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Inheritance and mechanism of resistance to herbicides inhibiting acetolactate synthase in *Sonchus oleraceus* L.

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Abstract A biotype of *Sonchus oleraceus* L. (Compositae) has developed resistance to herbicides inhibiting acetolactate synthase (ALS) following field selection with chlorsulfuron for 8 consecutive years. The aim of this study was to determine the inheritance and mechanism of resistance in this biotype. Determination of ALS activity and inhibition kinetics revealed that K_m and V_{max} did not vary greatly between the resistant and susceptible biotypes. ALS extracted from the resistant biotype was resistant to five ALS-inhibiting herbicides in an in vitro assay. ALS activity from the resistant biotype was 14 19, 2, 3 and 3 times more resistant to inhibition by chlorsulfuron, sulfometuron, imazethapyr, imazapyr and flumetsulam, respectively, than the susceptible biotype. Hybrids between the resistant and a susceptible biotype were produced, and inheritance was followed through the F_1, F_2 and F_3 generations. F_1 hybrids displayed a uniform intermediate level of resistance between resistant and susceptible parents. Three distinct phenotypes, resistant, intermediate and susceptible, were identified in the F_2 generation following chlorsulfuron application. A segregation ratio of 1:2:1 was observed, indicative of the action of a single, nuclear, incompletely dominant gene. F_3 families, derived from intermediate F_2 individuals, segregated in a similar manner. Resistance to herbicides inhibiting ALS in this biotype of *S. oleraceus* is due to the effect of a single gene coding for a resistant form of the target enzyme, ALS.

Key words *Sonchus oleraceus* L. · Acetolactate synthase · Inheritance · Herbicide resistance

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Introduction

The site of action of the commercially important sulfonylurea, imidazolinone and triazolopyrimidine herbicide classes (hereafter called ALS-inhibiting herbicides) is the plastidic enzyme acetolactate synthase (ALS; EC 4.1.3.18; Ray 1984). ALS is the first enzyme common to the biosynthesis of branched-chain amino acids. Over the past decade, ALS-inhibiting herbicides have become extensively used worldwide, mainly for selective weed control in a variety of crops. However, the widespread and persistent use of ALS-inhibiting herbicides has led to the appearance of weed biotypes resistant to these herbicides. The first case of resistance to a field-applied ALS-inhibiting herbicide was in *Lactuca serriola* L. (Mallory-Smith et al. 1990a). There have now been reports of several other weed species (mostly dicotyledonous) resistant to ALS-inhibiting herbicides in North America and Australia (reviewed in Saari et al. 1994). In most of the cases investigated, the biochemical mechanism of resistance to ALS-inhibiting herbicides is a herbicide-resistant ALS (Hall and Devine 1990; Devine et al. 1991; Saari et al. 1990 1992). However, many resistant biotypes of *Lolium rigidum* Gaud. have a susceptible ALS, but they are able to rapidly metabolise the herbicide to non-phytotoxic conjugates (Christopher et al. 1991; Cotterman and Saari 1992), most likely by enhanced cytochrome P_{450} activity (Christopher et al. 1994).

To date, published reports on the inheritance of sulfonylurea resistance in resistant weed populations which have developed in the field are limited. Studies on ALS herbicide-resistant *L. serriola* (Mallory-Smith et al. 1990b) and *Kochia scoparia* (L.) Schrad. (Thompson and Thill 1992, in Saari et al. 1994) have shown that resistance to ALS-inhibiting herbicides in these biotypes is controlled by a single nuclear gene with incomplete or complete dominance. Several studies on laboratory-generated ALS mutants have also shown a single major gene inheritance of resistance (Falco and Dumas 1985; Haughn and Somerville 1986; Harnett et al. 1987; LaRossa et al. 1987; Mourad et al. 1993).

In Australia we have recently documented resistance to ALS-inhibiting herbicides in two dicotyledonous weed species, *Sonchus oleraceus* L. and *Sisymbrium orientale* Torn. (Boutsalis and Powles 1994). In the present article, we report the biochemical mechanism and the mode of inheritance of resistance in a biotype of *S. oleraceus* resistant to ALS-inhibiting herbicides.

Materials and methods

ALS assays

Herbicides

Technical grade sulfonylurea, imidazolinone and triazolopyrimidine herbicides used for the enzyme assays were supplied by DuPont Agricultural Products (Newark, Del.) and American Cyanamid Co. (Princeton, N.J.).

Plant material

Seedlings of chlorsulfuron-susceptible (S) and -resistant (R) *S. oleraceus* (Boutsalis and Powles 1994) were grown in plastic trays (40 cm × 30 cm × 12 cm) containing pasteurised potting soil in a growth cabinet with day and night temperatures of 18.5°C and 14°C, respectively. Lighting was maintained at 490 μE m^{-2} s^{-1}, with a 14-h photoperiod.

ALS extraction and assay

The ALS extraction and assay protocols were modified from procedures described by Ray (1984) and Singh et al. (1988). Green leaf tissue from 3-week-old plants was ground in liquid nitrogen with 0.5 g polyvinyl polypyrrolidone. After grinding, extraction buffer (buffer:plant material, 2:1, v/w) containing 1 *M* potassium phosphate buffer (pH 7.5), 0.62% (w/v) dithiothreitol, 10 m*M* phenylmethylsulfonyl fluoride, 10 m*M* sodium pyruvate, 5 m*M* $MgCl_2$, 5 m*M* thiamine pyrophosphate (TPP), 100 μ*M* flavine adenine dinucleotide (FAD) and 10% (v/v) glycerol was added and the tissue homogenised for 30 s with an Ultra-Turrax homogeniser (Janke and Kunkel, Staufen, Germany). The homogenate was centrifuged at 30000 *g* for 20 min at 4°C and then filtered through one layer of miracloth (Behring Diagnostics, La Jolla, Calif.). The supernatant was brought to 60% (v/v) saturation by the dropwise addition of saturated ammonium sulfate to slowly stirring supernatant at 4°C and then stirred for 30 min. The crude enzyme pellet was collected by centrifugation for 30 min and then resuspended in 500 μl elution buffer (1 *M* potassium phosphate buffer (pH 7.5), 500 m*M* sodium pyruvate, 100 m*M* $MgCl_2$). The resuspended pellet was loaded on a sephadex G-25 column (Pharmacia PD-10) equilibrated with elution buffer, and eluted with 1.2 ml of elution buffer. Enzyme assays were performed in 400-μl well microassay plates (Labsystems).

Determination of ALS kinetic parameters

The assays were initiated with the addition of 20 μl crude enzyme fraction to 20 μl of sodium pyruvate and 60 μl of assay buffer (1 *M* potassium phosphate buffer (pH 7.0), 100 m*M* $MgCl_2$, 50 m*M* TPP and 100 μ*M* FAD). Enzyme activity for the pyruvate assays was expressed as nmol acetolactate mg^{-1} protein h^{-1} after determining acetoin content (Westerfield 1945) and protein concentration (Bradford 1976).

ALS inhibition by herbicides

The assays (104 m*M* sodium pyruvate) were conducted as previously described but with the addition of 20 μl of herbicide solution (prepared in 20 m*M* KH_2PO_4, pH 7.0). The assays were incubated at 35°C for 30 min. The enzymic reaction was terminated by the addition of 20 μl of 6 *N* H_2SO_4 to each well with a further 15-min incubation at 60°C. Finally, 95 μl of 0.55% (w/v) creatine and 95 μl of 5.5% (w/v) naphthol (in 5 *N* NaOH) were added. After a second 15-min incubation at 60°C, the absorbance was measured at 530 nm by a microassay plate reader (Titertek Multiskan MCC/340). Background optical densities (determined by adding 20 μl 6 *N* H_2SO_4 before the enzyme) were subtracted from the mean of four replicates. Activity was expressed as the percentage of activity in the samples containing no herbicide. I_{50} values were determined from the regression analysis of plots of ALS activity against log herbicide concentration. Both ALS kinetic and herbicide experiments were repeated in triplicate with four replications per experiment, and data were pooled with standard error of the means calculated for each concentration. The enzyme kinetic parameters, K_m and V_{max}, were estimated using Eadie-Hofstee plots.

Inheritance of ALS herbicide resistance

Parent plant material

Parent seeds of S and R *S. oleraceus* (Boutsalis and Powles 1994) were germinated on 0.6% (w/v) agar in a germination incubator at 20°C day /18°C night and 20 μE m^{-2} s^{-1} in a 12-h day/night cycle. After 5 days, seedlings were transplanted into two plastic trays (40 cm × 30 cm × 12 cm) containing potting soil and transferred to a glasshouse. When the seedlings were at the two-to-three-leaf stage, one tray, containing 30 S and 30 R plants, was sprayed with 23 g ai ha^{-1} chlorsulfuron plus 0.2% (v/v) non-ionic surfactant in a laboratory sprayer delivering 97 l ha^{-1}. After 3 weeks, 5 unsprayed S plants and 5 R herbicide survivors were transplanted into 25-cm-diameter pots and placed in the glasshouse. These plants were the parents used for the inheritance study.

Generation of F_1, F_2 and F_3 populations

S. oleraceus is self-fertile (Lewin 1948). The capitulum inflorescence of *S. oleraceus* commonly contains 140 florets (Salisbury 1942). Two distinct floret types are evident; inner florets and outer florets that have a purple tinge on the dorsal side (Lewin 1948). Studies in which either the inner or outer florets were removed and the capitula subsequently bagged to prevent unwanted pollen contamination have revealed that only the inner florets produce seed (data not shown). Also, Tsun-Shih et al. (1972) report that the flowers of *S. oleraceus* are partially sterile. This information was used to devise a method for artificially crossing *S. oleraceus* without the need for emasculation. One day prior to anthesis the sheath tissue of the capitula was cut, exposing the closed florets. Except for 4–6 purple tinged outer florets all of the other florets were removed and each capitulum immediately bagged to prevent contamination. By the next morning the selected florets had their ripened stigma protruding from the corolla. Crossing was performed by brushing the anthers of the R or S biotype against the exposed stigma of the S or R biotype, respectively. Control florets which were not cross-pollinated did not produce seed (data not shown). Mature F_1 seeds from florets cross-pollinated in this manner were harvested 2 weeks after crossing. Seeds from the parent S, R and the F_1 were germinated, transplanted into trays and transferred to the glasshouse, as described above. At the two-to-three-leaf stage the seedlings were sprayed with 4, 23 or 45 g ai ha^{-1} of the ALS herbicide chlorsulfuron plus 0.2% (v/v) non-ionic surfactant. Phenotypes were scored 28 days after treatment as either R, S or intermediate (I). Plants were designated as R if they showed no herbicide damage, S if they all died or I if they displayed severe stunting with only narrow and short new leaves produced from the meristem.

Three R♀ × S♂ F_1 and 3 S♀ × R♂ F_1 herbicide survivors were transplanted into 25-cm-diameter pots and transferred to the glasshouse. Just prior to anthesis each plant was covered with a plastic sleeve to ensure self-pollination. F_2 seeds collected from these plants and seed from the S and R parents were germinated and the seedlings treated with 23 g ai ha^{-1} chlorsulfuron plus 0.2% (v/v) non-ionic surfactant. Plant symptoms were scored 28 days after treatment.

To study segregation in the F_3 generation 19 F_2 herbicide survivors classed I and 17 classed as R were transplanted into 25-cm-diameter pots and allowed to self-pollinate as described for the F_2 production. Seeds were collected and F_3 seedlings treated with herbicide as described above.

Chi-square analysis of the segregation of ALS herbicide resistance in F_2 and F_3 plants was performed and a Chi-square homogeneity test conducted to compare the segregation ratios between families of the same generation (Fisher 1970).

Results

Mechanism of resistance to ALS-inhibiting herbicides

Eight annual consecutive field applications of chlorsulfuron have selected a biotype of *S. oleraceus* that is highly resistant to ALS-inhibiting herbicides (Boutsalis and Powles 1994). To establish the mechanism of ALS herbicide resistance in this biotype we first examined whether resistance could be endowed by an over-expression of ALS and whether the affinity for the substrate, pyruvate, differed between the enzyme from the two biotypes. We found that both the enzyme specific activity of ALS (V_{max}) and the affinity for pyruvate, measured as the K_m (the concentration of pyruvate required for 50% ALS activity), were similar between the two biotypes (Table 1). Thus, resistance to ALS-inhibiting herbicides in this biotype of *S. oleraceus* is not due to an over-expression of ALS, and there is no change in the affinity of the enzyme for pyruvate.

Any mutation of ALS that alters the herbicide binding site on the enzyme could cause resistance to ALS-inhibiting herbicides. To test this possibility, we performed in vitro assays with various ALS-inhibiting herbicides using the optimum pyruvate concentration (104 μ*M*). ALS from the R biotype was found to be highly resistant to the sulfonylurea herbicides chlorsulfuron and sulfometuron (Fig. 1a, b). The concentration of chlorsulfuron or sulfometuron required to inhibit in vitro ALS activity from the R biotype by 50% (I_{50}) was 14- and 19-fold greater, respectively, than that for the susceptible *S. oleraceus* biotype (Table 2). ALS from R plants was found to be less resistant to the imidazolinone herbicides imazethapyr and imazapyr and to the triazolopyrimidine herbicide flumetsulam, all of which also inhibit ALS (Table 2, Figs. 1c, 2) than to the sulfonylurea herbicides. The lower level of resistance of ALS to imidazolinone herbicides in vitro (Fig. 2) is correlated with the lower resistance exhibited by R *S. oleraceus* plants treated with these herbicides in vivo (Boutsalis and Powles 1994). Thus, the whole plant response of the resistant biotype to various ALS-inhibiting herbicides closely correlates with the response of the enzyme. This correlation strongly suggests that ALS herbicide resistance in this biotype of *S. oleraceus* is due to the possession of an ALS that is resistant to ALS-inhibiting herbicides.

Table 1 The catalytic properties K_m (pyruvate) and V_{max} of ALS isolated from leaves of resistant (R) and susceptible (S) biotypes of *S. oleraceus*. The catalytic properties were determined by regression analysis of Eadie-Hofstee plots from data obtained from three replicate experiments. Data is mean K_m and Vmax with standard error of the means

Biotype	K_m (μ*M*)	V_{max} (nmole mg^{-1} protein h^{-1})
R	13 ± 5	38 ± 12
S	16 ± 3	49 ± 15

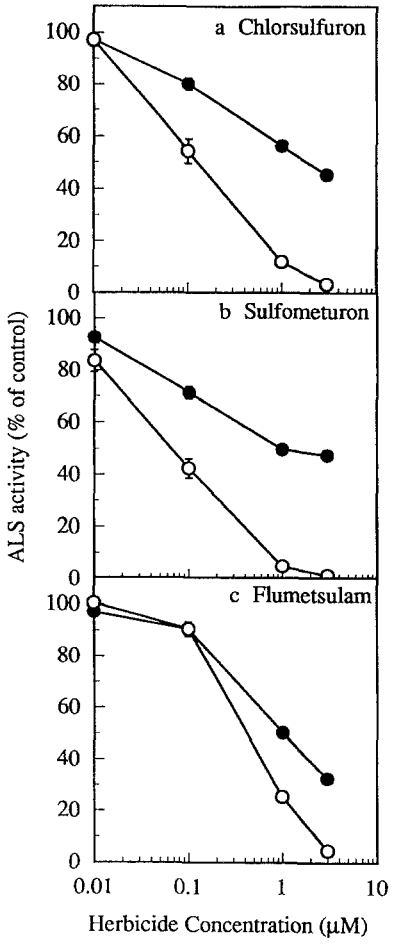

Fig. 1a–c Inhibition of ALS activity from a resistant (●) and a susceptible (○) *S. oleraceus* biotype by the sulfonylurea herbicides chlorsulfuron (**a**) and sulfometuron (**b**) and by the triazolopyrimidine herbicide flumetsulam (**c**). ALS activity is expressed as a percentage of activity in assays with no herbicide. Each point is the mean of triplicate experiments. *Vertical bars* represent the standard errors of the means (some error bars are obscured by point symbol)

Inheritance of resistance to ALS-inhibiting herbicides

Resistant *S. oleraceus* used as parents for this study were visually unaffected by 23 g ai ha^{-1} chlorsulfuron, whereas

Table 2 I_{50}[a] values for three classes of ALS-inhibiting herbicides: the sulfonylurea herbicides chlorsulfuron and sulfometuron, the imidazolinone herbicides imazethapyr and imazapyr and the triazolopyrimidine herbicide flumetsulam determined using crude extracts from resistant (R) and susceptible (S) *S. oleraceus*. I_{50}'s were computed by regression analysis

Herbicide	R	S	R/S ratio[2]
	I_{50} (μM)		
Chlorsulfuron	2.0	0.14	14
Sulfometuron	1.5	0.08	19
Imazapyr	84.1	28.2	3
Imazethapyr	49.4	27.1	2
Flumetsulam	1.0	0.31	3

[a] I_{50} is defined as the concentration of herbicide required to inhibit enzyme activity by 50%
[b] I_{50} ratio is calculated by dividing the I_{50} of R ALS by the I_{50} of S ALS

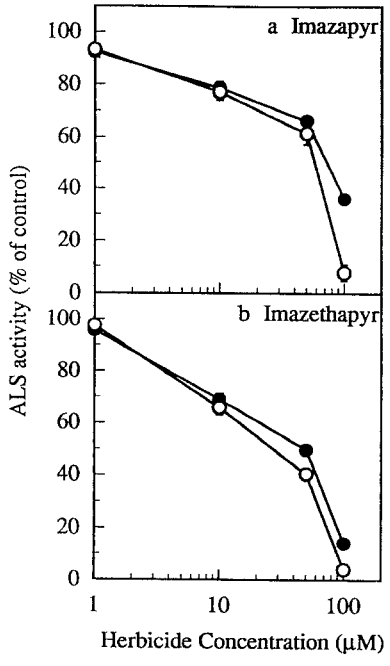

Fig. 2a, b Inhibition of ALS activity from a resistant (●) and a susceptible (○) *S. oleraceus* biotype by the imidazolinone herbicides imazapyr (**a**) and imazethapyr (**b**). ALS activity is expressed as a percentage of activity in assays with no herbicide. Each point is the mean of triplicate experiments. *Vertical bars* represent the standard errors of the means (some error bars are obscured by point symbol)

Table 3 Chi-square analysis of the segregation for chlorsulfuron resistance in F_2 families generated from self-pollination of three R♀ × S♂ and three S♀ × R♂ *S. oleraceus* F_1 plants, 28 days after treatment with 23 g ai ha^{-1} chlorsulfuron. Phenotype classes are R (as resistant parent), I (intermediate to both parents) and S (as susceptible parent)

Family	Phenotype				χ^2(1:2:1)	Probability
	R	I	S	Total		
F_2 (R♀ × S♂)						
1	38	73	36	147	0.06	0.97
2	37	72	45	154	1.48	0.48
3	24	47	29	100	0.86	0.65
F_2 (S♀ × R♂)						
1	32	86	29	147	4.37	0.11
2	38	73	46	157	1.59	0.45
3	25	51	23	99	0.72	0.92
Observed	194	402	208	804	0.49	0.78
Expected	201	402	201			
Test of homogeneity among F_2 families					8.59	0.13

Table 4 Chi-square analysis of the segregation for chlorsulfuron resistance in F_3 families generated from self-pollination of 18 intermediate F_2 *S. oleraceus* survivors of 23 g ai ha^{-1} chlorsulfuron 28 days after application

Family	Phenotype				χ^2(1:2:1)	Probability
	R	I	S	Total		
1	10	22	14	46	0.78	0.68
2	13	24	14	51	1.16	0.56
3	14	30	13	57	0.19	0.91
4	16	24	10	50	1.52	0.47
5	10	30	9	49	2.51	0.29
6	16	34	12	62	1.10	0.58
7	18	23	15	56	2.11	0.35
8	16	25	9	50	1.96	0.38
9	12	30	14	56	0.43	0.81
10	14	21	11	46	0.74	0.69
11	10	29	13	52	1.04	0.60
12	9	24	8	41	1.24	0.54
13	14	26	15	55	0.20	0.90
14	14	22	11	47	1.60	0.45
15	14	23	12	49	0.35	0.84
16	13	24	13	50	0.08	0.96
17	15	28	17	60	0.40	0.82
18	13	30	10	53	1.26	0.53
Observed	241	469	220	930	1.02	0.60
Expected	232.5	465	232.5			
Test of homogeneity among F_3 families					17.65	0.41

[a] See Table 3 for definition of phenotypes

as expected, all treated S plants were killed. All F_1 hybrids between R and S plants survived 4, 23 and 45 g ai ha^{-1} chlorsulfuron (data not shown). Furthermore, injury of reciprocal F_1 hybrids was uniform at each herbicide rate, indicating nuclear and not maternal inheritance of ALS herbicide resistance. At the higher herbicide rates F_1 hybrid growth was reduced more than that of the resistant parents, suggesting that the gene for ALS herbicide resistance is incompletely dominant. This differential growth reduction was used as the basis for scoring the intermediate phenotypes in the F_2 and F_3 generations.

Seeds from 6 self-pollinated F_1 parents were collected to generate six F_2 families. Treatment of the F_2 seedlings with 23 g ai ha^{-1} chlorsulfuron produced three distinct phe-

notypes: an R phenotype with slight injury; an I phenotype with severe stunting but no leaf necrosis or chlorosis, similar to the F_1 parents; and an S phenotype that was killed. ALS herbicide resistance in F_2 plants from each reciprocal cross segregated in a 1:2:1 (R:I:S) ratio (Table 3). Observed segregation ratios for each individual family (χ^2_5=8.59, P=0.13) and over the total 804 F_2 plants screened (χ^2_2=0.49, P=0.78) were not significantly different from the predicted 1:2:1 segregation ratios (Table 3). Hence, these studies establish that ALS-herbicide resistance in this biotype of *S. oleraceus* is endowed by a single, incompletely dominant gene. To further confirm single-gene Mendelian inheritance, we followed the inheritance of ALS-herbicide resistance into the F_3 generation. Eighteen F_2 I and 18 F_2 R plants were self-pollinated, producing 18 F_3 I and 18 F_3 R families. F_3 plants were herbicide-treated as described for F_2 plants. The 18 I F_3 families segregated in a 1:2:1 (R:I:S) ratio (Table 4). Chi-square analysis of the segregation of the F_3 I generation (χ^2_2=1.02, P=0.60) and of each individual family (χ^2_{17}=17.65, P=0.41) supported the hypothesis that ALS herbicide resistance in this biotype of *S. oleraceus* is endowed by a single, incompletely dominant, nuclear gene. No segregation for ALS herbicide resistance was observed for F_3 R plants.

Discussion

Biochemical mechanisms endowing herbicide resistance in plants can include increased metabolism, sequestration, reduced uptake and/or translocation and modification of the herbicide target site (Powles and Holtum 1994). For the ALS-inhibiting herbicides, both target site and non-target site resistance mechanisms have been reported (reviewed by Saari et al. 1994). Here, we have investigated whether ALS, the target site for ALS-inhibiting herbicides, from a resistant biotype of *S. oleraceus* has been modified and/or whether increased levels of the enzyme could endow resistance to ALS-inhibiting herbicides. Furthermore, we have identified the mode of inheritance of ALS herbicide resistance in R *S. oleraceus*.

Over-expression of ALS is not the mechanism of resistance in this biotype as there is similar ALS activity in both S and R *S. oleraceus* (Table 1). Other studies with ALS-resistant weed species have also shown that there is no difference in the specific activity of ALS between S and R biotypes (Christopher et al. 1992; Matthews et al. 1990; Saari et al. 1990 1992). That such a resistance mechanism is possible, however, has been established by mutagenesis, by which resistant *Daucus carota* lines were produced that have a 10-fold amplification of the ALS gene (Caretto et al. 1994).

Differences in the affinity of ALS from S and R plants for pyruvate could also contribute to ALS herbicide resistance. However, no significant difference in the K_m between ALS from R and S biotypes was measured (Table 1), suggesting the mutation(s) conferring ALS-herbicide resistance does not affect the affinity of the enzyme for pyruvate and possibly catalysis. This has also been reported for ALS-resistant *K. scoparia* (Saari et al. 1990).

The results presented in Figs. 1 and 2 establish that the mechanism of ALS herbicide resistance in this R *S. oleraceus* biotype is a herbicide-resistant ALS. The enzyme is resistant to sulfonylurea herbicides as shown by the high I_{50} values for chlorsulfuron (I_{50} ratio=14), the field selection agent, and for sulfometuron (I_{50} ratio=19). The R ALS is less resistant to the imidazolinone herbicides imazapyr and imazethapyr (I_{50} ratio=3 and 2, respectively) and to the triazolopyrimidine herbicide flumetsulam (I_{50} ratio=3). These in vitro enzyme results correlate well with the degree of resistance evident in vivo with whole plants treated with herbicide (Boutsalis and Powles 1994). High resistance to sulfonylurea herbicides and lower resistance to imidazolinone herbicides has also been reported for other ALS-herbicide resistant weeds (Devine et al. 1991; Christopher et al. 1992; Saari et al. 1992). It is possible that the specific mutation(s) conferring ALS-herbicide resistance in this biotype of *S. oleraceus* is the same as in other biotypes with a similar ALS herbicide-resistance spectrum.

Our studies on the inheritance of resistance to ALS-inhibiting herbicides in *S. oleraceus* establish that resistance is stably maintained through three successive generations. The segregation of ALS herbicide resistance showed Mendelian single-gene genetics. The absence of reciprocal differences in F_1 plants demonstrates that genetic control of ALS herbicide resistance is nuclear and not cytoplasmic. The intermediate phenotype observed for F_1, F_2 and F_3 heterozygous plants distinguishable from the parental phenotypes establishes that an incompletely dominant allele confers resistance to ALS-inhibiting herbicides. Chi-square analysis of the F_2 and F_3 generations and of the individual families (Tables 3, 4) supports a phenotypic 1:2:1 segregation ratio. Our findings from this analysis are strong evidence that ALS herbicide resistance in this biotype of *S. oleraceus* is endowed by a single, incompletely dominant nuclear gene and indicate that the R parents used in this study were homozygous for resistance. Homozygosity of R plants for ALS herbicide resistance is also supported by the low variation in percentage survival of plants treated with ALS-inhibiting herbicides (Boutsalis and Powles 1994).

The single gene inheritance of ALS herbicide resistance found in this resistant biotype of *S. oleraceus* is similar to ALS resistance reported elsewhere in plants. Resistance in ALS-resistant biotypes of *L. serriola* and *K. scoparia* in North America have shown that resistance segregated in a 1:2:1 (R:I:S) ratio in *L. serriola*, and in a 3:1 (R:S) ratio in *K. scoparia*. As the I phenotype was identified for *L. serriola*, ALS-herbicide resistance must be controlled by a single nuclear gene with incomplete dominance (Mallory-Smith et al. 1990b) while complete dominance is indicated for *K. scoparia* since no I phenotype was distinguished (Thompson and Thill 1992 in Saari et al. 1994). Studies on the inheritance of ALS herbicide resistant mutants of *Nicotiana tabacum*, *Arabidopsis thaliana*, algae, yeast and bacteria also indicate the presence of a dominant or incompletely dominant single gene endowing resistance in each

case (reviewed in Saari et al. 1994). Thus, the mode of inheritance of resistance reported in the literature for various organisms that have a resistant ALS is conferred by a single nuclear gene with varying degrees of dominance.

There is little published information on pollen dispersal of *S. oleraceus*. Lewin (1948) reported that bees and various flies, especially syrphids, visit *S. oleraceus* flowers. It follows that because the ALS gene is nuclearly encoded and therefore transferred by pollen, the potential for cross-pollination of susceptible plants with resistant pollen exists. Wind dispersal of pollen and achenes, coupled with the contamination of seed crops (Lewin 1948) from R plants, increase the potential risk of contamination of agricultural areas with R *S. oleraceus*.

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