increase fitness in specific environments, then alleles responsible for plastic responses are likely to be conditionally neutral. For example, if coping with a specific stress involves a phenotype that is costly in environments without that stress, then a mechanism causing that phenotype to only be expressed in the presence of stress (*i.e.* an induced phenotype) will circumvent the tradeoff. However, we lack information about how often responses to selection in a specific environment involve constitutive or environment-specific responses, or essentially, how often selection within a specific environment will act on plasticity.

We use artificially evolved glyphosate-resistant lineages of the common morning glory, *Ipomoea purpurea,* as a model for examining the fitness consequences of plastic changes in gene expression. There is a long history of using pesticide resistance evolution as models for adaptation (Ffrench-Constant 2013; Baucom 2019) and evidence that resistance to pesticides is costly in some systems (Bergelson and Purrington 1996; Ffrench-Constant and Bass 2017; Baucom 2019) but there is a significant gap in our understanding of the role of plasticity in shaping resistance evolution. Resistance to glyphosate (i.e., the active ingredient in the herbicide RoundUp) can involve either 'target-site' or 'non-target site' mutations. In the former case, the genes that are the target of the herbicide contain mutations that lead to resistance whereas in the latter case any other gene that is not the target of the herbicide may be involved (Baucom 2019). Although comparatively less is understood about non-target site resistance, this resistance mechanism can often be a subset of general plastic responses to abiotic stress which often involves multiple genes (Délye et al. 2013).

The common morning glory provides an excellent study system for examining questions about the role of adaptive versus maladaptive plasticity on the process of herbicide resistance evolution. Natural populations of *I. purpurea* vary in the level of glyphosate resistance (Kuester et al. 2015), with non-target site herbicide resistance the most likely explanation for resistance in this species (Van Etten et al 2020). There is strong evidence of fitness costs associated with resistance in *I. purpurea*, which is consistent with the idea that resistance incurs a trade-off (Debban et al. 2015; Van Etten et al. 2016). However, the specific role of plasticity in resistance evolution in *I. purpurea*, or any other weedy species is unknown.

In this study, we use seeds from an experimental evolution experiment designed to select for increased herbicide resistance in plants descending from a single population (Debban et al. 2015). We compared leaf transcriptomes of plants from the experimentally evolved resistant lineages and from control, non-evolved lineages that were exposed to glyphosate along with replicates of each selection line that were not treated with the herbicide. We used these transcriptomes to quantify how often plastic expression responses to glyphosate aligned with the expression changes that evolved during selection for resistance. We also investigated whether selection in the herbicide environment selected for increased plasticity or for constitutive changes that occur across environments. Overall, our results demonstrate a preponderance of adaptive gene expression plasticity in response to herbicide and that selection for increased resistance increases plasticity.

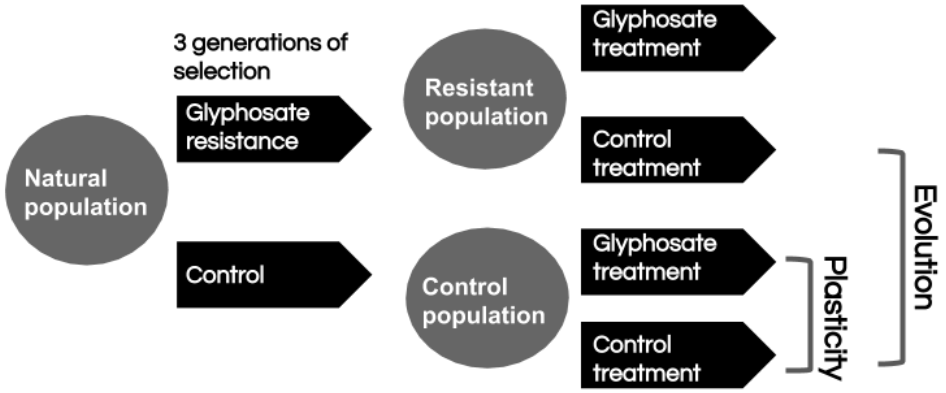

Figure 1: Conceptual figure of the experimental design

Results

Plastic and evolved expression responses to herbicide

Morning glory leaf gene expression responded plastically to herbicide, both 8 and 32 hours after herbicide application. Out of the 27,037 genes tested, 3,519 genes had higher expression and 2,841 genes had lower expression 8 hours after spray (FDR < 0.1) and 5,732 genes had higher expression and 5,285 genes had lower expression 32 hours after spray (FDR < 0.1). The log fold change of expression values between spray and not spray was correlated across time points (cor coeff = 0.614, $p < 0.001$, Figure S1). There were 137 genes with significant (FDR < 0.1) plastic responses to spray at both time points where the direction of response differed between the 8 hour and 32 hour time points. The genes with changes in the direction of plastic response were enriched for GO terms related to response to endoplasmic reticulum stress (FDR = 2.80×10^{-3}). Because of the similarity of response at 8 and 32 hours after spray, we pooled data for future analyses.

When data from both time points was pooled to estimate plastic responses to spray in general, 5,734 genes had lower expression in response to pesticide spray and 6,171 had increased expression in response to spray (FDR < 0.1, Figure 1). Genes with plastic responses to spray were enriched for a number of GO

biological processes at FDR < 0.05, including a number of terms related to chlorophyll and photosynthesis (Table S1).

Gene expression in *I. purpurea* leaves in non-herbicide conditions responded to selection for resistance. After three generations of selection for increased resistance, 166 genes showed decreased expression and 133 genes showed increased expression (FDR < 0.1, Figure 1). These genes were enriched for a number of GO biological processes including many related to protein phosphorylation and phosphorus metabolic processes (Table S2).

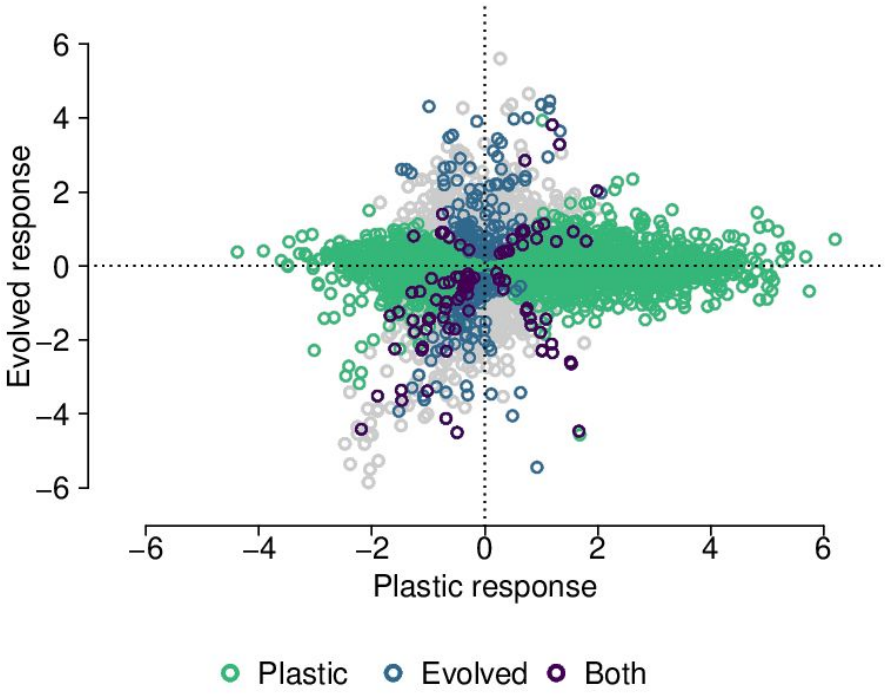

Figure 2: Plastic and evolved changes in expression: A plot of all genes colored by whether they have significant (FDR < 0.1) plastic expression responses, evolved expression responses or both. Each point represents one gene. The X axis shows the log-fold difference in expression between plants sprayed with herbicide and plants not sprayed with herbicide. The Y axis shows the log-fold expression difference between plants that were selected for herbicide resistance and plants that were not, with expression measured in the non-herbicide environment.

The prevalence of adaptive and maladaptive plasticity

There were 94 genes that had both significant plastic responses to spray and evolved responses to selection for resistance in unsprayed conditions (FDR < 0.1). Within these 94 genes, 68 had plastic responses in the same direction as evolved responses, consistent with adaptive plasticity (Figure 2). Overall, with 68 of the 94 genes showing plastic responses in the same direction as evolved responses, these results show that more of the expression responses in response to herbicide are adaptive than maladaptive. We only looked at selection in non-sprayed conditions to avoid confounding selective response and plasticity (Mallard et al. 2018).

The pattern of adaptive plasticity was also evident for gene expression in samples collected at 8 hours after spray, where 21 out of 56 genes with plastic and evolved responses showed adaptive plasticity, and at 32 hours after spray, where 44 of 71 genes with significant adaptive and plastic responses exhibited

adaptive plasticity. While there were more genes that showed evidence of either maladaptive or adaptive plasticity 32 hours after spray, the directionality of the response remained consistent, with only one of the genes changing direction from adaptive after 8 hours to maladaptive after 32 hours. This gene was a homolog of AT5G27730, a protein of unknown function.

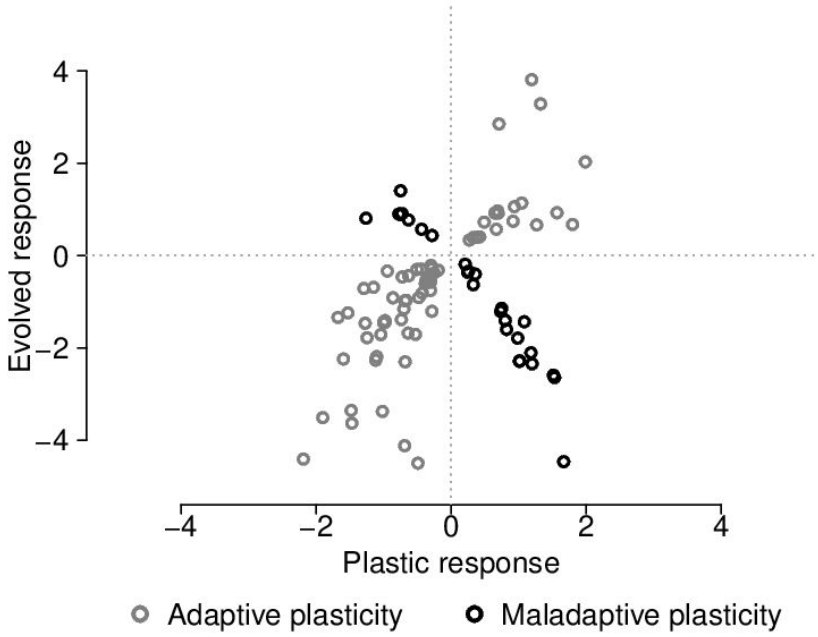

Figure 3: **Adaptive and maladaptive expression plasticity.** The x axis shows plastic responses to glyphosate, specifically the log fold change in expression between sprayed and nonsprayed conditions where positive values indicate increased expression in herbicide spray. The y axis shows the log fold difference in expression between lines selected for increased resistance to herbicide and lines that were not selected ('original lines'), where positive values indicate increased expression in the resistance selection lines. Each point represents a gene and all genes where there was both a significant plastic and an evolved response are shown (FDR < 0.1).

Specific genes showing adaptive and maladaptive plasticity for expression

It was possible to annotate 41 of the 68 genes with evidence of adaptive plasticity based on homology to *Arabidopsis thaliana* (Table S3). Some genes were connected to stress responses, like a homolog of ATR7, which regulates abiotic stress (Sujeeth et al. 2020), a homolog of PATL1 which is important for salt and freezing tolerance (Chu et al. 2018; Zhou et al. 2018), a homolog of PHT4;6 which is important for salt tolerance (Cubero et al. 2009), an ortholog of P5CDH which is important for many abiotic stress responses (Sharma and Verslues 2010; Liu et al. 2015; Ren et al. 2018) and a homolog of AtPTPN, which contributes to drought stress in *A. thaliana* (Zhang et al. 2020). However, many genes identified as having adaptive plasticity did not have homologs with obvious links to processes important for herbicide or other stress responses (Table S3).

We were able to annotate 25 of the 26 genes with evidence of maladaptive plasticity. Some of these genes showed connections to stress responses, such as a homolog of the gene BAK1 (AT4G33430.1)(Liu et al. 2020; Lv and Li 2020). However, most of the genes did not have clear connections to herbicide or other stress responses (Table S3).

Selection for herbicide resistance does not act only on expression in the presence of herbicide

One hypothesis for the prevalence of plasticity is that the evolution of plastic responses may allow organisms to escape trade-offs inherent when the optimum phenotype differs between environments. If this hypothesis is true, we would expect that the response to selection on resistance should cause a stronger evolutionary response in the herbicide environment than in the non-herbicide environment. However, there were 299 genes that showed evidence of selection in non-sprayed conditions and only 135 genes that showed evidence of selection in the herbicide environment (FDR < 0.1). Sixty-three of these genes showed a significant response to selection in both environments. However, for all genes, response to selection was correlated across spray and control environments (Correlation coefficient = 0.63, $p < 0.0001$, Figure 4A, Figure S3). These results suggest that even though selection occured only in the presence of herbicide, many of the genes showed evidence of significant responses to selection in non herbicide conditions as well.

However, despite evidence that the effects of selection for resistance affected the expression of more genes in control than herbicide environments, there was evidence that selection for resistance tended to increase plasticity compared to control lines. After selection for resistance, 13,690 genes had plastic expression responses to herbicide compared to 11,905 before selection (FDR < 0.1, Figure 4B). Similarly, 15,985 genes (59% of all genes studied) had a greater expression response to herbicide after selection for resistance.

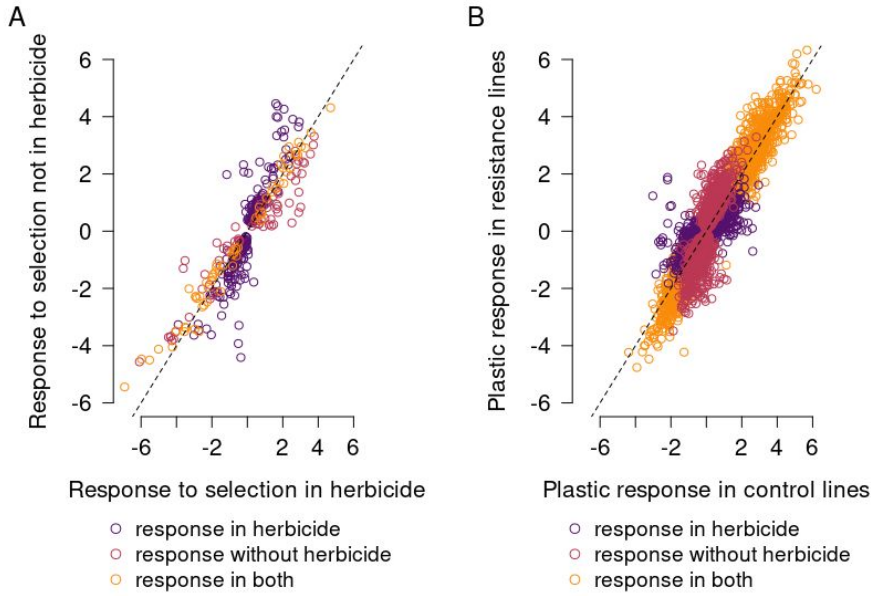

Figure 4: A) Response to selection of expression levels measured in herbicide and non-herbicide environments. Response is shown in a log 2 scale. The x=y line is plotted with a dashed black line. Points are colored by whether they show a significant response to selection in each or both conditions (FDR < 0.1). Genes without a response to selection in either environment are not shown. B) Plastic expression in control lines and lines selected for resistance. As in (A), response is shown on a log2 scale and the x=y line is plotted with a dashed black line. Points are colored by whether they show a significant plastic response to spray. Genes without a significant response in either set of lines are not shown.

Discussion

Here, we have shown that the gene expression plasticity of *Ipomoea purpurea* after glyphosate application is by and large adaptive. The plastic gene expression changes we identified generally align in the same direction as evolved changes after artificial selection for increased herbicide resistance. These results differ from those of other systems, where maladaptive gene expression plasticity appears to be more common (Ghalambor et al. 2015; Huang and Agrawal 2016; Ho and Zhang 2018; Ho and Zhang 2019). There are a number of potential explanations for this difference. First, the population studied here may exist in the condition space where plasticity is predicted to be adaptive (Scheiner 1993; Sultan and Spencer 2002; Price et al. 2003; Reed et al. 2010). For example, the ancestors of the lines used in this study were collected in 2000, after experiencing glyphosate application for at least 8 years (Baucom and Mauricio 2004), so fluctuating selection from herbicide use may have selected for plastic responses to herbicide. In addition, tolerance and resistance to glyphosate were present in *I. purpurea* before the use of glyphosate, suggesting that these plastic traits may be pleiotropic with plastic responses to other stresses (Baucom and Mauricio 2010).

There are a number of potential consequences of widespread adaptive plasticity. Adaptive plasticity could facilitate adaptation to new conditions by allowing populations to persist long enough for adaptation to occur. However, adaptive plasticity could also hinder local adaptation by masking underlying genetic variation from selection. Which process occurs depends on how close the plastic response is to the phenotypic optimum (Price et al. 2003; Ghalambor et al. 2007). In this study, despite the presence of adaptive gene expression plasticity, the population was still able to evolve increased resistance to herbicide (Debban et al. 2015), suggesting that the plastic responses do not completely move plants to the optimum trait value. Interestingly, the results here suggest that further adaptation to herbicide involved non-plastic expression changes that had effects in environments with and without herbicide.

It is unclear how the results of this study would change if evolutionary changes in expression were evaluated in natural populations that have evolved resistance instead of in artificially selected populations. It may be that by estimating evolved responses from an artificially selected population, we have underestimated the amount of plasticity that is adaptive. For example, a study comparing plastic responses to naturally evolved responses found that most plastic responses to dehydration in mice from a wet environment appeared adaptive based on expression comparisons between mice from wet and dry environments (Bittner et al. 2020).

There are some limitations to using leaf gene expression as a trait in studies of plasticity. First, leaves are made up of multiple tissues, so variation in different tissue types, instead of direct increases in the transcription of genes within cells, could drive changes in gene expression. Second, the expression level of different genes is not independent and observations of many genes changing in expression could stem from one or a few trans-regulatory factors (van Gestel and Weissing 2018). Incorporating information about how regulatory networks respond to environmental changes and stresses will be an important next step to understanding the links between selection, plasticity, and adaptation to stressful environments.

This is the first paper to directly investigate the adaptive potential of plastic expression changes in response to herbicide. Previous work has found evidence of gene expression changes in response to herbicide, often in genes not previously known to be important for herbicide response (An et al. 2014; Leslie and Baucom 2014; Duhoux et al. 2015; Serra et al. 2015; Zhao et al. 2017; Wright et al. 2018), but not directly investigated whether plastic responses appeared to be adaptive. One hint at the importance of maladaptive plasticity comes from the observation that a susceptible population of *Amaranthus tuberculatus* had increased

rapid (3 hour) expression response to herbicide compared to a naturally resistant population (Kohlhase et al. 2019), suggesting that plastic expression changes could be maladaptive.

In summary, we have shown that *I. purpurea* gene expression responds plastically to herbicide application and evolutionarily to selection for resistance. Crucially, plastic expression responses generally go in the same direction as evolved responses, consistent with the idea that this plasticity is adaptive. This is one of the first studies to show that expression changes in response to a stimulus are adaptive. We also showed that consistent selection for resistance can increase plasticity, suggesting plasticity is a key component of herbicide resistance in *I. purpurea*. All together, this work demonstrates the importance of adaptive plasticity for the evolution of resistance to environmental stressors.

Methods

Genetic material

All plants used in the experiment descended from a single population at the previous site of the University of Georgia Plant Sciences Farm in Oconee, GA in 2000. We haphazardly sampled seeds from 122 maternal individuals at approximately every 1 meter on a transect in this population. The offspring of this sample (Generation 0 or G0) were screened for high or low glyphosate resistance in a greenhouse (Baucom and Mauricio 2008) and the offspring of the top 20% highly resistant lines (24 families) were used to be the parents for two resistant selection lines (12 parents each) and 24 parents were randomly chosen from the whole population to be the parents of two control lines. Individuals from each set of parents were grown up (Generation 1 or G1) and another generation of artificial selection was performed in the resistant selection lines using a "family selection" design by propagating siblings of the individuals in the top 20% of herbicide resistance in the population. Random individuals within each control line were chosen for the next generation to generate Generation 2 or G2 seeds. Another identical round of selection was performed on the G2 individuals to generate G3 progeny. In a field trial, G3 plants from the resistant lines had higher survival than control lines after herbicide treatment (Debban et al. 2015).

After the field screening of G3 seeds, the 3 most resistant families were chosen from each of the selection lines and seeds from the G2 parents of these six families were crossed to each other (across lines) to generate outcrossed seeds and crossed to themselves to generate selfed seeds. The same procedure was used to generate seeds from the G2 control parents.

Experiment

We planted 20 replicate seeds from each maternal line into two blocks and two treatments (herbicide vs no herbicide) in a randomized block design at the Matthaei Botanical Gardens at the University of Michigan in the spring of 2015. Throughout the experiment, we watered plants when needed to prevent wilting. After 6 weeks of growth, we applied 0.84 kg ai/ha glyphosate to the plants in the herbicide treatment with a CO_2 sprayer. We used a relatively low level of herbicide to avoid killing the plants.

We collected leaf tissue from seeds at two time points post-herbicide application: eight and 32 hours after spraying. Within each time point we randomly chose two individuals per family and froze 1-2 young leaves in liquid nitrogen.

Transcriptome data generation

We extracted RNA from leaf tissue with Qiagen RNAeasy kits. We individually indexed libraries using the llumina TRUseq96 indexer mRNA stranded kit. Pooled libraries were run on 7 lanes of 50bp, single end sequencing on the Illumina HiSeq 4000 resulting in an average of 28 million reads per individuals.

We aligned single-end (1x52nt) adapter-trimmed Illumina RNA-seq reads separately for all samples to a pre-release assembly of the Ipomoea purpurea v 2.0 genome (https://genomevolution.org/coge/GenomeInfo.pl?gid=58735) using the splice aware STAR aligner in its basic two-pass mode (Dobin et al. 2013). Genome annotation for STAR was generated using GATK's CreateSequenceDictionary (v4, (McKenna et al. 2010)), samtools' v1.3 faidx function (Li et al. 2009) and STAR's genomeGenerate option together with an Augustus-derived GFF3 annotation (Stanke et al. 2008) provided with the v.2.0 assembly. The resulting sorted bam alignment files were used for measuring RNA-seq digital gene expression using the provided GFF3 gene annotation and the featureCounts tool in the SubRead package (Liao et al. 2019).

Orthologs in *A. thaliana* were found based on protein homology (TAIR10 v.20101214, https://www.arabidopsis.org/download_files/Proteins/TAIR10_protein_lists/TAIR10_pep_20101214), with the blastp mode of blastall (NCBI v.2.2.26) used to find the closest matching thaliana protein to each Augustus-predicted I purpurea protein sequence with an upper limit p-value for reporting a homolog of 1e-6.

Differential expression analysis

We used Edgr's filterByExpr function with the default settings (minimum 10 counts-per-million per gene) to filter the gene set to 25,534 genes (Chen et al. 2016) We calculated normalization factors that scale each sample by raw library size with Edgr's calcNormFactors function (Anders and Huber 2010; Bullard et al. 2010; Robinson and Oshlack 2010). Read counts were transformed with the voom function in Limma and we used Limma to run linear models that estimated differential expression between samples. (Law et al. 2014; Ritchie et al. 2015; Phipson et al. 2016).

We conducted the following comparisons for differential expression:

1. Identifying genes with plastic responses by comparing non-selected control lines in herbicide spray (n=16) to non-selected control lines not sprayed with herbicide (n=15). This comparison identifies plasticity in the original population before selection but note that these lines were grown in lab conditions for three generations, so drift or lab selection could affect our observations of plasticity. We also conducted these comparisons within the two timepoints tested, so we compared leaves collected 8 hours after spray (n=8) to leaves from unsprayed plants collected at the same time (n=8) and leaves collected 32 hours after spray (n=8) to leaves from unsprayed plants collected at the same time (n=7).
2. Identifying genes with evolved responses after selection for herbicide resistance by comparing resistance selection lines not sprayed (n=15) to original lines not sprayed (n=15).
3. Identifying genes with evolved expression responses in the herbicide environment after selection for herbicide resistance by comparing resistance selection lines in spray conditions (n=15) to original lines in spray conditions (n=15).

We conducted GO enrichment with PANTHER on pantherdb.org (Mi et al. 2019) using the Fisher's exact test with a false discovery rate analysis. We compared genes that had increased expression in herbicide (3,789 annotated genes) or decreased expression (N annotated genes) to all expressed genes from the sample (12,702 annotated genes).

Acknowledgements

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Supplemental figures

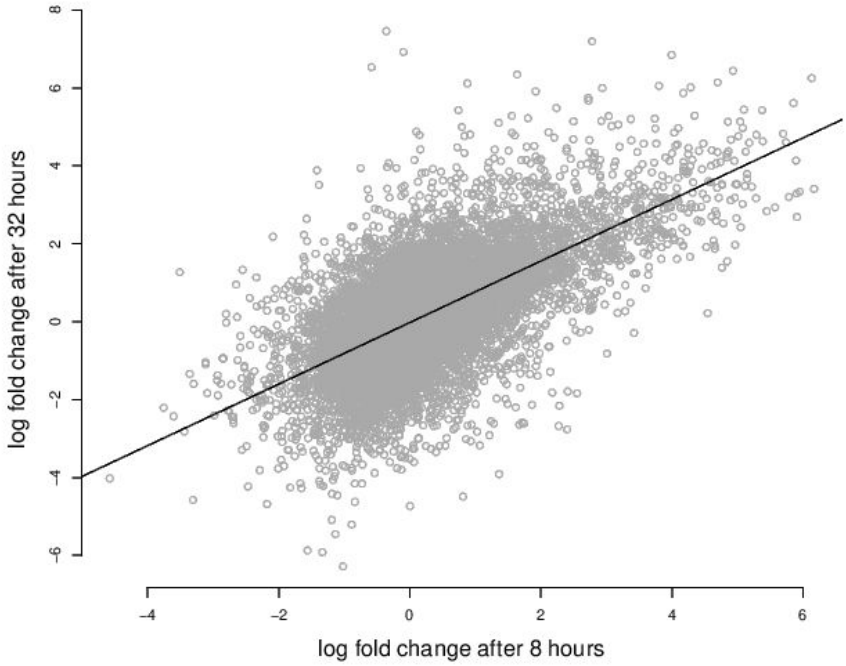

Figure S1: Plastic responses to herbicide spray 8 and 32 hours after treatment. The x axis shows the log-fold difference in expression in sprayed and unsprayed conditions 8 hours after treatment and the y axis shows the same value 32 hours after treatment. The black dotted line shows a line with a slope of 1 and intercept of 0. The correlation coefficient is 0.616. All genes are shown.

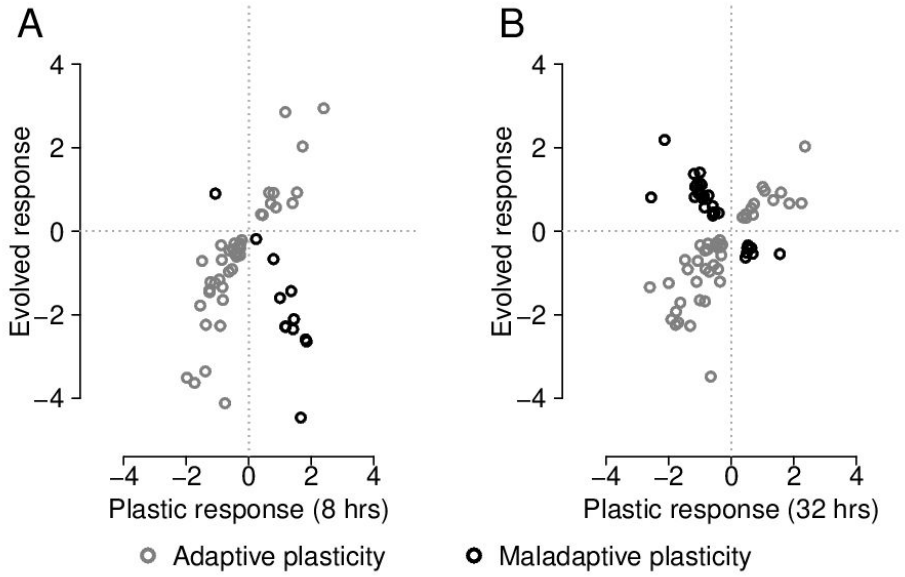

Figure S2: Adaptive and maladaptive expression plasticity at 8 hours (A) and 32 hours (B) after herbicide treatment. In both panels, the x axis shows plastic responses to glyphosate, specifically the log fold change in expression between sprayed and nonsprayed conditions where positive values indicate increased expression in herbicide spray. The y axis shows the log fold difference in expression between lines selected for increased resistance to herbicide and lines that were not selected ('original lines'), where positive values indicate increased expression in the resistance selection lines. Each point represents a gene and all genes where there was both a significant plastic and an evolved response are shown (FDR < 0.1).

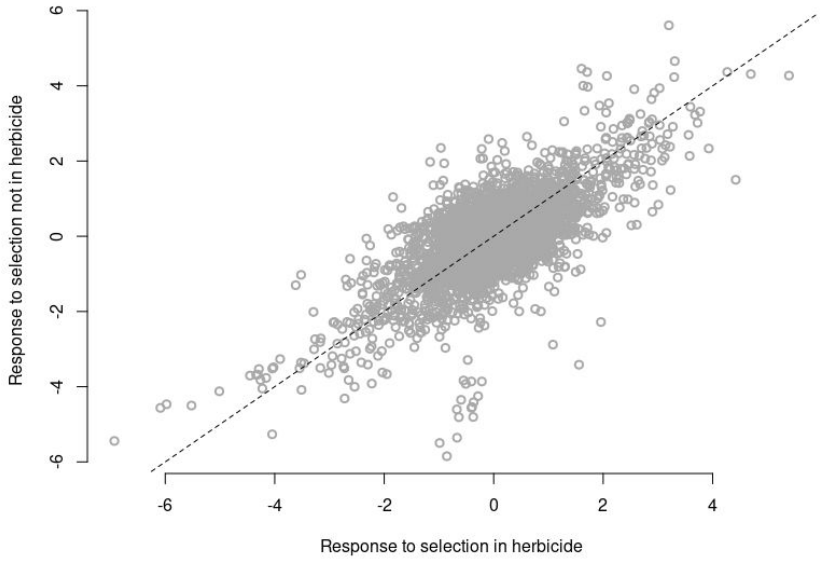

Figure S3: The relationship between response to selection across environments. The x axis shows the log-fold difference in expression in herbicide conditions between lines selected for herbicide resistance and control (unselected) lines while the y axis shows the log-fold difference in expression in non-herbicide conditions between lines that were selected for herbicide resistance and control lines. Each point represents one gene and all genes in the study are shown. The black dotted line shows a line with a slope of 1 and intercept of 0. The correlation coefficient is 0.616. All genes are shown.

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