Glyphosate induces transient male sterility in *Ipomoea purpurea*

Regina S. Baucom, Rodney Mauricio, and Shu-Mei Chang

Abstract: Plant death is the most common effect resulting from the application of glyphosate, the active ingredient in the herbicide Roundup®. Individual seedlings of the morning glory, *Ipomoea purpurea* L. Roth, however, have been shown to exhibit tolerance to glyphosate, surviving after what should have been a lethal dose. Those that grow and reach reproductive maturity often exhibit deformed anthers within what appear to be normally developed flowers. *Ipomoea purpurea* has a mixed mating system and normally has hermaphroditic flowers that are capable of both selfing and outcrossing. The deformed anthers do not produce pollen, essentially converting a hermaphroditic flower to a female. Here we describe this morphological change and investigate the reproductive consequences of anther deformation. First, there is phenotypic variation for the propensity of an individual to exhibit male sterility through deformed anthers in response to treatment, but a series of field and greenhouse studies suggest that this variation is not genetic. The male sterility is also transient; within an individual, the frequency of flowers with deformed anthers declines over time. Although flowers with deformed anthers do not produce pollen, we observed mixed effects on female function of such flowers. In the greenhouse, flowers with deformed anthers that were hand-pollinated produced as many seeds as flowers with normal anthers, suggesting no effect on female fertility. In the field, however, plants with a higher proportion of anther deformation set significantly fewer seeds than those untreated, suggesting either reduced female fertility, or a reproductive penalty in flowers with deformed anthers due to the inability to self pollinate. Thus, the presence of this trait could alter the selfing to outcrossing ratio in populations that are sprayed with the herbicide. Individuals that exhibited a higher proportion of anther deformation also produce fewer total flowers than untreated plants, suggesting that anther deformation is part of a suite of responses to damage by glyphosate.

Key words: anther, glyphosate, male sterility, pollen, reproduction.

Résumé : L'application de glyphosate, agent actif de l'herbicide Roundup®, conduit généralement à la mort de la plante. On a cependant démontré que des plantules individuelles de la gloire du matin, *Ipomoea purpurea* L. Roth, montrent une tolérance au glyphosate en survivant à une dose qui aurait dû s'avérer létale. Les plantes qui se développent et atteignent la maturité montrent souvent des anthères déformées dans ce qui apparaît comme une fleur au développement normal. L'*I. purpurea* possède un double système de croisement impliquant normalement des fleurs hermaphrodites capables d'autofécondation et de fécondation croisée. Les anthères déformées ne produisent pas de pollen, convertissant ainsi la fleur hermaphrodite en fleur femelle. Les auteurs décrivent cette modification morphologique et examinent les conséquences reproductives de la déformation des anthères. D'abord, on observe une variation phénotypique pour la propension d'un individu à afficher une stérilité mâle par des anthères déformées suite au traitement, mais une série d'études en serres et aux champs suggère que cette variation ne soit pas génétique. La stérilité mâle est également transitoire; chez un individu, la fréquence des fleurs avec anthères déformées diminue avec le temps. Bien que les fleurs avec anthères déformées ne produisent pas de pollen, les auteurs observent des effets mixtes sur la fonction femelle de telles fleurs. En serres, les fleurs avec anthères déformées soumises à une pollinisation manuelle produisent autant de graines que des fleurs avec des anthères normales, suggérant l'absence d'effet sur la fertilité femelle. Aux champs,cependant, les plantes avec une forte proportion de déformation des anthères produisent significativement moins de graines que les plantes témoins, ce qui suggère soit une réduction de la fertilité femelle ou soit une pénalité reproductive chez les fleurs avec anthères déformées, liée à l'incapacité d'autofécondation. Ainsi, la présence de ce caractère pourrait altérer le ratio d'auto fécondation vs la fécondation croisée dans les populations traitées avec l'herbicide. Les individus qui montrent une plus forte proportion de déformation des anthères produisent également un nombre total de fleurs plus faible que les plants témoins, ce qui suggère que la déformation des anthères constitue une partie des dommages causés par le glyphosate.

Mots-clés : anthère, glyphosate, stérilité mâle, pollen, reproduction.

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Introduction

The introduction of crops that can tolerate the application of glyphosate (RoundUp®, Monsanto, St. Louis, Miss.) has dramatically increased application of this herbicide (Young 2006). Unfortunately, increased use of any single herbicide can increase the frequency of resistant or tolerant weed species in field populations. In fact, there are presently 13 plant species considered resistant to glyphosate, (www.weedscience.org), and others that show tolerance this herbicide (Cerdeira and Duke 2006).

Ipomoea purpurea L. Roth (Convolvulaceae), the common or tall morning glory, is a species often found in cotton, maize, and soybean fields that have been sprayed with glyphosate (Baucom and Mauricio 2004, 2008). Baucom and Mauricio (2004) showed that tolerant lines of *I. purpurea* maintained a fitness advantage when sprayed with glyphosate, and were expected to increase in frequency in fields where glyphosate was used. Baucom and Mauricio (2004) also identified a significant constraint on the evolution of tolerance: tolerant lines had lower fitness than susceptible lines in the absence of glyphosate. Further examination of the plants in Baucom and Mauricio's (2004) field experiment revealed another possible constraint on the spread of tolerant genotypes: tolerant individuals exhibited some floral abnormalities that could represent a significant barrier to the spread of tolerance to herbicide.

Ipomoea purpurea has perfect flowers and is capable of reproducing both through selfing and outcrossing (Ennos 1981). *Ipomoea purpurea* plants sprayed with glyphosate show a distinctive pattern of damage: vegetative chlorosis followed by leaf necrosis and plant death. Tolerant individuals show leaf damage and necrosis, but the plant is able to recover from the vegetative damage to regrow, eventually becoming reproductive. Two morphological traits are apparent after tolerant plants have regrown: sprayed plants produce many more vegetative stems than controls, and we observe that many flowers have deformed anthers within normally hermaphroditic flowers.

A reduction in male fertility in response to glyphosate application in tolerant individuals could represent a significant barrier to the spread of tolerance to the herbicide. In this paper, we describe the floral abnormalities in depth and the extent of changes in both male and female fertility. Phenotypic and genotypic variation for deformed anthers is also assessed to determine the relative contributions of genotype and environment to the trait.

Materials and methods

Experimental details

Greenhouse experiment

In a previous greenhouse experiment (hereinafter, GH experiment), half-sib families that exhibited either the least change in height or the greatest change in height when treated with 1.121 kg a.e.·ha^{-1} of glyphosate were considered as either "tolerant" or "susceptible" lines, respectively. From field-collected sibling seeds of these families, six tolerant lines were crossed with six susceptible lines; reciprocal pollinations were included. One of the six susceptible lines did not produce enough seed and was, therefore, excluded from this study. These crosses produced 30 full-sib F_1 families and 30 full-sib F_1 reciprocals. Seed from each full-sib F_1 family and its reciprocal was planted in the greenhouse and plants were allowed to self to generate 30 inbred F_2 families and 30 inbred F_2 reciprocal families. An F_2 family resulted from bulking selfed seed from several full-sib F_1 plants.

On 8 February 2005 we planted 9 replicate plants from 11 of the parental lines and 6 replicate plants from each F_1 and F_2 family and their reciprocals for a total of 819 individuals in a completely randomized design, in the greenhouse. Individuals were allowed to grow until 1 April 2005, at which time we applied glyphosate at a rate of 1.121 kg·ha^{-1} with a pressurized CO_2 plot sprayer (R & D Sprayers, Opelousas, La.). Flowering began on 20 June 2005. Every 3 d from this date we counted the number of flowers each individual had produced and the number of flowers that exhibited anther deformation, for a total of 17 census dates. Anther deformation was scored as present if the anthers were considered abnormally small. Flowering data was collected until the end of the experiment on 16 August 2005.

We estimated the proportion of time each individual plant exhibited anther deformation, as well as the proportion of the total number of flowers that exhibited deformed anthers. The proportion time each individual exhibited anther deformation was estimated by summing the number of times the plant exhibited anther deformation and dividing it by the total number of times that plant was assayed for the trait, over the course of the experiment. The proportion of anther deformation was estimated by dividing the number of deformed flowers by the total number of flowers produced.

To determine whether flowers with deformed anthers produced less pollen than normally developed flowers, we collected one anther from four individuals that exhibited anther deformation and one anther from four individuals that produced normal anthers, every 3 weeks starting 27 June over four collection dates; 32 different individuals were assayed, such that no one individual plant was resampled over collection dates. Each plant sampled had been treated with glyphosate. Pollen grains from these anthers were suspended on glass slides and fixed in a basic fuchsin jelly (Kearns and Inouye 1993). Slides were photographed using a Leica DC200 camera fitted onto a Leica MZ6 dissecting microscope. Pollen grains in the captured images were counted using the Count Particle function in the imaging software "NIH-Image J" developed by National Institute of Health (available from rsb.info.nih.gov/ij/download.html).

We performed pollinations over the course of this experiment to determine whether flowers with deformed anthers produced fewer seeds than those that appeared normal. Every third day from the onset of flowering, the status of randomly chosen flowers was marked as either having deformed anthers or normal, and each flower was pollinated using a mix of pollen from three unsprayed control plants. A total of 495 flowers were pollinated; 203 of these flowers were scored as normal and 292 had deformed anthers. The number of seeds per hand-pollinated flower was counted once fruits had matured.

Field experiment 1

The objectives of this experiment were to determine whether glyphosate treatment induced deformed anthers in the study population and to assess the potential for maternal line effects, and thus, genetic variation for the production of deformed anthers. A split-plot design with five replicate blocks was utilized. Within a block, whole plots were randomly assigned to either glyphosate treatment or no herbicide treatment. In each whole-plot replicate, 32 half-sibling families (i.e., maternal lines) were randomized and five scarified seeds were planted per line. Replicates of each family were randomly planted with 1 m^2 spacing within and between rows. We applied glyphosate at a rate of 1.121 $kg \cdot ha^{-1}$ with a hand-held CO_2 pressurized plot sprayer calibrated to a spray volume of 20 gallons per acre, to half of the experimental individuals within each block on 17 July 2002.

The replicates of each maternal line used in this experiment were generated by selfing the families in the greenhouse to minimize the effect of the maternal environment on experimental individuals. Five individuals from each maternal line were randomized within the greenhouse and progeny from each individual was bulked according to family.

We collected flowering data on all experimental individuals in both the control and glyphosate plots over the course of the experiment. Individuals began flowering on 4 August, and every 3 d until the end of the experiment we counted the flower production and marked the presence of the trait. On each sampling date, the presence or absence of the deformed anthers was determined for three flowers on each plant. The trait was scored as present if the anthers were distinctly smaller than normal, and lacking in pollen grains in at least one flower. From these data we estimated the proportion time each individual exhibited the deformed anthers by summing the number of times the plant exhibited the trait and dividing the sum by the total number of times that plant was assayed for the trait over the course of the experiment.

Field experiment 2

We investigated the anthers of flowers in a second field experiment where we used a set of carefully generated genotypes. To produce experimental individuals, six susceptible and six tolerant half-sib family lines were crossed in two six-by-six incomplete diallels. The six tolerant lines were crossed amongst each other within one diallel; the six susceptible lines were crossed similarly in the second diallel. Anthers were removed from each pollen donor and recipient and stored the night before crosses were made, to prevent self-pollination, according to established protocol (Chang and Rausher 1998). These crosses produced 60 full-sib families within 12 maternal and 12 paternal half-sib families.

On 9 July 2004 we planted seven replicates of each family, including reciprocal crosses, into two spatial blocks of a randomized block design in an agricultural field on the University of Georgia's Plant Sciences Farm. We applied glyphosate at a rate of 1.121 kg a.e.$\cdot ha^{-1}$ with a pressurized CO_2 plot sprayer (R & D Sprayers) on 15 August 2004 to experimental plants within each block.

We collected flowering data on all experimental individuals over the course of the experiment. For nine census dates from the onset of flowering we counted the number of flowers each individual produced, noting both the number of fully developed flowers and the flowers that exhibited deformed anthers. Deformed anthers were scored as present if the anthers were distinctly smaller than normal. We collected fruits and counted all viable seeds.

We estimated the proportion time each individual exhibited anther deformation, as well as the proportion of the total number of flowers produced that were scored as anther-deformed. The proportion of anther deformation was estimated by dividing the number of flowers with deformed anthers by the total number of flowers produced.

Data analysis

Greenhouse experiment

To determine whether flowers with deformed anthers produced less pollen or produced fewer seeds than normally developed flowers after the application of glyphosate, a Wilcoxon's signed rank test was performed on both the pollen count data and the seed count data from the GH experiment, with data grouped by the status of the flower (either anther-deformed or normal). To determine whether the proportion of flowers with deformed anthers changed over time, a repeated measures analysis of variance was used with the proportion of flowers with deformed anthers produced each census date as the dependent variable; the proportion of flowers with deformed anthers was first averaged for each sire and $\log_e$-transformed prior to analysis. To test for the presence of genetic variation underlying the anther deformation, the $\log_e$-transformed proportion time of anther deformation and the total proportion of flowers with deformed anthers were the dependent variables in analyses of variance performed according to generation in the multi-generational GH experiment data; parental line was the predictor variable in an analysis of the parents used to generate the F_1's. Dam, sire, and the dam × sire interaction were the predictor variables analyzed within the F_1 generation. In the analysis of the F_2 progeny, the dam used to generate the F_1 family of each F_2 family was the predictor variable. In these analyses, all factors were considered fixed as the experimental individuals used were from lines chosen for their relative level of tolerance or susceptibility to glyphosate.

Field experiment 1

To determine whether glyphosate induced the deformed anthers in the study population and to assess whether maternal lines varied for the proportion time the trait was present, a mixed model analysis of variance was performed using the PROC MIXED command in SAS (version 8.0, SAS Institute Inc., Cary, N.C.). The proportion of time individuals exhibited anther deformation was the response variable, with maternal line, treatment, and block as the independent variables. Maternal line, block, and the block × treatment interactions were considered random, and treatment was considered a fixed effect with the $\log_e$-transformed proportion time individuals exhibited anther deformation as the dependent variable. We chose to use a mixed model for this analysis, as maternal lines used in this field study were randomly chosen from a natural population, i.e., not chosen based on a priori knowledge of the tolerance level of the lines.

Field experiment 2

Data were analyzed using PROC GLM of SAS. Both the

Table 1. Mixed analysis of variance showing the effect of maternal line, treatment with glyphosate, and experimental block on the proportion of time plants exhibited deformed anthers (Proportion time DA) in Field Experiment 1.

Source of variation	Proportion time DA	
Random effects	Variance estimate (10^{-5})	χ^2
Maternal line	6.3±3.0	11.9***
Block	0.53±8.8	0
Block×treatment	15.8±12.6	22.2***
Residual	174.4±7.5	
Fixed effect	df	*F*
Treatment	1, 4	449.9***

Note: **, $P < 0.001$; ***, $P < 0.0001$.

proportion time individuals exhibited anther deformation and the total proportion of flowers with deformed anthers produced per individual were first $\log_e$-transformed and then analyzed separately with dam, sire, the dam by sire interaction (all nested within diallel), diallel and block as the independent variables.

To determine whether the proportion of flowers with deformed anthers changed over time, a repeated measures analysis of variance was used with the proportion of flowers with deformed anthers produced each census date as the dependent variable; the proportion of flowers with deformed anthers was first averaged for each sire and $\log_e$-transformed prior to analysis.

We assessed whether individual seed production and flower production were correlated with the proportion of flowers with deformed anthers by performing the CORR procedure in SAS on $\log_e$-transformed proportion of flowers with deformed anthers and the total numbers of seed and flowers produced. All traits were transformed to improve normality of the residuals and homogenize the variance to meet the assumptions of the ANOVA.

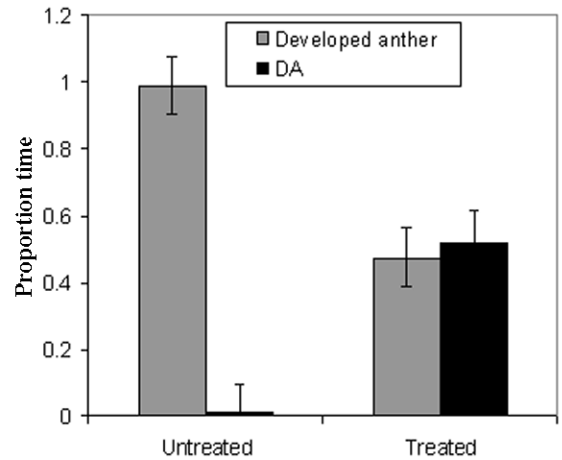
Results

Application of glyphosate in Field Experiment 1 induced deformed anthers (Table 1). Although we found a very low frequency (<1%) of flowers in the control plots with this phenotype, the flowers of sprayed plants exhibited deformed anthers on over 50% of the sampling dates (Fig. 1).

Careful examination of the flowers we scored as having deformed anthers allowed us to describe the phenotype (Fig. 2). The anther deformation was variable, ranging from stamens that completely lacked anthers to anthers that were shrunken and lacked obvious pollen grains. In comparison, normal *I. purpurea* pollen is plentiful and white, and can be seen without magnification. The corollas of flowers with deformed anthers were often shrunken in size compared with normal flowers, but a shrunken corolla was not always an indicator of anther deformation. In flowers with deformed anthers, the gynoecium appeared normal.

Glyphosate application significantly altered the pollen production of *I. purpurea* flowers (data not shown). In the GH experiment, most of the flowers scored as having deformed anthers produced no pollen. In comparison, an

Fig. 1. Proportion of sampling dates on which the experimental individuals exhibited normally developed or deformed anthers (DA) over the course of the first field season in both the untreated and glyphosate-treated plots, averaged across maternal lines (±SE).



anther scored as normal after glyphosate application produced an average of 235 grains of pollen.

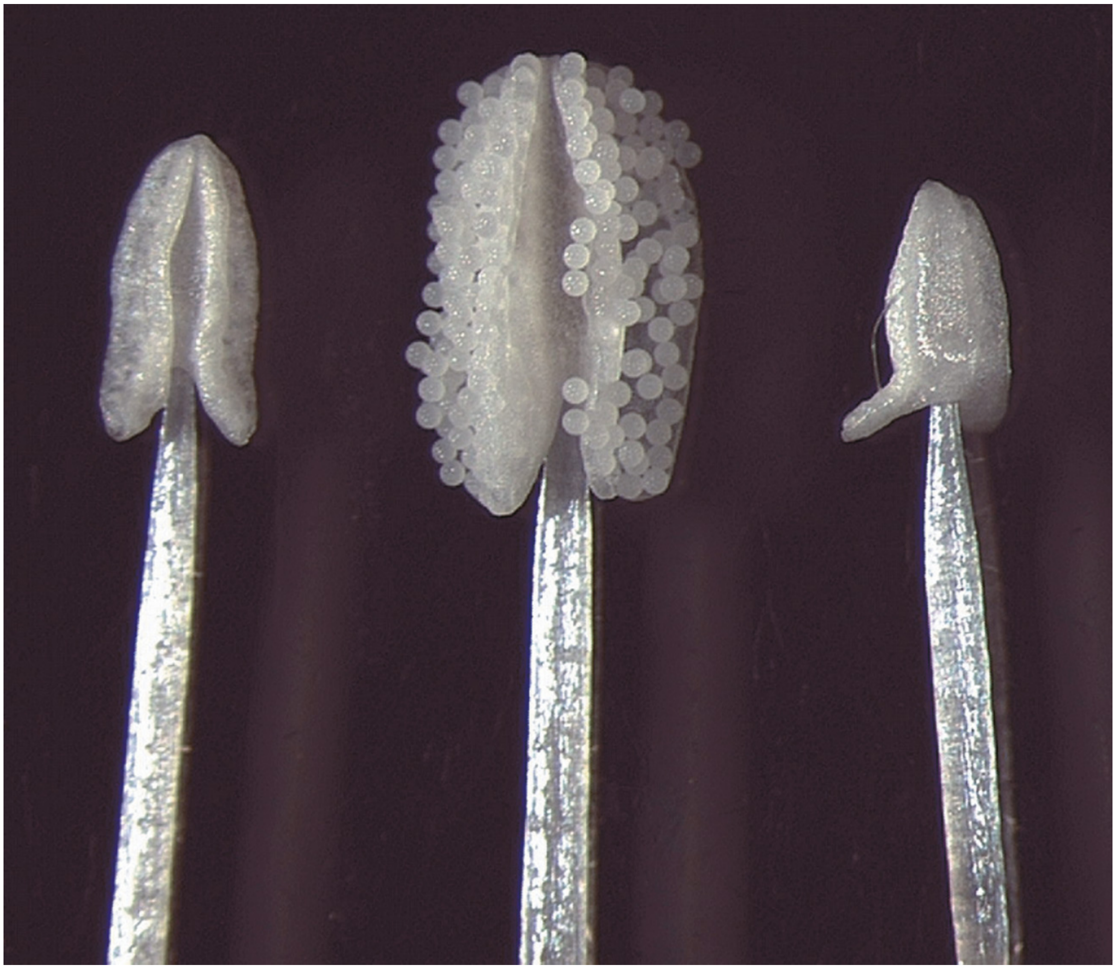
Although the flowers exhibiting anther deformation were male-sterile, they were able to produce, on average, a similar number of seeds as normal flowers when hand-pollinated with control pollen (Wilcoxon's signed rank test $P = 0.39$; mean ± SE: flowers with developed anthers, 0.86 ± 0.11, flowers with deformed anthers, 0.78 ± 0.08).

In the field, however, the proportion of anther deformation was negatively correlated with both the seed production (Pearson's correlation coefficient: –0.6303, $P < 0.0001$; Fig. 3*a*) and the total number of flowers an individual produced (Pearson's correlation coefficient: –0.6633, $P < 0.0001$; Fig. 3*b*). The total number of seeds an individual produced was positively correlated with the total number of flowers it produced after treatment (Pearson's correlation coefficient: 0.8490, $P < 0.0001$).

From both field and GH experiment observations, the anther deformation phenotype is transient; over time, even plants that had been sprayed and had flowers with deformed anthers eventually began producing normal flowers. The proportion of flowers exhibiting anther deformation declined over census dates in both the field experiment and in the GH experiment (Field $F_{[8,72]} = 28.27$, $P < 0.0001$; GH experiment $F_{[16,48]} = 8.75$, $P < 0.0001$). In the field experiment, approximately 70% of the flowers had deformed anthers at the first two census dates, but only 20% by the last census date. Similarly, in the GH experiment, sprayed plants produced 100% of flowers with deformed anthers at the first census but only 35% at the last census (Figs. 4*a* and 4*b*).

We determined whether variation existed among maternal lines for the proportion time plants exhibited the deformed anthers using data from Field Experiment 1 and found evidence for a significant maternal line effect ($\chi^2 = 11.9$, $P = 0.0001$). Given that we selfed replicates of each maternal line in the greenhouse for one generation, potentially removing maternal effects, this result suggested that genetic variation for the trait existed within the population (Table 1) with the chance that the maternal environment might be partially responsible for such pattern.

Fig. 2. The phenotype 'deformed anther'. From left to right: the adaxial side of a deformed anther, a developed anther, and the abaxial view of an deformed anther. Developed and deformed anthers were removed from the open flowers of plants that were treated with glyphosate.



To further investigate the genetics underlying the deformed anthers, we assessed both the proportion of time that individuals exhibited the trait and the proportion of flowers with deformed anthers produced by individuals treated with glyphosate using data from the Field Experiment 2 and the GH experiment. Contrary to the initial results that suggested genetic variation was manifested through maternal line variation for the proportion time the deformed anthers were present, we found no evidence of any significant dam, sire, or dam × sire effects for deformed anthers in Field Experiment 2 (Table 2). Using the multi-generational data from the GH experiment, we found a significant parental line effect (F = 2.34, P = 0.021) for the proportion of time plants exhibited deformed anthers but no significant parental line effect for the proportion of flowers exhibiting deformed anthers (Table 3*a*). There was no evidence for dam or sire effects, providing no evidence for genetic variation underlying the trait in the F_1 generation (Table 3*b*). There was a significant effect of the proportion of time plants had deformed anthers in the F_2 generation (F = 1.89, P = 0.048; Table 3*c*).

Discussion

Although the most commonly reported effect of spraying with the herbicide glyphosate is plant death, other possible responses exist; plants can be resistant and (or) tolerant to glyphosate (Cerdeira and Duke 2006). Baucom and Mauricio (2004) documented tolerance to glyphosate such that genotypes of *I. purpurea* were able to survive treatment, with renewed vegetative growth and eventual production of flowers. In this paper, we report a subtle effect of glyphosate treatment in *I. purpurea*: male sterility.

Our experiments suggest that this male sterility, caused by the lack of proper development of anthers, is the result of glyphosate treatment and is found in plants that are tolerant of the herbicide. In the GH experiment, the deformed anthers produced almost no pollen grains. In the field, plants with deformed anthers were present in the glyphosate treatment plots approximately 55% of the time, while the control plants almost never exhibited this phenotype.

Floral abnormalities, in response to glyphosate application, have been reported in cotton plants that have been genetically engineered to be resistant to to the herbicide (Pline et al. 2003). Sprayed transgenic plants exhibited shorter stamens and lower total pollen deposition on the stigma than those not treated. In addition, this pollen was collapsed and highly vacuolated, with a 60% lower rate of germination than pollen grains from nontreated transgenic plants (Pline et al. 2003). The pollen of transgenic maize has also been

Fig. 3. The relationship between (*a*) total seed production and proportion deformed anthers and (*b*) total flower production and proportion of flowers with deformed anthers for individuals that survived application of glyphosate in the field experiment.

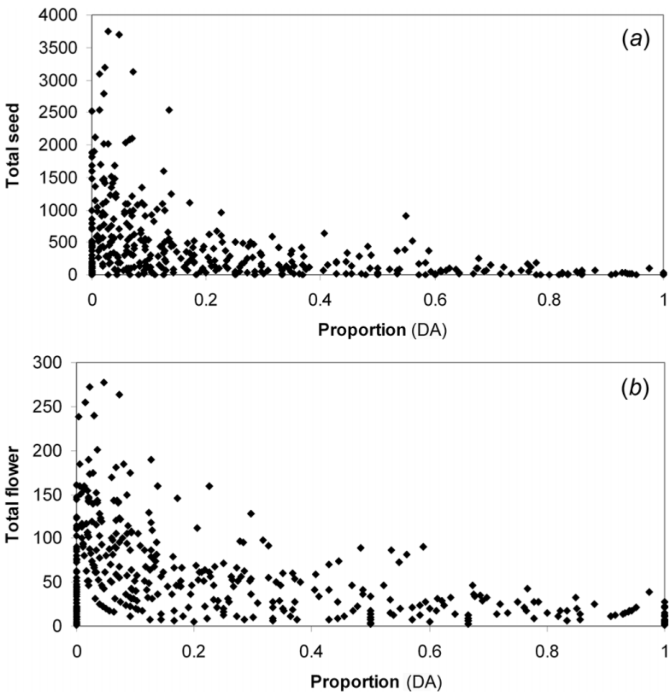
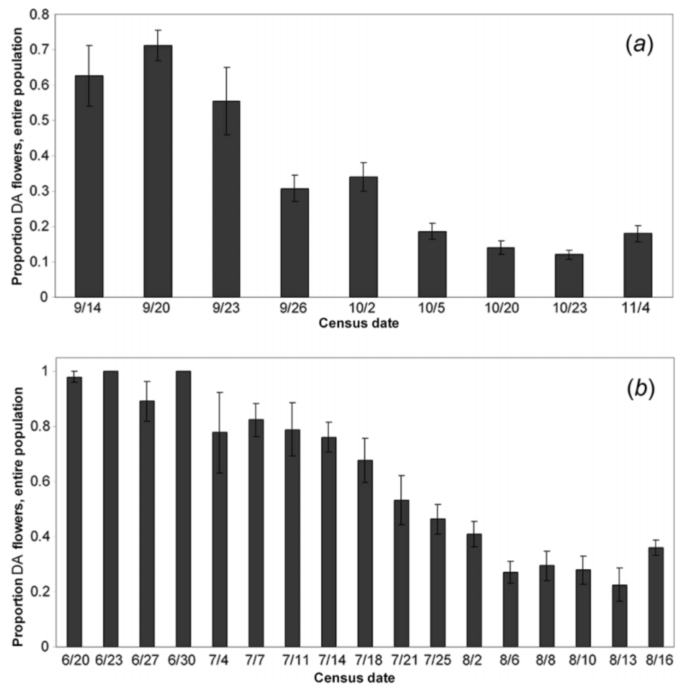


Fig. 4. The proportion of the total number of flowers that exhibited deformed anthers over time in the (*a*) field experiment and the (*b*) GH experiment. In both (*a*) and (*b*), the proportion of flowers with deformed anthers was averaged for each sire within each census date (±SE).



found to be decreased after application of glyphosate, but this decrease did not affect kernel set or yield (Thomas et al. 2004).

Previous studies have demonstrated that pollen development is often disrupted when plants are under stress. Specifically, water stress during meiosis has been shown to inhibit the development of pollen grains in cereal crops (Saini 1997). Although we do not have evidence for a mechanism of the anther deformation in sprayed *I. purpurea* individuals, glyphosate causes stress to plants and also accumulates in meristemic regions and reproductive organs (Gougler and Geiger 1981; Pline et al. 2002; Viator et al. 2003). Studies of both glyphosate-resistant and glyphosphate-susceptible cotton have shown greater accumulation of shikimic acid in reproductive tissues than vegetative tissues, suggesting that reproductive tissue might be more sensitive to glyphosate than vegetative tissue (Pline et al. 2002).

Although male fertility is dramatically reduced after glyphosate application, female function of *I. purpurea* flowers was largely unaffected in the GH experiments. We found that hand-pollinating flowers with deformed anthers, using pollen from a normal flower, produced a similar number of seeds per pollination as the hand-pollinated normal flowers of plants that had not been treated with glyphosate. Similarly, studies on genetically engineered glyphosate-resistant cotton also showed that hand pollination between glyphosate-treated GR cotton plants resulted in a reduced number of seeds per boll when the male parent was treated with glyphosate, but not the female parent (Pline et al. 2003). This suggests that while male reproductive structures are affected by glyphosate, female structures are more robust to its application.

In the field, however, the seed production of sprayed *I. purpurea* plants is negatively correlated with the proportion of flowers with deformed anthers. Specifically, individuals with a higher proportion of flowers with deformed anthers produce fewer seeds than individuals with a lower proportion. There are several hypotheses to explain this finding. First, it is possible that glyphosate does impact female function, but this pattern is difficult to detect in the benign environment of the greenhouse. Similarly, Relyea (2005) found that natural stressors increased the negative impacts of RoundUp® on North American tadpoles. Second, the relationship between proportion of flowers with deformed anthers and fitness could be due to a decline in the ability to self-pollinate in those individuals that are male-sterile, since a portion of *I. purpurea*'s fitness is due to self-fertilization (Ennos 1981). Third, it is also possible that flowers with deformed anthers attract fewer pollinators than normal flowers; flowers with deformed anthers often have a shrunken and disfigured corolla.

One of the ultimate goals for this study was to understand how the production of deformed anthers might fit in the broad context of the evolution of plant tolerance to herbicide. Several experiments reported here provide results relevant to this goal. Initially, our data suggested the presence of genetic variation underlying the proportion time the deformed anthers were present. Analyses of Field Experiment 1 showed that this trait varied according to maternal line, suggesting that control of the trait was due to either nuclear or maternal cytoplasmic factors. This conclusion was drawn because we produced self-pollinated seed of the field-collected maternal lines in the greenhouse for one generation to reduce potential maternal effects in our Field

Table 2. Analysis of variance showing effects of diallel, block, dam, sire, and the interaction of dam and sire on both the proportion of time anthers were deformed (Proportion time DA) and the proportion of the total number of flowers that exhibited deformed anthers (Proportion DA) in Field Experiment 2.

	Proportion time DA			Proportion DA		
Source of variation	df	Type III SS	*F*	df	Type III SS	*F*
Diallel	1	0.0001	0	1	0.0366	3.02
Block	1	0.0006	0.22	1	0.0067	0.56
Dam (diallel)	10	0.0287	1.02	10	0.0829	0.68
Sire (diallel)	10	0.0386	1.37	10	0.1100	0.91
Dam × sire (diallel)	38	0.1258	1.17	38	0.0093	0.77
Error	360	1.0162			4.3520	

Note: **, $P < 0.001$; ***, $P < 0.0001$.

Table 3. Analysis of variance on both the proportion of time anthers were deformed (Proportion time DA) and the proportion of the total number of flowers that exhibited DA (Proportion DA) in the multi-generational greenhouse study using (*a*) the field-collected parental lines (*b*) the F_1 generation and (*c*) the F_2 generation.

	Proportion time DA			Proportion DA		
(*a*) Source of variation	df	Type III SS	*F*	df	Type III SS	*F*
Parental line	10	0.0654	2.34*	9	0.0748	1.06
Error	61	0.1707		31	0.2442	
	Proportion time DA			Proportion DA		
(*b*) Source of variation	df	Type III SS	*F*	df	Type III SS	*F*
Dam	10	0.0081	0.44	10	0.0768	1.2
Sire	9	0.0202	1.12	9	0.0529	0.83
Dam × sire	39	0.0781	0.99	39	0.3080	1.11
Error	188	0.3789		129	0.9152	
	Proportion time DA			Proportion DA		
(*c*) Source of variation	df	Type III SS	*F*	df	Type III SS	*F*
Dam	10	0.3680	1.89*	10	0.1078	1.25
Error	232	0.4526		157	1.3537	

Note: Dam and sire in (*b*) refer to the effects of dam and sire used to generate the F_1 progeny. Dam in (*c*) refers to the maternal parent of the F_1's that were selfed to produce the F_2's. *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$.

Experiment 1. However, further analysis of the trait using carefully generated genetic lines from crossing schemes failed to show a significant effect of dam or sire in both Field Experiment 2 and the GH experiment. This suggests that the presence of variation in the deformed anthers in Field Experiment 1 was perhaps more likely due to relict maternal effects from using field-collected, once-selfed maternal lines rather than due to genetic factors. Maternal environmental effects have been documented to persist through more than one generation (Roach and Wulff 1987), thus one round of selfing might not have been sufficient to decrease this source of maternal effect in our field data. That we see a significant parental line and F_2 effect in the multi-generational greenhouse study also corroborates the idea that maternal effects underlie the presence of this trait. This is because replicates of the parental lines used in this study were the seed of field-collected plants and were subject to the maternal environment of the collected plant. In addition, the F_2 individuals used in the GH experiment derived from a single F_1 plant of each family line, thus, the full-sib F_2's were subject to the same maternal environment within the greenhouse. Based on multiple lines of evidence, the most parsimonious explanation is that the maternal environment, whether in the field or even slight environmental heterogeneity in the greenhouse, lead to variation in the glyphosate-induced deformed anthers observed in this group of studies, rather than genetics.

In conclusion, we find that the male sterility studied in this series of experiments is environmentally induced and transient, as the flowers that develop late tend to produce normal flowers with both male and female function. Male sterility, although temporary, can have a significant effect on the mating system dynamics of this plant given that *I. purpurea* normally exhibits a 30% selfing rate (Ennos 1981). The inability to self, owing to undeveloped anthers, would alter the normal selfing to outcrossing ratio in the population, and potentially lead to 100% outcrossing in plants exhibiting deformed anthers, at least temporarily. The increased outcrossing rates during this male sterile stage could facilitate gene flow from nearby, nonsprayed populations or populations that have recovered from spraying. It could also temporarily boost the pollen success of individuals that recover faster than others and, hence, change the genetic composition of the populations. Thus, glyphosate can have a significant effect on the evolution of resistance and tolerance to glyphosate through even transient male sterility.

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