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Genetics and Inheritance of Nontarget-Site Resistances to Atrazine and Mesotrione in a Waterhemp (*Amaranthus tuberculatus*) Population from Illinois

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A waterhemp population (McLean County resistant, MCR) from McLean County, Illinois is resistant to both mesotrione and atrazine by elevated rates of herbicide metabolism. Research was conducted to investigate the inheritance of these resistance traits. Resistant and sensitive plants were crossed to obtain reciprocal F_1 populations, which were then used to create pseudo-F_2 and backcross (to sensitive parent; BC_S) populations. The various populations were evaluated with whole-plant herbicide efficacy studies in a greenhouse. The responses of the F_1 populations to both mesotrione and atrazine were intermediate when compared with parental populations. In the case of atrazine, BC_S and F_2 populations segregated 1 : 1 and 1 : 3, respectively, for susceptibility (S) : resistance (R), at a dose that controlled the sensitive parent but not the F_1 or resistant parent. For mesotrione, variability was observed within the F_1 populations, suggesting that mesotrione resistance is multigenic and the resistant parents used in the cross were not homozygous at the resistance loci. Furthermore, at low mesotrione doses, more F_2 plants survived than expected on the basis of a single-gene trait, whereas at high doses, fewer F_2 plants survived than expected. Dry weight data confirmed the conclusions obtained from survival data. Specifically, atrazine responses segregated into two discrete classes (R and S) in both the F_2 and BC_S populations, whereas mesotrione responses showed continuous distributions of phenotypes in F_2 and BC_S populations. We conclude that metabolism-based atrazine resistance in MCR is conferred by a single major gene, whereas inheritance of mesotrione resistance in this population is complex.

Nomenclature: Atrazine; mesotrione; common waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea and Tardif AMATU.

Key words: Genetic inheritance, herbicide metabolism, HPPD, monogenic, multigenic.

Waterhemp has been a prevalent agronomic weed species in the midwestern United States since the 1990s. Indigenous to Illinois, waterhemp is a dioecious plant with wind-pollinated flowers and rapid growth rate due to being C_4 (Steckel 2007). It is a prolific reproducer, with a female plant capable of producing one million seeds (Steckel et al. 2003). The biological attributes of waterhemp combine to make this species particularly adept at evolving resistance to herbicides (Tranel et al. 2011).

A population (designated MCR, for McLean County resistant) of waterhemp evolved resistance to atrazine and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides (Hausman et al. 2011). Atrazine disrupts electron transport by outcompeting plastoquinone for the secondary electron-accepting plastoquinone-binding site on the D1 protein of photosystem II (PSII) in chloroplasts, causing cellular damage by oxidative stress (Hess 2000). Resistance to atrazine and similar PSII inhibitors is common, having been documented in at least 72 weed species (Heap 2014). HPPD-inhibiting herbicides act as competitive inhibitors of HPPD, which is a key enzyme in the biosynthesis of carotenoids and tocopherols. The loss of these compounds leads to photooxidative damage of chlorophyll and photosynthetic membranes, resulting in what is commonly referred to as bleaching of new leaf tissue (Mitchell et al. 2001). Resistance to HPPD inhibitors has been reported only in waterhemp and Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Hausman et al. 2011; Jhala et al. 2014).

There are two general mechanisms of herbicide resistance in plants: (1) target-site alterations, such as mutations affecting herbicide binding kinetics or amplification of the target-site gene, and (2) nontarget-site (NTS) mechanisms based on differences in, e.g., herbicide metabolism, translocation, or sequestration (Powles and Yu 2010; Yuan et al. 2007). Many herbicide resistances elucidated thus far are due to point mutations in target-site genes that confer single-gene inheritance and have partial or full dominance at typical herbicide use rates. In contrast, NTS resistance is less well understood and can be controlled by one or multiple genes (Délye 2013). For example, both single-gene and

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multiple-gene inheritance has been suggested in different cases of NTS atrazine resistance, but in neither case is the underlying mutation(s) known (Anderson and Gronwald 1987; Patzoldt et al. 2003). Elucidating NTS resistance is difficult because of the long lists of candidate genes (Délye 2013). Regardless of the underlying mutation(s) responsible for NTS resistance, a better understanding of the inheritance of the resistance trait provides insights into resistance evolution, genetic structure of weed populations, adaptation dynamics, and resistance management (Neve et al. 2009).

Previous research on the MCR population indicated that resistances to both atrazine and mesotrione are mediated by NTS mechanisms. Specifically, enhanced herbicide metabolism by cytochrome P450 and glutathione *S*-transferase (GST) activity conferred resistance to mesotrione and atrazine, respectively (Ma et al. 2013). The objective of this research was to determine the inheritance of these two resistance traits and gain insight into the number of genes involved.

Materials and Methods

Parental, F_1, Pseudo-F_2 (ψ-F_2), and Backcross (BC) Plants. The originating populations used in this study were MCR and Wayne County sensitive (WCS), respectively. The former was described by Hausman et al. (2011) and is resistant to both mesotrione and atrazine, and the latter is known to be sensitive to these herbicides (Patzoldt et al. 2006). Plants from the original MCR field collection were grown from seed in a greenhouse and selected with an application of an HPPD inhibitor (mesotrione, 105 g ai ha^{-1} [Callisto, Syngenta, P.O. Box 18300, Greensboro, NC 27419] or topramezone, 18 g ai ha^{-1} [Impact, AMVAC, 4100 E. Washington Blvd., Los Angeles, CA 90023]) plus atrazine (560 g ai ha^{-1} [AAtrex 4L, Syngenta]) when they were 10 to 15 cm tall. Survivors were used as resistant (R) parents for crosses. Two pairwise crosses, each between two R parents, yielded progenies designated NH2(R) and NH3(R), which were used as R control populations for dose–response and segregation experiments. WCS seeds were obtained from random mating of plants from the original WCS field collection, and were used as sensitive (S) parents in crosses and as the S control population in dose–response and segregation experiments.

F_1 populations derived from five parental crosses, three R by S and two S by R (female parent listed first), were designated as NH4(F_1), NH5(F_1), and NH6(F_1); and NH9(F_1) and NH10(F_1), respectively, and used in the studies. F_1 plants that survived mesotrione (158 or 210 g ha^{-1}, applied to 10- to 15-cm-tall plants) were utilized in subsequent crosses. Most F_1 plants evaluated at these rates survived, although they were severely injured (data not shown). The dioecious nature of waterhemp precludes selfing of F_1s to make true F_2s; therefore, F_1 plants were intermated to make ψ-F_2 populations (hereafter referred to simply as F_2 populations). F_1 males were allowed to also pollinate WCS females to produce BC_S (backcross to S) populations. Four backcross populations, designated NH6-1(BC_S), NH6-2(BC_S), NH4-3(BC_S), and NH4-4(BC_S), and four F_2 populations, NH6-5(F_2), NH6-6(F_2), NH4-7(F_2), and NH4-8(F_2), were derived from NH6 or NH4 F_1 plants and selected for further analysis on the basis of seed availability. All crosses were performed in greenhouse rooms, and intermated plants were enclosed within a tent constructed with a 198-cm by 183-cm pollination bag (Vilutis & Co., 22535 S. Center Rd., Frankfort, IL 60423).

Evaluation of Herbicide Response. Before germination, all seeds were suspended in 0.1 g L^{-1} agar solution at 4 C for at least 4 wk to enhance germination (Bell et al. 2013). Seeds from the various populations were germinated on water-saturated filter paper in petri dishes incubated in a germination chamber (CMP4030 model, Conviron 572 S. Fifth St., Suite 2, Pembina, ND 58271) set for 15/35 C day/night with a 12 : 12-h photoperiod. Seedlings were transferred into either 164 cm^3 Cone-tainers (Ray Leach SC10 "Cone-tainer", 31933 Rolland Dr., Tangent, OR 97389) for segregation analysis, or 12- by 12-cm trays for herbicide dose–response experiments. Those planted in 12- by 12-cm trays were later transplanted into 720 cm^3 pots when seedlings were about 2 cm tall. Cone-tainers were used rather than pots for the segregation analysis because they facilitated analysis of hundreds of plants in a limited amount of greenhouse space. Both the pots and the Cone-tainers contained growth medium consisting of a 3 : 1 : 1 : 1 mixture of LC1 (Sunshine Mix #1/LC1, Sun Gro Horticulture, 770 Silver St., Agawam, MA 01001), soil, peat, and torpedo sand. Slow-release complete fertilizer (Osmocote 13–13–13 slow-release fertilizer, The Scotts Company, 14111 Scottslawn Rd., Marysville, OH 43041) was mixed into the growth medium before planting at a rate of 5 kg m^{-3}. For plants grown in Cone-tainers, about 80 mg of additional fertilizer was added to the top of the growth medium in each Cone-tainer about

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a week after transplanting. Greenhouse conditions were maintained at 28/22 C day/night with a 16 : 8-h photoperiod. Natural sunlight was supplemented with mercury halide lamps to provide a minimum of 800 μmol m^{-2} s^{-1} at the plant canopy.

Herbicide applications were made using a compressed air research sprayer (DeVries Manufacturing, 86956 State Hwy. 251, Hollandale, MN 56045) fitted with a Teejet 80015 EVS nozzle (Teejet Technologies, P.O. Box 7900, Wheaton, IL 60187) calibrated to deliver 185 L ha^{-1} at 275 kPa. The nozzle was maintained approximately 45 cm above the plant canopy. Mesotrione spray solutions contained methylated seed oil (1% v/v) and liquid ammonium sulfate (2.5% v/v), and atrazine was applied with crop oil concentrate (1% v/v). Plants grown in pots (for dose–response study) were sprayed when they were 10- to 15-cm tall, whereas plants grown in Cone-tainers (for segregation analysis) were sprayed when they were 5 to 7 cm. Because of the difference in plant size at the time of application, herbicide efficacies at a given dose are not directly comparable between the two studies.

F_1 Whole-Plant Dose Responses. Uniform plants 10- to 15-cm tall were selected from F_1, NH3(R), and WCS populations and left nontreated or treated with either atrazine (10 to 31,600 g ha^{-1}) or mesotrione (0.1 to 1,000 g ha^{-1}). The rates for mesotrione and atrazine were equally spaced along a logarithmic scale with a base of 3.16. After herbicide application, plants were returned to the greenhouse and placed in a completely randomized design. For atrazine treatments, two runs (experimental repeats in time) were used, and there were at least four replicates (single plants) of each treatment per run for the F_1 (NH5[F_1] and NH6[F_1]) and WCS populations. Seed supply was limited for NH3(R) during atrazine treatments so two replicates were used in run 1 and four replicates were used for run 2. For mesotrione, three runs were used, and each treatment contained six replicates per run. For mesotrione, all runs included WCS(S), F_1, and NH3(R) populations. Run 1 and run 2 included the F_1 populations NH5(F_1), NH6(F_1), NH9(F_1), and NH10(F_1). Seed supply was limited for NH4(F_1); however, since some of the evaluated BC_S and F_2 populations were derived from this F_1, a third run was conducted that included NH4(F_1), NH5(F_1), and NH6(F_1).

At 12 and 21 d after treatment (DAT) for atrazine and mesotrione respectively, plant injury was visually evaluated and recorded using a scale ranging from 0 (no green tissue) to 100 (no injury). Fewer DATs were used for atrazine because of its faster herbicidal activity. Aboveground plant tissue was then harvested and dried at 65 C for at least 4 d before dry weights were collected. The dry weight data (m) and the visual data (v) were combined to obtain an adjusted dry weight (y) using the following function (Guo et al. 2015):

$$y = mv/100 \quad [1]$$

Adjusted dry weights were expressed relative to the mean of the corresponding population's no-herbicide control.

For both dose responses, a linear model was used to compare the response of each population across runs using R software (R version 3.1.1, R Core Team 2013). There was no significant interaction between run and population, so the combined data were fit to dose–response curves for each herbicide. The combined data were analyzed using a nonlinear regression model with the dose–response curve package in R software based on the four-parameter Weilbull model:

$$y = c + (d - c)\exp\{-\exp[b(\log x - \log ED_{50})]\} \quad [2]$$

where b is the slope of the curve, c is the lower limit, d is the upper limit, and the ED_{50} value is the herbicide dose causing a 50% reduction in adjusted dry weight (Knezevic et al. 2007). Resistance ratios were calculated as the ED_{50} of the R or F_1 population divided by the ED_{50} of the S population.

Degree of dominance (D) was calculated for pooled F_1 populations on the basis of the formula given by Stone (1968):

$$D = (2W_3 - W_2 - W_1)/(W_2 - W_1) \quad [3]$$

where W_1, W_2, and W_3 are the log(ED_{50})s of the sensitive parent, resistant parent, and F_1 population, respectively.

Segregation Analysis in BC_S and F_2 Populations. Preliminary studies were conducted for both atrazine and mesotrione to determine appropriate herbicide rates for segregation analysis (data not shown). An atrazine rate of 985 g ha^{-1} was chosen because it effectively distinguished S plants from F_1 and R plants. For mesotrione, because of the lower magnitude of resistance and because of a less uniform response within F_1 populations (see Results and Discussion), it was not possible to identify a single high rate that would consistently distinguish F_1 and R plants, nor a single low rate that would

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distinguish F_1 and S plants. Therefore, multiple rates (from 75 to 120 g ha^{-1} for high rates, and from 4 to 25 g ha^{-1} for low rates) were included in each run, and the rates that best distinguished F_1 plants (from R plants at the high rates and from S plants at the low rates) were used for segregation analysis. In the end, mesotrione segregation at a high rate was assessed in two runs that included two F_2 populations each at rates ranging from 95 to 120 g ha^{-1}; and at a low rate (10 g ha^{-1}) in three runs that each included two F_2 populations and two BC_S populations. For each herbicide rate at each run, included BC_S and F_2 populations were each represented by 20 to 49 and 72 to 107 plants, respectively. Each rate at each run also included 10 to 18 S plants, 10 to 29 F_1 plants, and 10 to 21 R plants. At 12 and 14 DAT for atrazine and mesotrione respectively, each F_2 and BC_S plant was visually evaluated and assessed as dead or alive (new growth evident).

A chi-square goodness-of-fit test (χ^2) was used to compare the observed and expected plant survival frequencies based on a single-gene, qualitative trait model. The model was rejected if $P < 0.05$. For mesotrione, corrections to the expected survival frequencies were made on the basis of observed survival of the F_1 and parental populations at the same mesotrione rate and in the same run, assuming a single-gene, qualitative trait model (Busi et al. 2013; Han et al. 2014). For example, the expected survival frequency of an F_2 population was calculated as:

$$\text{Exp } F_2 = 0.25 \times \text{Obs R} + 0.5 \times \text{Obs } F_1 + 0.25 \times \text{Obs S} \quad [4]$$

where Obs is the observed frequency of survival in R, F_1, or S populations. Corrections were not necessary for the atrazine segregation study because there was 100% survival of R and F_1 populations, and 0% survival of the S population at the rate used.

In one run of the experiment for each herbicide (985 g atrazine ha^{-1}; 10 g mesotrione ha^{-1}), all aboveground plant tissue was harvested and dried at 65 C for at least 4 d, and dry weights recorded. The dry weight data were used in frequency distribution analysis to better visualize the segregation of the populations. Dry weights of parental populations did not have a normal distribution, but did demonstrate a normal distribution after natural-log transformation (Kolmogorov–Smirnov, P values = 0.21–0.84); therefore, natural-log transformations of dry weights were used on all populations.

Results and Discussion

Whole-Plant F_1 Dose Response. Resistance in the R parent relative to the S parent used to generate F_1 populations was 41- and 16-fold for atrazine and mesotrione, respectively (Tables 1 and 2). Even though atrazine resistance in the MCR population was reported previously (Hausman et al. 2011), the magnitude of resistance was not reported. The magnitude of atrazine resistance observed herein for the MCR population is similar to that reported for ACR (38-fold) and SegR (16-fold), two other waterhemp populations with NTS atrazine resistance (Patzoldt et al. 2003; 2005), and much less than that observed (> 1,000-fold) in waterhemp populations with target-site atrazine resistance (Foes et al. 1998; Patzoldt et al. 2003). Hausman et al. (2011) previously reported that mesotrione resistance in the MCR population was 35-fold relative to WCS. This resistance ratio is about twice that observed herein. Differences between the dose–response experiments (for example, our study used adjusted dry weights, factoring in visual observations, whereas their resistance magnitude was based solely on dry weights) could account for the different resistance ratios.

For each of the two herbicides, the different F_1 populations evaluated had similar responses, with ED_{50} values having overlapping 95% confidence intervals (data not shown). Data therefore were pooled among the two F_1 populations evaluated for atrazine response (R by S cross only) and among the two (S by R) or three (R by S) F_1 populations evaluated for mesotrione response (Tables 1 and 2). The responses of the F_1 populations were intermediate to those of the R and S parental populations for both atrazine and mesotrione (Figure 1). Resistance ratios for F_1 populations relative to the S parent were 8 for atrazine and about 3 for mesotrione (Tables 1 and 2). Resistance to mesotrione in reciprocal F_1 populations did not differ, indicating that mesotrione resistance is nuclearly inherited and had no maternal effects. In the case of atrazine, the dose response was performed on F_1 populations from only one crossing direction (R parent as female). However, resistance in BC_S populations (described below), obtained from crosses in which the F_1 was the male parent, indicated that atrazine resistance also was nuclearly inherited.

Although inheritance of both atrazine and mesotrione resistances were close to additive ($D = 0$), atrazine resistance was slightly greater than additive ($D = 0.12$) and mesotrione resistance was somewhat

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Table 1. Whole-plant responses to atrazine of resistant NH3(R) and sensitive WCS parents and their F_1 progeny. F_1 progeny includes both NH5(F_1) and NH6(F_1).

Population	ED_{50} (g ha^{-1})[a]	R/S[b]
R × S(F_1)	310 (163–457)[c]	8
WCS	38 (8–65)	—
NH3(R)	1,576 (−310–3,462)[d]	41

[a] The effective dose at which plants showed a 50% reduction, which was determined using a combination of dry weights and visual observations of herbicide responses.

[b] ED_{50} of resistant or F_1 population divided by ED_{50} of the sensitive population.

[c] Numbers in parentheses denote 95% confidence interval ± 1 standard error.

[d] The large confidence interval for NH3(R) is at least partially attributed to failure of the highest atrazine rate evaluated to provide complete control; see Figure 1.

Table 2. Whole-plant responses to mesotrione of resistant (NH3[R]) and sensitive (WCS) parents and their F_1 progeny. R × S(F_1) includes NH4(F_1), NH5(F_1), and NH6(F_1); S × R(F_1) includes NH9(F_1) and NH10(F_1).

Population	ED_{50} (g ha^{-1})[a]	R/S[b]
R × S(F_1)	1.9 (1.4–2.3)[c]	2.7
S × R(F_1)	1.8 (1.2–2.4)	2.6
WCS	0.7 (0.4–0.8)	—
NH3(R)	10.9 (5.0–15.2)	15.6

[a] The effective dose at which plants showed a 50% reduction, which was determined using a combination of dry weights and visual observations of herbicide responses.

[b] ED_{50} of resistant or F_1 population divided by ED_{50} of the sensitive population.

[c] Numbers in parentheses denote 95% confidence interval.

less than additive ($D = -0.29$). On the basis of the degree of dominance calculations, atrazine resistance can be described as incompletely dominant, whereas mesotrione resistance can be described as incompletely recessive (Stone 1968). However, the degree of dominance for mesotrione may not have been accurately revealed in our study because of lack of homogeneity of the parental R plants and potential interactions of multiple genes that might control the trait (described below).

From a practical standpoint, both atrazine and mesotrione resistance in MCR functionally can behave as a dominant or recessive trait, depending on the herbicide rate (as well as other factors such as plant size at time of application). The relatively high magnitude of atrazine resistance in the MCR population, along with potentially a higher degree of dominance compared with mesotrione resistance, suggests that the atrazine resistance trait would be more easily selected under field conditions with normal herbicide use rates (i.e., 1,000 g atrazine ha^{-1}). In contrast, evolution of mesotrione resistance may be more dependent on applications of the herbicide below the recommended rate of 105 g ha^{-1}. In a study by Busi et al. (2013), using low doses of herbicides allowed for the apparent selection of multiple genes contributing to herbicide resistance. Although herbicide efficacy under greenhouse conditions does not necessarily equate to that under field conditions and the atrazine and mesotrione experiments were not performed simultaneously, it is clear in Figure 1 that atrazine was more detrimental than was mesotrione to F_1 plants at or near the field use rates (compare F_1 responses to 1,000 g atrazine ha^{-1} vs. 100 g mesotrione ha^{-1}).

Inheritance of Herbicide Resistance in Segregating Populations. *Atrazine.* Segregation of atrazine resistance was evaluated at an atrazine dose (985 g ha^{-1}) in which resistance was functionally dominant (i.e., F_1 plants survived). Segregation in both of the F_2 populations evaluated did not deviate from the 3 : 1 (R : S) ratio expected for a single dominant gene in either of two experimental runs (Table 3). Similarly, two BC_S populations did not deviate from the expected 1 : 1 ratio. One BC_S population (NH4-4[BC_S]) significantly deviated from the expected 1 : 1 ratio in the first run ($P = 0.03$) but not in the second run when more plants were evaluated ($P = 0.09$).

Frequency distributions of dry weight data from individual plants showed discrete phenotypic classes, consistent with atrazine resistance being a qualitative trait (Figure 2). Dry weight distributions between the R parent and F_1 population significantly overlapped, comprising a single phenotypic class. This was not surprising since the atrazine rate was chosen such that resistance was functionally dominant. The F_2 population had two phenotypic classes: about 25% of the plants had dry weights similar to those of the S parent and about 75% of the plants had dry weights similar to those of the R parent and F_1 plants. Plants in the BC_S population also exhibited these two phenotypic classes, with about half of the plants in each class.

Atrazine resistance in MCR likely is due to increased GST-mediated detoxification of the herbicide (Ma et al. 2013). Increased GST-catalyzed metabolism has been previously documented in velvetleaf (*Abutilon theophrasti* Medik.) populations (Gary et al. 1996; Gronwald et al. 1989), and inheritance of resistance in this species also followed that of a single, incompletely dominant gene (Anderson and Gronwald 1987). As previously

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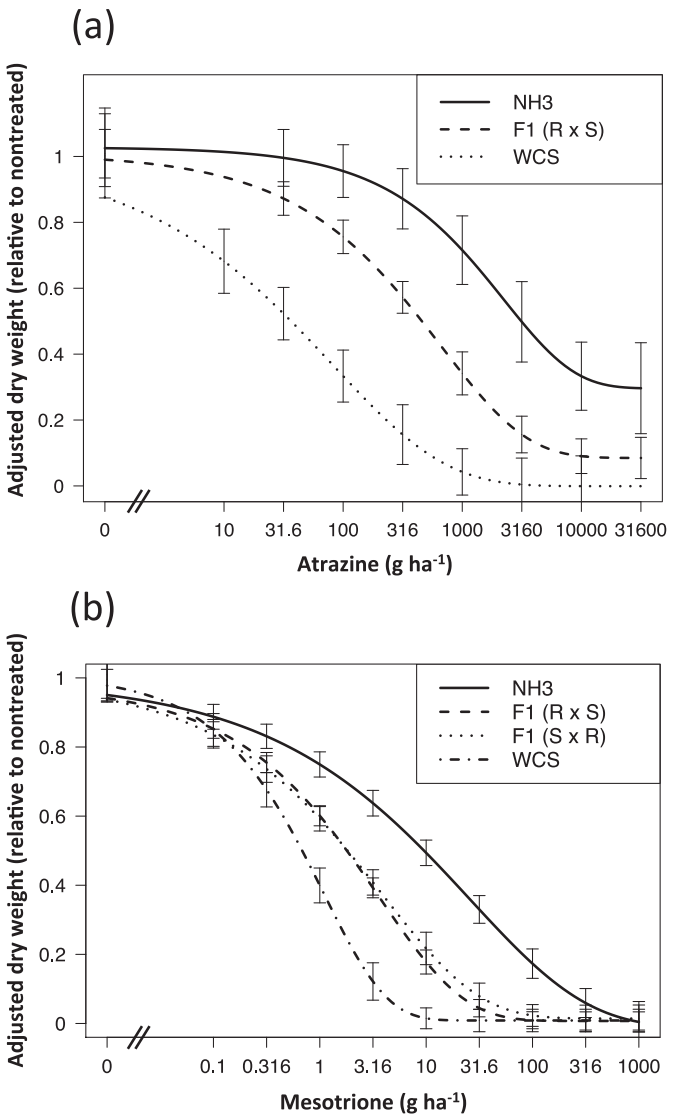

Figure 1. (a) Atrazine dose–response curves for atrazine-sensitive population, WCS (n = 8), atrazine-resistant population, NH3(R) (n = 6), and F_1 hybrid. The F_1 (R by S) data were pooled from NH5(F_1) (n = 12) and NH6(F_1) (n = 12). Vertical bars indicate ±1 standard error (SE) of the means at each dose. (b) Mesotrione dose–response curves for mesotrione-sensitive population, WCS (n = 18), mesotrione-resistant population, NH3(R) (n = 18), and reciprocal F_1 hybrids. The F_1 (R by S) curve was obtained by pooling NH4(F_1) (n = 6), NH5(F_1) (n = 18), and NH6(F_1) (n = 18); and the F1 (S by R) curve by pooling NH9(F_1) (n = 12) and NH10(F_1) (n = 12). Vertical bars indicate ±1 SE of the means at each dose.

mentioned, two other waterhemp populations, ACR and SegR, also have NTS atrazine resistance. Inheritance of NTS atrazine resistance has not been reported for ACR, but in SegR it apparently is incompletely dominant and multigenic (Patzoldt et al. 2003). Because inheritance was analyzed at a single rate, we cannot rule out the presence of a second, minor gene contributing to atrazine resistance in MCR (the effects of a second gene may only be apparent at higher or lower rates). Nevertheless, inheritance was distinctly different from that described for SegR, and consistent with a single major gene. Future research to compare in parallel the atrazine resistance inheritance patterns of ACR, MCR, and SegR populations may provide further insights into the diversity of NTS atrazine resistance genes and mechanisms in waterhemp.

Mesotrione. As with atrazine, segregation was evaluated at a mesotrione rate at which resistance was functionally dominant. However, because of variability within the F_1 populations (Figure 2) and the relatively low level of resistance, it was not possible to find a single rate that distinguished all S from all F_1 plants. For this reason, and because preliminary observations suggested multigenic inheritance, segregation of mesotrione resistance also was evaluated at a high rate, at which resistance was functionally recessive.

Although multiple rates were used in the low-rate analysis, 10 g ha^{-1} was the minimum dose that most effectively controlled the S plants in all runs. At this rate, only one S plant (2%) survived in all three runs, whereas 20% of S plants survived at the next lowest rate of 8 g ha^{-1} (data not shown). Segregation of BC_S and F_2 plants therefore was evaluated only at the 10 g ha^{-1} rate. At this rate, survival of F_1s ranged from 35 to 76% across runs, and expected survival percentages of BC_S and F_2 populations were adjusted on the basis of the lack of 100% F_1 survival. Segregation in each F_2 population evaluated deviated from the corrected 3 : 1 (R : S) ratio expected for a single dominant gene in all three experimental runs (Table 4). Similarly, all BC_S populations deviated from the corrected expected 1 : 1 ratio in all three runs (Table 4). Survival of the BC_S populations ranged from 2 to 17%, which was less than the expected survival percentages (18 to 41%). In contrast, survival of F_2 plants was higher than expected (43 to 65%), ranging from 75 to 90%. Frequency distributions of dry weight data from segregating populations did not display discrete phenotypic classes. For example, dry weights of F_2 plants treated with 10 g mesotrione ha^{-1} exhibited a bell-shaped distribution, which spanned almost the entire range of dry weights collectively spanned by plants from the S and R parents (Figure 2).

In the high-rate analysis, rates in the range of 95 to 120 g ha^{-1} best distinguished R and F_1 plants and therefore were used for segregation analysis in the F_2 populations. Segregation in each of the F_2 populations

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Table 3. Chi-square analysis for goodness of fit of the observed segregation of atrazine resistance in F_2 and BC_S populations. The herbicide rate was chosen, on the basis of previous experiments, to control the sensitive (S, WCS) parental population but not the resistant (R, NH2[R]) parental or F_1 (NH10[F_1]) populations. Expected survival is based on a single resistance gene that is dominant at the herbicide rate used.

Run	Rate (g ha^{-1})	Population	No. of plants	Observed survival	Expected survival	χ^2	P
1	985	F_2					
		NH6-6(F_2)	90	71	68	0.73	0.39
		NH4-7(F_2)	84	58	63	1.42	0.23
		BC_S					
		NH4-4(BC_S)	20	5	10	5	0.03
		NH6-1(BC_S)	24	11	12	0.17	0.68
		F_1	19	19			
		S	14	0			
		R	17	17			
2	985	F_2					
		NH6-6(F_2)	91	63	68	1.61	0.20
		NH4-7(F_2)	89	69	67	0.30	0.58
		BC_S					
		NH4-3(BC_S)	28	16	14	0.57	0.45
		NH4-4(BC_S)	43	16	22	2.81	0.09
		NH6-1(BC_S)	49	24	25	0.02	0.89
		F_1	29	29			
		S	18	0			
		R	18	18			

evaluated consistently deviated from the corrected 1 : 3 (R : S) ratio expected for a single recessive gene (Table 4). Survival percentages of F_2 populations ranged from 0 to 5%, which were much less than the 25% expected for a single-gene, qualitative trait model, even when taking into account the correction for less than 100% survival of R (e.g., run 1, 120 g ha^{-1}) or more than 0% survival of the F_1 (e.g., run 2, 95 g ha^{-1}). Collectively, segregation analysis of mesotrione resistance at both low and high rates indicated that this resistance is likely multigenic. Herbicide resistance with multigenic inheritance previously has been reported in other weed populations (e.g., Busi et al. 2011; Faulkner 1974; Han et al. 2014; Neve and Powles 2005).

Although our study of mesotrione resistance inheritance is confounded by the apparent lack of a starting homogenous R population, our conclusion that mesotrione resistance does not follow simple, single-gene inheritance is still supported. For example, if resistance to mesotrione was controlled by a single gene, then R parent heterozygosity would not explain the range of phenotypes that was observed in the F_1 (i.e, with a single, partially dominant gene model, all F_1 plants from a particular cross will either be uniformly intermediate or segregate 1 : 1). Furthermore, even if the original R parent was heterozygous, each F_1 plant used to make BC_S and F_2 populations was selected for resistance. Again assuming a single-gene, qualitative trait model, each F_1 used in the subsequent crosses therefore should have been heterozygous at the single resistance locus, thereby keeping valid our segregation analysis of the BC_S and F_2 populations.

Attempts to fit the observed segregation ratios of mesotrione resistance with multiple-gene models obtained limited success. For example, although one could invoke multiple additive genes to account for the high proportion of F_2 survivors in the low-rate study, the high mortality of BC_S plants conflicted with the models. Furthermore, the various assumptions required to correct expected ratios (because of variation of the F_1 populations) and the numerous potential interactions among multiple loci (Han et al. 2014) would render any conclusions of specific multigene models speculative at best. Variation within F_1 populations additionally suggests that somewhat different results might be obtained depending on the specific F_1 plant(s) used to make F_2 and BC_S populations. Although multigenic inheritance is one explanation for the observed segregation, other phenomena, including epigenetic effects or conditional dominance, could be at play (van Heyningen and Yeyati 2004).

Mesotrione resistance in MCR has been attributed to P450-based herbicide detoxification (Ma et al. 2013). Although one or more P450 genes could be resistance loci, it is also possible that a resistance locus is a gene encoding a transcription factor of the P450 gene(s). One can envision numerous other

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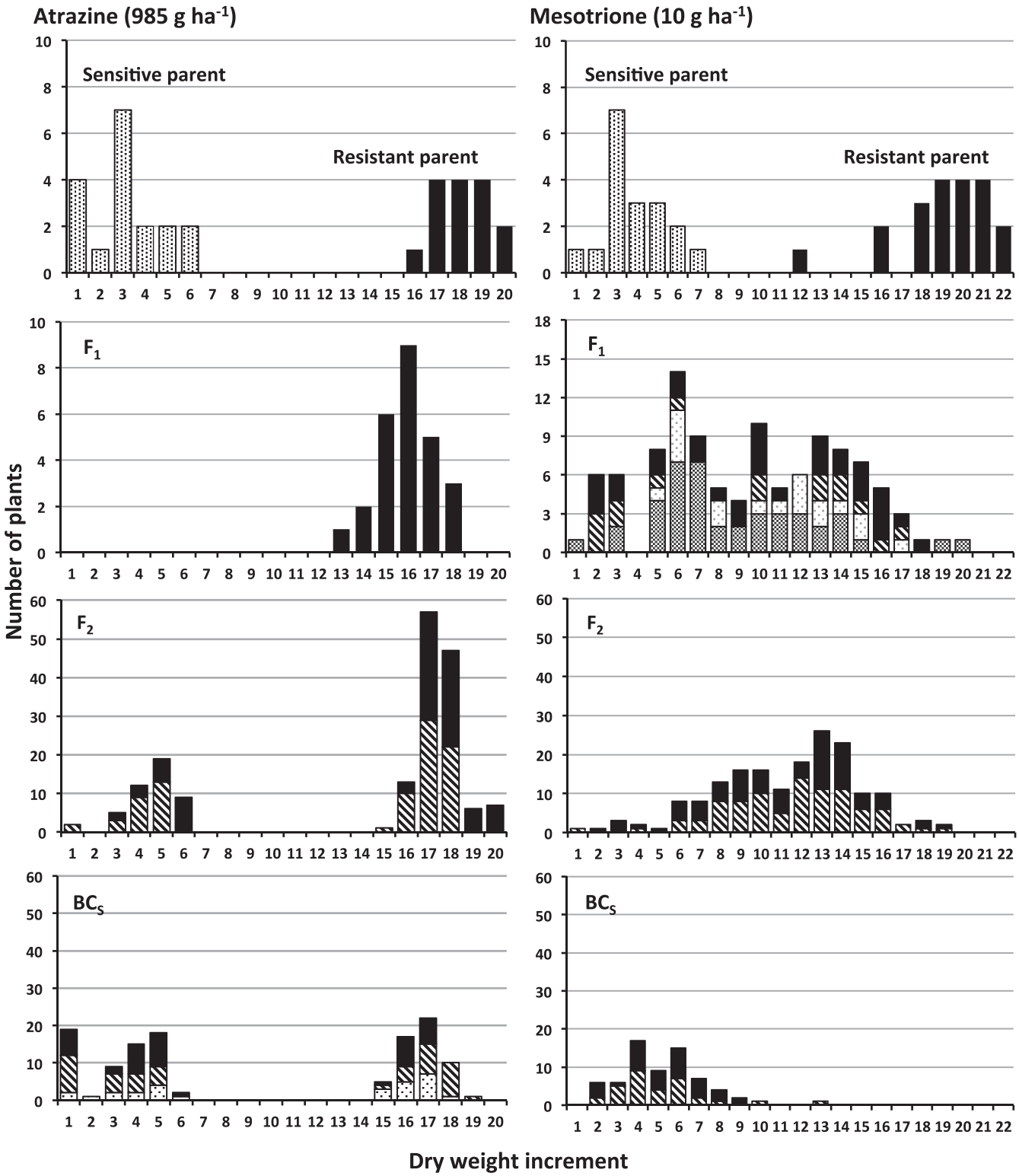

Figure 2. Distributions of plant responses to atrazine or mesotrione. Natural-log-transformed dry weights of individual plants were grouped into incremental bins of 0.1. The *y*-axis indicates the number of plants in a given bin. Multiple populations were pooled for some crosses, with each population represented by a different pattern within the bars.

loci that could modulate P450 activity, or work independently from or in concert with a P450 to confer mesotrione resistance. The identification of complex inheritance of mesotrione resistance in MCR indicates that much more research is needed to fully understand the P450-based herbicide detoxification in this population.

In addition to atrazine and mesotrione resistance, the MCR population also has both target site and NTS resistance to acetolactate synthase (ALS)

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Table 4. Chi-square analysis for goodness of fit of the observed segregation of mesotrione resistance in F_2 and BC_s populations. Segregation analysis was performed at high rates of mesotrione, at which resistance was recessive, and a low rate, at which resistance was dominant. Expected survival was based on a single-gene model, and was corrected for the number of survivors observed in sensitive (S, WCS) and resistant (R, NH3[R]) parental and F_1 (NH10[F_1]) populations that were included in each experimental run.

Run	Rate	Population	No. of plants	Observed survival	Expected survival	χ^2	P
Low rate	g ha^{-1}						
1	10	F_2					
		NH6-6(F_2)	107	95	59	49.35	<0.001
		NH4-8(F_2)	81	66	45	22.95	<0.001
		BC_S					
		NH6-2(BC_s)	48	8	14	4.06	0.04
		NH4-4(BC_s)	48	7	14	5.43	0.02
		F_1	10	6			
		S	10	0			
		R	10	10			
2	10	F_2					
		NH6-5(F_2)	98	74	42	43.27	<0.001
		NH4-7(F_2)	87	65	37	36.57	<0.001
		BC_S					
		NH6-1(BC_S)	43	1	8	6.95	0.01
		NH4-3(BC_S)	46	2	8	5.60	0.02
		F_1	17	6			
		S	17	0			
		R	19	19			
3	10	F_2					
		NH6-5(F_2)	91	82	59	24.05	<0.001
		NH4-8(F_2)	83	75	53	25.85	<0.001
		BC_S					
		NH6-1(BC_S)	41	6	17	11.79	<0.01
		NH4-3(BC_S)	32	5	13	7.37	<0.01
		F_1	17	13			
		S	18	1			
		R	21	21			
High rate							
1	100	F_2					
		NH6-5(F_2)	98	2	25	27.6	<0.001
		NH4-7(F_2)	87	3	22	21.6	<0.001
		F_1	17	0			
		S	17	0			
		R	19	19			
1	120	F_2					
		NH6-5(F_2)	98	5	16	9.44	<0.01
		NH4-7(F_2)	87	0	15	14.4	<0.001
		F_1	17	0			
		S	17	0			
		R	18	12			
2	95	F_2					
		NH6-6(F_2)	97	5	27	24.1	<0.001
		NH4-8(F_2)	72	1	20	24.5	<0.001
		F_1	21	1			
		S	14	0			
		R	15	15			
2	110	F_2					
		NH6-6(F_2)	98	4	21	17.52	<0.001
		NH4-8(F_2)	72	1	15	17.17	<0.001
		F_1	20	0			
		S	14	0			
		R	14	12			

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inhibitors (Guo et al. 2015; Hausman et al. 2013). The genetic control of NTS ALS-inhibitor resistance is currently unknown in MCR. It is also unknown whether there is any overlap among the mechanisms or genes associated with NTS mesotrione, atrazine, and ALS-inhibitor resistances. One of the concerns of NTS herbicide resistance is that it can lead to unpredictable cross-resistance to different herbicide groups (Délye 2013). A better understanding of the specific genes and mechanisms controlling herbicide resistances in the MCR population may provide insights into the evolutionary process by which they were selected. Notable progress is being made toward identification of genes involved with evolved metabolic herbicide resistance (Cummins et al. 2013; Duhoux et al. 2015; Gaines et al. 2014; Iwakami et al. 2013, 2014).

In conclusion, herein we demonstrated that atrazine resistance is mediated by a single major, incompletely dominant nuclear gene. In contrast, mesotrione resistance in this population is more complex and might be multigenic. The more complex inheritance associated with mesotrione resistance suggests that this trait may spread more slowly, and its evolution may be fostered by the application of reduced herbicide rates.

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