

A Common Ragweed (*Ambrosia artemisiifolia*) Biotype in Southwestern Québec Resistant to Linuron¹

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Abstract: The degree of resistance to linuron of a common ragweed biotype was investigated. Suspected linuron-resistant plants collected from a carrot field near Sherrington, Québec, were subjected to increasing rates of linuron under glasshouse conditions. Resistance to linuron of the common ragweed biotype was suspected because 33% of plants survived to reproduction after they were sprayed at a rate of 4.5 kg ai/ha, two times the dose rate recommended for linuron in carrots, and also because 3% of plants survived to reproduction after they were sprayed at a rate of 22.5 kg ai/ha, 10 times the recommended dose. Susceptible plants collected from a field with no prior history of linuron use were all killed when sprayed at the lowest dose rate recommended, 1.125 kg ai/ha. The herbicide-resistance ratio was 29.0 for linuron, and for cross-resistance to atrazine, the ratio was 1.3, indicating that these plants exhibit greater resistance to linuron than to atrazine.

Nomenclature: Linuron; common ragweed, *Ambrosia artemisiifolia* L. #³ AMBEL; carrot, *Daucus carota* L.

Additional index words: Urea herbicides, cross-resistance, atrazine.

INTRODUCTION

Common ragweed is a widespread summer annual weed in southern regions of the Canadian provinces of Québec and Ontario and in most of the eastern United States (Bassett and Crompton 1975). This highly successful pioneer species is most often found in frequently disturbed habitats such as road sides, waste places, and agricultural fields. In southwestern Québec, common ragweed is an especially troublesome weed in vegetable crops such as carrot (*Daucus carota* L.), onion (*Allium cepa* L.), and cabbage (*Brassica oleracea* L.). In weed surveys in the late 1990s, 25% of carrot fields and 50% of cabbage fields in southwestern Québec were found to be heavily infested with common ragweed (Phytodata, Inc., and southwestern Québec carrot growers, personal communication). In 1999, approximately 3,500 ha of carrot and 1,500 ha of cabbage were grown in this region of Québec, with total losses due to common ragweed infestations alone estimated at \$Canadian (CDN) 1 million annually. Losses were as high as \$CDN1,000 per

hectare in carrot and \$CDN500 in cabbage (Phytodata, Inc., personal communication). These losses included (1) additional costs for mechanical and manual hoeing of common ragweed, (2) reduced carrot stand density as a result of crop seedlings being mistakenly removed instead of common ragweed seedlings during hand-weeding and manual hoeing operations, (3) lower productivity due to direct competition for available resources by this weed, and (4) losses due to interference with mechanical crop-harvesting operations by common ragweed plants escaping control.

Common ragweed can be controlled successfully using many herbicides, including 2,4-D, atrazine, bentazon, dicamba, diuron, linuron, and MCPA. However, relatively few herbicides are registered for safe use in carrot, onion, or cabbage crops (OMAF 2004), although there have been efforts to find alternative herbicides selective for carrots (Bellinder et al. 1997; Cisneros and Zandstra 2002). Herbicides registered for use in these vegetables generally have a narrow spectrum of crop safety, and few can be used to effectively control common ragweed once the crop has emerged (OMAF 2004; WSSA 2002).

The substituted urea herbicide linuron has been used extensively in carrot production since the 1960s for control of annual broad-leaved weeds (Bell et al. 2000; Bellinder et al. 1997; Dickerson and Rahn 1963; Hogue 1972; Kuratle and Rahn 1968; Trevett and Gardner

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³ Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised 1989. Available only on computer disk from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

1963). Linuron is registered in the Eastern Canadian provinces of Québec and Ontario for use in carrot as either a preemergence (0.55 to 1.125 kg ai/ha) or a post-emergence treatment (1.125 to 2.25 kg ai/ha), alone or in combination with preplant-incorporated herbicides such as trifluralin or preemergence herbicides such as prometryne (OMAF 2004). However, more than 85% of carrot production in Québec and Ontario occurs on high-organic matter (muck) soils (NRC 2004), in which trifluralin is ineffective and not recommended (Bellinder and Ellerbrock 2004). Linuron is the only postemergence herbicide registered in eastern Canada for the control of common ragweed in carrots (OMAF 2004).

Since the late 1980s, there has been a gradual decline in the ability of linuron to effectively control common ragweed in some southwestern Québec vegetable fields. Current applications of pre- and postemergence registered rates of linuron do not provide economically acceptable levels of common ragweed control, especially since plants can establish throughout the growing season (Bassett and Crompton 1975). Some producers in the region have resorted to using multiple early-season, reduced-rate applications of linuron to increase control, targeting the more susceptible cotyledon or one- to two-leaf stage of the weed. Results, however, have not been satisfactory. Linuron is currently still being used in Québec carrot, onion, and cabbage production systems because there are few other acceptable control strategies available against common ragweed in these crops. However, dependence on a single herbicide within these vegetable systems is precarious because of the possible development of herbicide resistance in the local flora.

There are relatively few reports of weed resistance to linuron and no reports of common ragweed resistance. Therefore, the objectives of this research were to (1) determine the degree of resistance to linuron of a common ragweed population from a carrot field in which the weed was suspected of escaping control from this herbicide and (2) evaluate cross-resistance of this population to atrazine, a widely used herbicide in the corn (*Zea mays* L.) phase of the rotation sequence in this region.

MATERIALS AND METHODS

Experiment 1. Glasshouse Trials Using Linuron Only. In the spring and summer of 1999, suspected linuron-resistant common ragweed seedlings (R-group) were collected from a 10.8-ha field in Sherrington, Québec, Canada, where significant control failures against this weed had been reported. The field had a deep (over 1.5 m in depth), well-drained, and decomposed organic

(muck) soil sitting on a medium loam mineral soil (limno humic mesisol), with an organic matter content of 75% and a soil pH of 5.6. The typical 4-yr crop rotation cycle on this field had been corn, onion, and two consecutive years of carrot. Linuron was applied regularly on this field as a postemergence treatment in carrots, but the efficacy of linuron in suppressing common ragweed had progressively decreased over the last 10 years (L. Brodeur, personal communication). A second group of common ragweed seedlings were collected on the same day as seedlings from the Sherrington site from a field located at the Emile A. Lods Agronomy Research Centre, Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, Québec, about 45 km northwest of the Sherrington site. The soil type was a St. Bernard fine sandy loam (orthic melanic brunisol) with an organic matter content of 3% and a soil pH of 6.7. This other field had no prior history of linuron use, so the resident common ragweed population was assumed to be susceptible to linuron (S-group). Plants of common ragweed from both fields were collected at the two- to four-leaf stage, their roots thoroughly cleaned of the original soil, and plants were transplanted into a potting medium composed of two-thirds commercial potting mix⁴ and one-third black potting soil. Plants were arranged in groups of three in 13- by 15-cm Styrofoam flats, with each flat comprising one experimental unit. Flats were placed in a glasshouse and subjected to a 16-h photoperiod, either as natural light or supplemented with artificial lighting (400-W high-power sodium lamps), 28/19 ± 3 C day/night temperatures, and watered as required. One hundred milliliters of 10-30-10 fertilizer was added twice during the first week of growth to each seedling, after which 20-20-20 fertilizer was used weekly. Thirteen days after transplanting, resistant and susceptible plants that were 5 to 10 cm in height (6- to 12-leaf stage) were treated with one of six linuron⁵ concentrations: 0 (0×), 1.13 (0.5×), 2.25 (1×), 4.50 (2×), 6.75 (3×), 9.00 (4×), and 22.50 (10×) kg ai/ha. The maximum post-emergence recommended rate of linuron in carrots is 2.25 kg ai/ha (OMAF 2004). Linuron was sprayed using a spray chamber equipped with a flat-fan nozzle⁶ delivering 260 L/ha at 250 kPa. Control plants (0×) were sprayed with distilled water only. Immediately after spraying, plants were returned to the glasshouse under

⁴ Premier® Pro-mix BX®, Premier Horticultural Ltd, 1785 55th Avenue, Dorval, QC H9P 2W3, Canada.

⁵ Lorox 50DF, E.I. Dupont De Nemours and Co., Barley Mill Plaza, P.O. Box 8003, Wilmington, DE 19880.

⁶ XR-Teejet 8002VS, Teejet Spray Nozzles, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189-7900.

the same environmental conditions described earlier. The experiment was set up as a randomized complete block (RCBD) with six replications of treatments. The experiment was repeated in time with the first spray application occurring in late May 1999 (trial 1) and the second application taking place in early August 1999 (trial 2). For trial 2, a new cohort of common ragweed seedlings was collected in late July from the same two fields used in the first trial. Seedlings were collected and grown as described for trial 1. Environmental conditions in the glasshouse for the second trial were similar to those described for the first trial.

Mortality, number of staminate inflorescences, and height of common ragweed plants were recorded 31 d after spraying. Plant height was measured from the crown to the shoot apex. Live plants were then uprooted and excised at the crown. Root material was harvested by thoroughly rinsing the soil off the excised main rootball. Shoots and roots from each plant were placed in separate paper bags and oven-dried at 60 C for 3 d before weighing.

Experiment 2. Glasshouse Trials Using Linuron and Atrazine. The linuron rates used for experiment 1 were too high to establish a dose–response curve for the S-biotype; thus, a second experiment was conducted using lower linuron rates and also to test for cross-resistance to atrazine, a widely used herbicide in these fields during the corn phase of the rotation.

In February 2000, R-group seeds were collected from the same Sherrington field as in experiment 1. Since at this time of the year the field was snow-covered, pieces of frozen soil were removed from the field, placed in flats, and left at 4 C for 3 d in a refrigerator to allow for gradual thawing. The flats were then placed in a heated glasshouse to stimulate germination of common ragweed seeds. S-group seeds were also collected at this time from dead common ragweed plants protruding above the snowline in the same field as for experiment 1. In the glasshouse, the S-group seeds were immersed in water for about 1 h before sowing in the same soil medium described in experiment 1. Emerged seedlings from each field at the two- to three-leaf stage were transplanted in groups of three in 13- by 15-cm Styrofoam flats, with each flat comprising one experimental unit. Flats were placed in a glasshouse and subjected to a 16-h photoperiod (natural light supplemented with artificial lighting of 400-W high-power sodium lamps), $26/17 \pm 3$ C day/night temperatures, and watered as required. One hundred milliliters of 10-30-10 fertilizer was added twice during the first week of growth to each seedling, after

which 20-20-20 fertilizer was used weekly. Fourteen days after transplanting, plants 5 to 8 cm in height (6- to 10-leaf stage) were treated with one of six linuron concentrations: 0×, 1/16×, 1/8×, 1/4×, 1/2×, and 1× for the S-group plants, and 0×, 1/2×, 1×, 2×, 4×, and 8× for the R-group plants. A similar set of plants was treated with one of the following concentrations of atrazine⁷: 0×, 1/16×, 1/8×, 1/4×, 1/2×, 1×, and 2× for the S-group plants, and 0×, 1/2×, 1×, 2×, 4×, and 8× for the R-group plants. The ‘×’ atrazine rate of 1.0 kg ai/ha is the minimum recommended dose when used alone in maize for postemergence control of annual broad-leaved weeds (OMAF 2004). Both linuron and atrazine were sprayed using the same equipment and conditions described in experiment 1. Control plants (0×) were sprayed with distilled water only. Immediately after spraying, plants were returned to the glasshouse and grown under the same environmental conditions described earlier.

The experimental design for this experiment was a RCBD with five replications of treatments. The experiment was repeated in time and space, with a 1-wk interval between herbicide applications (trials 1 and 2). Plants for the two trials were placed on separate benches in the same glasshouse. Mortality of common ragweed R-group and S-group plants was recorded 20 and 32 d after herbicide application for the linuron and the atrazine trials, respectively.

Mortality data for experiment 1 and experiment 2 were subjected to analysis of variance (ANOVA),⁸ whereas height, shoot biomass, and number of male inflorescence for experiment 1 were subjected to an analysis of covariance, where the 0× rate served as the covariable. Root biomass data were not subjected to an ANOVA because they were heterogeneous. If results for both trials in either experiment were not significantly different ($P > 0.05$), data were pooled prior to analysis. To obtain homogeneous variances, data were transformed as follows: for experiment 1, mortality and number of male inflorescence were ln-transformed and height data were square-transformed; for experiment 2, mortality data for the R biotype for both the linuron and atrazine trials were square-root transformed, and S biotype data for the atrazine trial were also square-root transformed. Variable responses to increasing rates of linuron

⁷ Atrazine 480, United Agri Products, 7251 West 4th Street, Greeley, CO 80634.

⁸ SAS Statistical Software Package, Version 6.12, SAS Institute, Campus Drive, Cary, NC 27513.

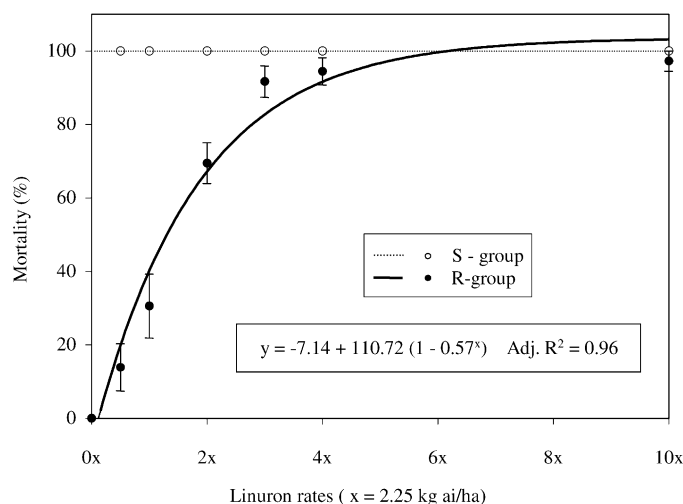


Figure 1. Percentage mortality (\pm SE) of linuron-susceptible (S) and linuron-resistant (R) common ragweed to varying linuron application rates for the first glasshouse experiment.

were further analyzed by regression.⁹ Data for the linuron trials in experiment 2 were ln-transformed for better fit. The herbicide rate required to achieve 50% mortality (LC_{50}) was then estimated. Resistance ratios (RR) were determined by dividing the LC_{50} for the R-biotype by the LC_{50} of the S-biotype.

RESULTS AND DISCUSSION

In experiment 1, all S-plants were killed at each of the linuron rates used (Figure 1), including the 0.5 \times rate (0.91 kg ai/ha), which closely corresponds to the minimum recommended rate for linuron in carrot (OMAF 2004). In contrast, R-plants were substantially less susceptible to linuron than S-plants, with only 14% control of R-plants at the 0.5 \times rate and almost 3% of R-plants surviving the 10 \times rate (Figure 1). Mortality in S-plants was also faster than that observed in R-plants, with death of S-plants occurring within 3 to 4 d of herbicide application, whereas R-plants that were killed died 6 to 7 d after treatment (data not shown). The maximum recommended linuron rate of 2.25 kg ai/ha (1 \times) resulted in 30% mortality of R-plants, although shoot biomass was reduced by more than 50% in both trials (Figure 2), and root biomass was reduced by 89% and 65% for trials 1 and 2, respectively, compared with the control treatment (0 \times) (Figure 3).

At the recommended 1 \times linuron rate, some plants in trial 2 had higher aboveground biomass (Figure 2), height, and number of staminate inflorescences (data not

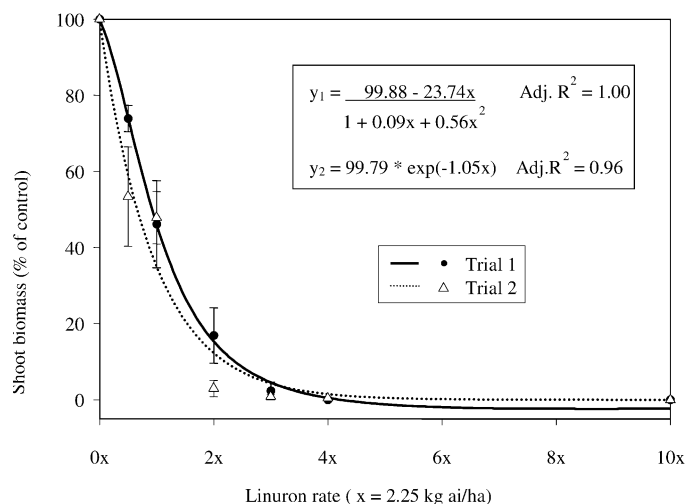


Figure 2. Shoot biomass (\pm SE; as a percentage of control) of linuron-resistant common ragweed to varying linuron application rates for the first glasshouse experiment. The two trials were not pooled as a result of significant differences ($P < 0.05$) in the analysis of covariance (ANCOVA) test.

shown) than that predicted by regression analysis. This finding indicates that recommended linuron rates used to control common ragweed may now actually provide enough of a stress to stimulate plant growth in the same manner as mechanical mowing has been shown to rapidly stimulate lateral shoot production in this weed species (Bachand and Christin 1996; Vincent and Ahmim 1985). Common ragweed is known to recover relatively quickly from many stresses (Bachand and Christin 1996; Ballard et al. 1996); therefore, reducing the population of this weed is more desirable than simply reducing its biomass. This explains why we focused results on the

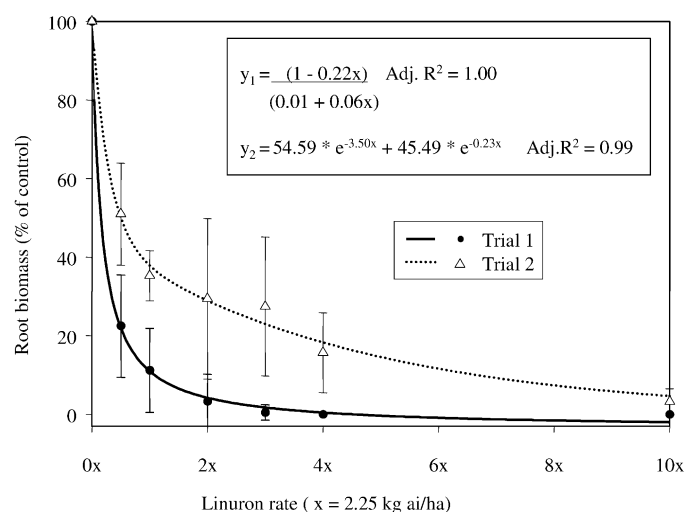


Figure 3. Root biomass (\pm SE; as a percentage of control) of linuron-resistant common ragweed to varying linuron application rates for the first glasshouse experiment. The two trials were not pooled because of nonhomogeneity of variances.

⁹ Sigma Plot 2000, Jandel Scientific. P.O. Box 7005, San Rafael, CA 94912-8920.

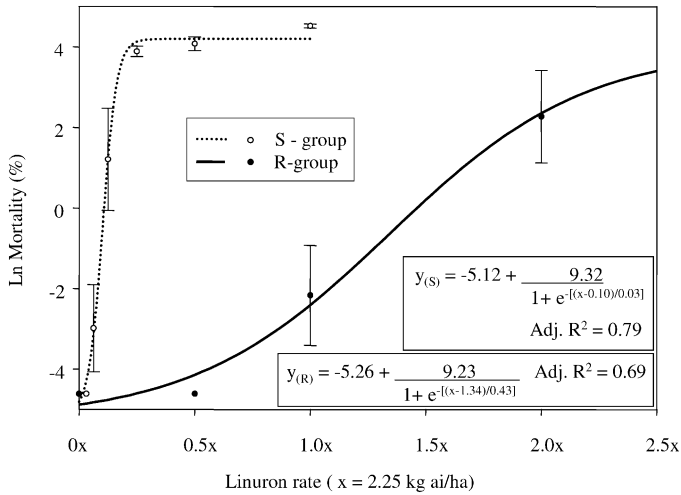


Figure 4. Percentage mortality (\pm SE) of linuron-susceptible (S) and linuron-resistant (R) common ragweed to varying linuron application rates for the second glasshouse experiment.

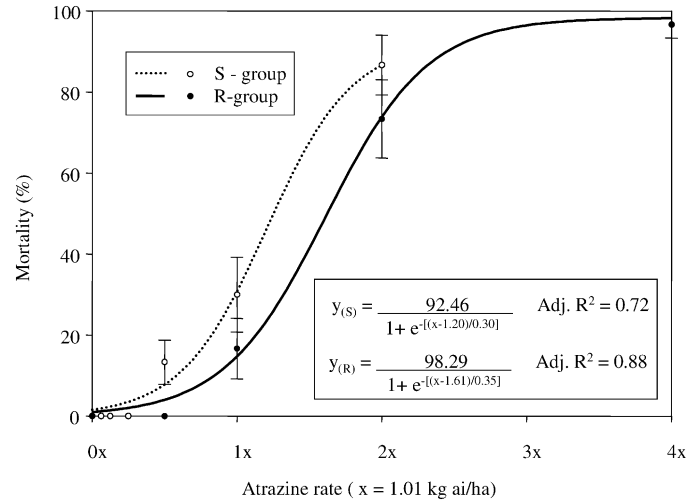


Figure 5. Percentage mortality (\pm SE) of linuron-susceptible (S) and linuron-resistant (R) common ragweed to varying atrazine application rates for the second glasshouse experiment.

effect of linuron on mortality of common ragweed plants rather than on biomass.

Linuron inhibits photosynthesis by binding to the Q_B -binding niche on the D1 protein of photosystem II complex in the chloroplast thylakoid membranes, thereby blocking electron transport from Q_A to Q_B (WSSA 2002). This results in stoppage of CO_2 fixation and production of ATP and $NADPH_2$, which are all required for plant growth. Linuron affects photosystem II in a similar manner to atrazine (Coupland 1991; Gronwald 1994; Moss and Rubin 1993; but see Smeda et al. 1993), and many linuron-resistant weed biotypes are cross-resistant to atrazine (Fuerst et al. 1986; Masabni and Zandstra 1999a). In experiment 2, lower linuron rates were used to determine the herbicide rate–response curve for the S-biotype that was required for calculating the linuron RR and also to verify for possible cross-resistance to atrazine. The RR for linuron in this study was 29.0 (Figure 4), which is considerably greater than the postemergence linuron RR of 3.4 obtained by Fuerst et al. (1986) for smooth pigweed (*Amaranthus hybridus* L. #AMACH) exposed to triazines and the postemergence ratio of 1.91 reported by Beuret (1989) for common groundsel (*Senecio vulgaris* L. #SENVU) treated with linuron. The RR for atrazine in this study was 1.3 (Figure 5), a value substantially lower than the postemergence atrazine RR values reported by Fuerst et al. (1986) of 530 for smooth pigweed, 49 for common lambsquarters (*Chenopodium album* L. #CHEAL), 890 for canola (*Brassica napus* L. var. Atratower), and 1,500 for common groundsel. Our findings indicate that the Sherrington, Québec, population of common ragweed studied is

much more sensitive to linuron applications than to atrazine, although both herbicides presumably were widely used in this field. Fuerst et al. (1986) showed that resistance to atrazine was much greater for smooth pigweed than resistance to linuron in a weed population that had been repeatedly sprayed with atrazine. However, Masabni and Zandstra (1999a) found that a common purslane (*Portulaca oleracea* L. #POROL) biotype resistant to linuron had developed an even greater preemergence and postemergence resistance to atrazine, even though the weed population had repeatedly been exposed to linuron and not to atrazine. Fuerst et al. (1986) noted that the precise point-mutation conferred to resistant biotypes by exposure to these herbicides could vary, thereby explaining discrepancies in resistance responses of the various weed populations tested. However, Smeda et al. (1993) reported no concomitant reductions in photosynthetic electron transport or cell growth rates in potato (*Solanum tuberosum* L.), despite mutant cells exhibiting extreme (250-fold greater) levels of resistance to atrazine, compared with wild-type cells.

The findings reported in this study indicate that some common ragweed biotypes in southwestern Québec have developed resistance to linuron and also cross-resistance to atrazine. This is the first reported case of common ragweed resistance to linuron. To date, 51 cases of resistance to urea and amide herbicides in 20 weed species have been reported, including resistance to linuron (Heap 2004). Weed populations from unrelated plant families and from various regions of the world have also exhibited resistance to linuron, including redroot pigweed (*Amaranthus retroflexus* L. #AMARE) and Powell am-

LITERATURE CITED

- aranth (*Amaranthus powellii* S. Wats. #AMAPO) in Canada, horseweed (*Conyza canadensis* (L.) Cronq. #ERICA) in France, wild poinsettia (*Euphorbia heterophylla* L. #EPHHL) in Ecuador, and common lambsquarters in Norway (Heap 2004; Holt and LeBaron 1990). Linuron-resistant biotypes of common purslane and common groundsel have been found in carrot fields of Michigan (Masabni and Zandstra 1999a) and Switzerland (Beuret 1989), respectively. Common ragweed has also developed resistance to S-triazine herbicides such as atrazine, cloransulam-methyl, cyanazine, simazine, and many Group 2 herbicides (Heap 2004; Patzoldt et al. 2001). Kuratle et al. (1969) reported that common ragweed did not metabolize linuron into nontoxic derivatives as well as carrot, thus suggesting that the selectivity of this herbicide is due to differential metabolism. Both Oettmeier et al. (1982) and Fuerst et al. (1986) reported that the linuron resistance observed in some weed biotypes was due to an alteration of the herbicide binding site within the chloroplast, although Fuerst et al. (1986) concluded that the biotypes used by the two research groups differed in the exact mutation that conferred resistance. Moreover, Fuerst et al. (1986) cautioned that the absorption, translocation, and metabolism of the herbicides used may also have played a role in the observed resistance. Beuret (1988, 1989) demonstrated that linuron resistance in horseweed was likely not due to a chloroplastic mutation and that resistance of common groundsel to this herbicide may be a result of the slow penetration or rapid metabolization of the chemical. Sequence analysis of the chloroplast D1 protein by Masabni and Zandstra (1999b) showed that linuron-resistant common purslane plants had undergone a serine to threonine substitution at position 264.
- The exact mechanism(s) of linuron resistance involved in the common ragweed-resistant biotype investigated in this study is not known and warrants further study.
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