# Diclofop-resistance in a Lolium multiflorum biotype from Oregon

**J. W. Gronwald, K.C. Betts, N. J. Ehlke, and D. L. Wyse.** Plant Science Research Unit, USDA-ARS and the Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota. USA.

**Summary**
**An Italian ryegrass (Lolium multiflorum Lam) biotype found in a wheat field in Oregon exhibited a 100-fold increase in tolerance to diclofop compared to a typical susceptible biotype. Resistance to diclofop was not due to a change in diclofop retention, absorption, translocation or metabolism, but rather to the presence of an altered form of ACCase which was less susceptible to inhibition by diclofop and other aryloxyphenoxypropionic acid herbicides. An intermediate level of tolerance was expressed at the whole plant level in the F1 population obtained by reciprocal crosses of the susceptible and resistant biotypes. Chi-squared analyses of the segregation of the resistance trait in F2 populations indicated that resistance was controlled by a single nuclear gene exhibiting partial dominance.**

## Introduction

Diclofop has been widely used for more than a decade to control Italian ryegrass, a serious annual weed in the wheat-growing regions of the Pacific Northwest. In 1987, a diclofop-resistant biotype of Italian ryegrass was found in an Oregon wheat field (4). Diclofop had been applied to the field for seven consecutive years prior to the discovery of resistance. We obtained seed of the resistant biotype with the goal of determining the level of resistance, the mechanism of resistance, and the mode of inheritance of the trait.

## Materials and Methods

Growth response studies to determine the herbicide concentration (kg $ha^{-1}$) required to reduce shoot dry weight by 50% ($GR_{50}$ values) were conducted. Resistant and susceptible plants in the three-leaf stage were sprayed with commercially-formulated herbicides (diclofop, haloxyfop, quizalofop, sethoxydim) using a stationary pot sprayer and a carrier volume of 96 L $ha^{-1}$. Spray mixtures of sethoxydim and haloxyfop also contained 1.5% (v/v) crop oil concentrate. Shoot dry weight was determined two weeks after herbicide application. Acetyl-CoA carboxylase (ACCase) activity was measured in extracts from etiolated shoots from resistant and susceptible biotypes. Etiolated tissue was homogenized for 2 min in a mortar and pestle containing the homogenization medium described by Matthews et al. (3). The homogenate was centrifuged (30,000g, 30 min) and desalted on a sephadex G-25 column. ACCase activity was assayed in the crude extract as described by Burton et al. (1). Herbicide concentrations required to inhibit ACCase activity by 50% ($I_{50}$ values) were determined. F1 families were obtained by reciprocal crosses of the resistant and susceptible biotypes. F2 plants were generated by sib-mating of F1 plants.

## Results and Discussion

The level of tolerance to diclofop and two other aryloxyphenoxypropionic acids was determined for the susceptible

and resistant biotypes (Table 1). The $GR_{50}$ value for diclofop in the resistant biotype was greater than the highest rate applied, 15 kg $ha^{-1}$, indicating that the resistant biotype was at least 100-fold more tolerant of diclofop than the susceptible biotype. The diclofop-resistant biotype also exhibited resistance to haloxyfop and quizalofop, but the level of resistance was less than for diclofop. As compared to the susceptible biotype, the resistant biotype was 29- and 16-fold more tolerant to haloxyfop and quizalofop, respectively. The resistant biotype exhibited only a two-fold increase in tolerance to sethoxydim (data not shown).

Diclofop retention, absorption, translocation and metabolism were examined in the susceptible and resistant biotypes. There was little or no difference in these parameters between the two biotypes (data not shown) which suggested that resistance resided at the herbicide target site, ACCase (2). ACCase activity measured in etiolated leaf extracts from the resistant biotype was less sensitive to inhibition by diclofop compared to ACCase activity measured in extracts from the susceptible biotype (Table 1). As found at the whole plant level, the degree of resistance expressed at the enzyme level was greater for diclofop than haloxyfop and quizalofop.

**Table 1. Effect of Aryloxyphenoxypropionic Acids on Growth and ACCase Activity of Resistant and Susceptible Biotypes.**

| Herbicide | $GR_{50}$[a] | | $I_{50}$[b] | |
|---|---|---|---|---|
| | Susceptible | Resistant | Susceptible | Resistant |
| Diclofop | 0.15 | >15.0 | 0.3 | 8.3 |
| Haloxyfop | 0.01 | 0.29 | 1.8 | 16.4 |
| Quizalofop | 0.005 | 0.08 | 0.07 | 0.7 |

[a]Herbicide concentration in kg $ha^{-1}$ required to reduce shoot dry weight by 50%.
[b]Herbicide concentration (μM) required to inhibit enzyme activity by 50%.

Crosses were made between the resistant and susceptible biotypes to determine the mode of inheritance of resistance. The F1 plants obtained from reciprocal crosses exhibited an intermediate level of diclofop tolerance ($GR_{50}$ = 5.6 kg $ha^{-1}$) compared to that found in the resistant and susceptible biotypes. There was no difference in the response of either reciprocal cross indicating that the resistance trait was not maternally-inherited. The F2 populations obtained by sib-mating were treated at the three-leaf stage with 6.5 kg $ha^{-1}$ of diclofop. Three different phenotypes were identified; susceptible (S), intermediate (I), and resistant (R) (Table 2). The observed segregation ratio of the S, I, and R phenotypes in the F2 population was in agreement with the predicted segregation ratio of 1:2:1 for a trait inherited as a single, partially dominant, nuclear gene.

**Table 2. Chi-square Analysis of the Segregation of Phenotypes in the F2 Population.**

| | Phenotype | | |
|---|---|---|---|
| | Resistant | Intermediate | Susceptible |
| | ----------No. of Individuals---------- | | |
| Observed | 26 | 45 | 25 |
| Predicted | 24 | 48 | 24 |

(P = 0.80)

It is concluded that resistance to diclofop and other aryloxyphenoxypropionic acids in the Italian ryegrass biotype from Oregon is due to the presence of a tolerant form of ACCase. The results of the inheritance studies are consistent with the resistance trait being controlled by a single nuclear gene that encodes ACCase.

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**References**

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