Two Recessive Gene Inheritance for Triallate Resistance in *Avena fatua* L.


Extensive use of the preemergence herbicide triallate over the last three decades has selected for resistant (R) *Avena fatua* L. populations in several areas of the United States and Canada. R plants are also cross-resistant to the unrelated pyrazolium herbicide difenzoquat. We made reciprocal crosses between inbred R and susceptible (S) lines to determine the genetic basis of triallate resistance. Seeds from parental lines and F₂ populations were treated with soil applications of 0.275, 0.55, or 1.1 kg/ha triallate in the greenhouse and plant heights recorded after 37 days. Surviving F₂ plants were selfed and the resulting F₃ families were screened with 1.1 kg/ha triallate. In the F₃ populations, assortment of S and R phenotypes fit a 15:1 segregation ratio, suggesting that resistance was controlled by the two independently segregating recessive genes *TRR1* and *TRR2*. None of the 912 F₃ progeny from 51 R F₂ individuals was susceptible to triallate treatment, further supporting a two-gene mode of inheritance. There was a possible maternal effect on susceptibility at the highest triallate rate tested.

Widespread use of herbicides has selected for resistant (R) populations of more than 200 weed species in many agronomic and noncrop situations (Heap 2001). Triallate, a thiocarbamate herbicide used for *Avena fatua* (wild oat) control, has been used successfully in small grain production fields for more than 25 years. However, *A. fatua* populations not controlled by field rates of triallate were first documented in 1990 in Alberta (O’Donovan et al. 1994) and have since been confirmed in Montana (Kern et al. 1996a; Malchow 1995). Dose response analyses of several *A. fatua* accessions from Montana indicated that R plants were 6- to 20-fold more tolerant to triallate than susceptible (S) accessions. Further, the R accessions were resistant to the related thiocarbamate herbicide diallate as well as difenzoquat, an unrelated pyrazolium herbicide (Kern et al. 1996a).

Our investigations of the mechanism of resistance indicated that neither reduced triallate uptake nor altered translocation patterns were responsible for the resistance phenotype (Kern et al. 1996a).

Triallate and other thiocarbamate herbicides inhibit fatty acid elongation in sensitive species. Of interest, these compounds also require in vivo metabolic activation in order to become toxicologically active (Casida et al. 1974). Triallate is converted to triallate sulfoxide by enzymes with either cytochrome P-450-like oxygenase (Schuphan et al. 1979) or peroxxygenase (Blee and Durst 1987) activities. Because of the diverse nature of enzymes mediating xenobiotic oxidation in plants, more than one enzyme system is probably required for triallate sulfoxidation.

In previous studies we showed that triallate sulfoxidation rates were 10- to 15-fold slower in R plants than in S plants (Kern et al. 1996b). This apparently novel mechanism of herbicide resistance was confirmed by our findings that synthetic triallate sulfoxide was metabolized at the same rate and to the same end products in R and S plants, and that triallate sulfoxide was equally phytotoxic to both R and S plants (Kern et al. 1996b).

In the field, extensive herbicide use has most often selected for individual plants with mutations conferring resistance that are partially or completely dominant (Jasieniuk et al. 1996). Of the 216 confirmed herbicide-resistant weed biotypes worldwide as of 1998 (Heap 2001), only trifluralin resistance in *Setaria viridis* (Jasieniuk et al. 1994) and diclofop-methyl resistance in *Avena sativa* (Warkentin et al. 1988) are known to be conferred by recessive gene(s). Most mathematical models predicting the evolution of herbicide resistance indicate that resistance can develop in as few as 4-6 years when herbicide selection pressure is high and resistance is conferred by a single dominant gene (Jasieniuk et al. 1996; Maxwell et al. 1990). In contrast, herbicide resistance conferred by one or more recessive genes is predicted to take considerably longer to develop unless the target species is highly selfing. The existing populations of triallate-resistant *A. fatua* in Montana and Alberta were selected by 16–20 years of continuous triallate use before being noticed by producers (Malchow 1995).

Because of the apparently novel mechanism of resistance and its unusually slow rate of development, we conducted reciprocal crossing studies to determine the inheritance of triallate resistance in *A. fatua*. Knowledge of the genetic basis of this resistance may provide some insight into how plants respond to selection pressures, and the information can be used in the development of resistance management strategies.

Materials and Methods

S seeds were collected from a field-grown population of the inbred *A. fatua* line AN265 (Jana and Naylor 1976) at the Arthur H. Post Research Farm near Bozeman, MT in 1992. The R line was derived from a field population designated FG93R22 that was collected near Fairfield, MT in 1993 from plants surviving treatment with 1.1 kg/ha triallate the preceding spring. The field had been treated with triallate annually for approximately 16 years (Malchow 1995). The FG93R22 population, which contained about 80% R individuals, was further selected for resis-
stance through two additional generations. Greenhouse soil mix [1:1:1 Bozeman silt loam:washed sand:peat moss (v/v/v)] was treated with 1.1 kg/ha triallate and mixed thoroughly in an electric soil mixer. Treated soil was spread uniformly 2 cm deep above 300 FG93R22 seeds sown on 5 cm of untreated soil mix in 55 cm × 35 cm × 10 cm flats. Flats were held in the greenhouse with a 14-h daylength under mercury vapor lamps supplemented with natural sunlight and day/night temperatures of 22°C/16°C, fertilized weekly, and watered as needed. The surviving individuals (about 200 plants out of 300) were grown to maturity, self-pollinated, and a subset of their progeny subjected to an identical triallate treatment. Seedlings from all non-dormant seeds survived herbicide treatment (about 1000 seedlings), confirming homozygosity for resistance. The resulting plants were self-pollinated and their bulked progeny used as the R parental line. Parental lines and all subsequent generations were selfed by enclosing panicles within glassine bags prior to another dehiscence. The R parental line was shown to be 17-fold more tolerant to triallate than the S parent (Kern et al. 1996a).

To produce F1 seed, parental plants were grown in growth cabinets at 16°C under 16 h days. Irradiance was 600 μE/m²/s PPFD from fluorescent and incandescent bulbs. To ensure asynchronous anther dehiscence of pollen donor and maternal plants, two cabinets with different photo-period timings were utilized. For pollen donor plants, the day cycle was initiated 2 h earlier than for maternal plants. This time difference created a period during which emasculation and pollen transfer could be performed with minimal risk of self-pollination of maternal plants. All florets on the main panicle of maternal plants were emasculated and cross-pollinated, while all other panicles were selfed. Emasculation and crossing were conducted over a period of 7 days, during which time stigmas on emasculated panicles remained receptive. Selfed seeds from parental plants were later screened to confirm homozygosity for resistance or susceptibility. Two F1 types, FG93R22/AN265 and AN265/FG93R22, were produced by making reciprocal crosses between four plants of each parental line. Due to the small number of F2 seeds produced, no F1 progeny were tested for triallate response.

F2 seeds were produced by selfing 21 F1 plants grown under the greenhouse conditions described above. To break seed dormancy, seeds were rinsed in 10% (v/v) commercial bleach solution for 15 min and then vigorously rinsed with deionized water prior to planting. Seeds from each cross were planted singly in 15 cm diameter plastic pots. After plants reached the boot stage, selfing was assured by enclosing individual panicles in glassine bags.

A total of 1139 F2 seedlings derived from F1 hybrids were screened to determine S:R segregation ratios using a modified form of the dose response assay previously described by Kern et al. (1996a). F2 and parental seeds were dehulled (lemma and palea removed by hand) and the caryopses planted in greenhouse soil treated with 0, 0.275, 0.55, or 1.1 kg/ha triallate as described above. Previous studies (Kern et al. 1996a) showed that these treatment rates differentiated between R and S seedlings by allowing germination but inhibiting growth of S seedlings. Growth of R plants was not affected by the 0.275 or 0.55 kg/ha treatment rates and was slightly inhibited by the 1.1 kg/ha treatment rate. The heights of the parental and F2 plants were recorded 37 days after planting. A randomized nested block design was used for these experiments.

The 51 surviving F2 plants were replanted into 25 cm diameter pots and selfed as described above. The resulting 912 F2 progeny were treated with 1.1 kg/ha triallate as before and plant heights were recorded after 32 days. The frequencies of R and S seedlings from each population were determined as described above and chi-squared tests used to test goodness-of-fit to common Mendelian ratios in the individual S plant used as the pollen donor and maternal plants. All progeny from selfed F2 S plants was not affected by the 0.275 or 1.1 kg/ha triallate treatment rate. This apparent maternal effect conferring higher susceptibility could be due to the presence of modifying genes in the individual S plant used for the S × R cross. In contrast, the chi-squared value for F2 (S × R) plants treated with 1.1 kg/ha triallate was 10.62, indicating a significant divergence from the 15 S:1 R ratio. Tests of data independence across crosses and across rates showed that the parental source of resistance was significant only at the 1.1 kg/ha treatment rate. This apparent maternal effect conferring higher susceptibility could be due to the presence of modifying genes in the individual S plant used for the S × R cross. For the F2 generation, all progeny from selfed F2 S × R (318 individuals) and R × S plants (594 individuals) were resistant to 1.1 kg/ha triallate, confirming a two-gene recessive model for triallate inheritance.

Although herbicide resistance as a two-gene recessive trait is unusual, several characteristics of the weed/herbicide combination investigated here may have contributed to its selection in this case. First, A. fatua is primarily a self-fertilizing plant (Iman and Allard 1965), in which a recessive trait would be fixed more quickly than in an outcrossing species (Jasieniuk et al. 1996). Second, the relatively high herbicide selection pressure against homozygous dominant and heterozygous S

<table>
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<tr>
<th>Source</th>
<th>Treatment rate (kg/ha)</th>
<th>S Resistant (%)</th>
<th>R Resistant (%)</th>
<th>χ²</th>
<th>P</th>
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<tr>
<td>F1 (S × R)</td>
<td>0.275 181 9 0.74 .39</td>
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<td>0.55 181 9 0.74 .39</td>
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<td>1.1 181 1 10.62 .00</td>
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<tr>
<td>F2 (S × R)</td>
<td>0.275 177 13 0.11 .74</td>
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<td>0.55 179 10 0.30 .59</td>
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<td>1.1 181 9 0.74 .39</td>
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Table 1. Segregation of triallate resistance in A. fatua F2 families

Results and Discussion

After triallate treatment, the mean plant height of the R parental line (45.06 ± 0.41 cm) was significantly greater than the S parental mean (0.66 ± 0.04 cm) over all treatment rates, as determined by Student’s t test (P < .01). Treatment of F2 progeny resulted in a bimodal plant height distribution. F2 plant heights greater than 30 cm were not significantly different from parental R plant heights (Student’s t test; P < .01 for all treatment rates) and were therefore classified as R. Parental and F2 plants less than 10 cm tall were classified as S for all triallate treatment rates. No F2 plant heights between 10 and 30 cm were observed.

Ratios of S:R F2 plants from each reciprocal cross were tested for goodness-of-fit to common Mendelian ratios at each triallate treatment rate (Table 1). In all but one case (S × R, 1.1 kg/ha), segregation for triallate resistance was not significantly different from a 15 S:1 R ratio. At 0.275 kg/ha triallate, S × R and R × S reciprocal crosses had chi-squared values of 0.74 and 0.11, respectively, yielding nonsignificant P values. Similarly the 0.55 kg/ha treatment rate gave chi-squared values of 0.74 and 0.30 for S × R and R × S crosses, respectively, and P values exceeded .38 in both cases. The chi-squared value for F2 (R × S) plants treated with 1.1 kg/ha triallate was 0.74, with a nonsignificant P value. In contrast, the chi-squared value for F2 (S × R) plants treated with 1.1 kg/ha triallate was 10.62, indicating a significant divergence from the 15 S:1 R ratio. Tests of data independence across crosses and across rates showed that the parental source of resistance was significant only at the 1.1 kg/ha treatment rate. This apparent maternal effect conferring higher susceptibility could be due to the presence of modifying genes in the individual S plant used for the S × R cross. For the F2 generation, all progeny from selfed F2 S × R (318 individuals) and R × S plants (594 individuals) were resistant to 1.1 kg/ha triallate, confirming a two-gene recessive model for triallate inheritance.

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plants would select for homozygous recessive R individuals. Third, the apparently unique mechanism of triallate resistance in *A. fatua,* that is, a marked decline in the rate of triallate sulfoxidation (Kern et al. 1996b), is consistent with a recessive (loss of function) model. The results reported here show that, at least for the one R line tested, triallate resistance in *A. fatua* is a recessive trait. We speculate that mutations in two nuclear genes encoding oxidative enzymes or parts of enzyme complexes—TRR1 and TRR2—are required to confer resistance. Of interest, producers have observed that population densities of R plants decline to low levels within a few years after the discontinuation of triallate use, suggesting that such mutations may also be associated with a loss of fitness.

All populations of triallate-resistant *A. fatua* that we (Kern et al. 1996a) and others (O’Donovan et al. 1994) have tested were cross-resistant to the unrelated herbicide difenzoquat. Fields in Montana from which the triallate-resistant populations arose had rarely if ever been treated with difenzoquat (Malchow 1995), and so the relationship between the resistance mechanism(s) for these dissimilar herbicides has remained unclear. Rashid et al. (1998) suggested that increased gibberellin levels in R plants from Canada may be involved in the resistance mechanism for both herbicides. Based on compartmental analyses of difenzoquat efflux from suspension cultured cells, we concluded that resistance was due to tight binding of the herbicide in cell walls, effectively excluding it from in R plants to triallate and cross-resistance to difenzoquat (Malchow 1995). Wild oat resistance to triallate in Montana (Master’s thesis), Bozeman, MT: Montana State University-Bozeman.


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**Inheritance of Disease**

**Lesion Mimic Leaf Trait in Groundnut**

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In groundnut, two identical mutants with disease lesion mimic leaf trait were isolated independently from two different parents through induced mutagenesis and in vitro culture technique. The leaf chlorophyll content in both the mutants was found to be drastically reduced. The segregation pattern in the *F*₂ and *F*₃ generations for normal and mutant traits fitted a 13:3 ratio, indicating that the disease lesion mimic trait in the mutants was due to suppressive gene action. Both mutants were allelic for the disease mimic trait.

In groundnut (*Arachis hypogaea* L.), the occurrence of several chlorophyll mutants was reported either spontaneously (Hammons 1973) or through induced mutagenesis (Patil 1966). In our groundnut breeding program, two phenotypically identical mutants were isolated and designated as TG 18AM and TAG 24M. They were derived through gamma ray treatment of seeds of TG 18A (Chandra Mouli and Kale 1982) and in vitro plant development through somatic embryogenesis from cultured immature embryonal axes (Eapen S, personal communication) of cv. TAG 24 (Patil et al. 1995). Leaves of both the mutants mimic the symptoms of groundnut rust disease (*Puccinia arachidis* Speg.). Several independent disease lesion mimics (DLM) have been identified in many species including groundnut (Branch 1998; Dansl et al. 1996; Greenberg and Ausubel 1993; Jothal et al. 1995). Lesion mimics are normally inherited either as recessive or dominant traits (Jothal et al. 1995). The objective of the present study was to investigate the inheritance and the allelic relationship of the DLM leaf trait in both the mutants in groundnut.

**Materials and Methods**

Crosses were made between (1) TG 18AM and normal leaf genotypes TG 18A, TAG 24, TAG 28A ( *A. hypogaea* ssp. *fastigata*) and ICGV 86564 (*A. hypogaea* ssp. *hypogaea*), (2) TAG 24M and TAG 24, and (3) TG 18AM and TAG 24M. From *F*₁ to *F*₃ generations, plants were advanced as plant to row progenies and segregation for normal and mutant phenotypes were scored. The distribution of DLM scores was fitted to expected ratios by using the chi-square test. The chlorophyll content in the mutants and their parents was estimated (Arnon 1949) by taking fresh leaf discs from upper and lower leaves from 65-day-old field-grown plants.

**Results and Discussion**

Mutants TG 18AM and TAG 24M were characterized by erect habit, sequential flowering, and rose seed coat, resembling their...