

Dominance variation across six herbicides of the *Arabidopsis thaliana* *csr1-1* and *csr1-2* resistance alleles

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Abstract: Dominance of a resistance trait can be defined as a measure of the relative position of the phenotype of the heterozygote RS compared with the phenotype of the two corresponding homozygotes, SS and RR. This parameter has been shown to have primary importance in the dynamics of pesticide resistance evolution. Literature on insecticide resistance suggests that dominance levels in the presence of insecticide vary greatly from completely recessive to completely dominant. With insecticides, both the chemical applied and the dosages used have been demonstrated to affect the dominance. By contrast, almost all herbicide resistances have been found to be inherited as partially to totally dominant traits. This discrepancy between weeds and insects may partly result from the methodologies applied to measure the dominance, ie a single dose for herbicide *versus* several doses for insecticide. Using two well-known resistances (*csr1-1* and *csr1-2*) to acetolactate synthase (ALS) inhibitors in *Arabidopsis thaliana* (L) Heynh (mouse-ear cress), we used several herbicide doses to determine the dominance level to six ALS-inhibiting herbicides. The dominance level in the presence of herbicide varied from completely dominant to completely recessive, depending on the resistance allele and the herbicide tested. The dominance of the *csr1-1* and *csr1-2* resistance alleles ranged from 0 (completely recessive) to 1.1 (dominant) and from 0 to 0.3 (partially dominant), respectively. The recessivity of some resistance alleles in the presence of herbicide could lead to the development of improved resistance management in order to delay or avoid herbicide resistance evolution, especially in the control of outcrossing weed species.

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Keywords: herbicide resistance; acetolactate synthase inhibitors; resistance ratio; high dose; heterozygote; management; recessive trait

1 INTRODUCTION

Over the last 25 years, herbicide resistance has increased dramatically worldwide, repeating previous trends observed in insects and pathogens.^{1,2} As a consequence, numerous models have been developed to propose strategies to delay pesticide resistance. The dominance level of a pesticide resistance allele has been identified as a key parameter in models analysing the forces affecting the evolution of pesticide resistance.³ The fate of resistance alleles depends on their selective advantage or disadvantage (cost) in the presence or the absence of pesticides, as well as the dominance of these effects.⁴ The level of resistance conferred by a resistant allele is usually evidenced by a shift in the mortality/dose response. The dominance level of a resistance allele can be described theoretically as a measure of the relative position of the phenotype of the RS heterozygote relative to that of the two corresponding homozygotes (RR resistant and SS susceptible individuals).⁵ Although dominance level

in the presence of herbicide has limited incidence on the spread and establishment of resistance genes in selfing weed species,⁶ migration of RR seeds or gene flow by pollen from a resistant population to a susceptible population promotes the formation of RS plants in outcrossing weed species. It has been clearly established that the diffusion of a resistance allele will be much faster if it is expressed as a dominant (heterozygous RS individuals = homozygous RR resistant individuals) rather than a recessive trait (heterozygous RS individuals = homozygous SS susceptible individuals).⁷

In a review, Gould⁸ noted that the pattern of dominance of resistance alleles in the presence of pesticide tends to vary from insects to weeds. In insects, resistance alleles display a range of dominance, from completely recessive to completely dominant, in the presence of insecticide. For example, insecticide resistance provided by a major gene (an insensitive acetylcholinesterase) in the mosquito

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Culex pipiens L was found to be either recessive or dominant, depending on insecticide, insecticide dose, environmental parameters⁹ and genetic background.¹⁰ In contrast, for herbicide resistance, the literature indicates that almost all resistances are inherited as partially to totally dominant traits.^{6,11}

However, most studies reporting the degree of dominance of a herbicide-resistant trait in weeds were primarily designed to assess the inheritance of the resistance mutation. As a consequence, a single threshold herbicide dose is generally used. In theory, as illustrated in Fig 1, this single dose approach may not be appropriate to correctly assess dominance: when the lowest dose inhibiting the growth of SS plants is applied (dose A in Fig 1), RS and RR plants may both have normal growth. In this case, the resistant trait appears dominant. If the herbicide dose used increases (dose B in Fig 1), both RS and SS plants may exhibit no growth while RR continue to exhibit normal growth. In this case, the resistant trait appears to be recessive. In other words, the applied dose may affect apparent dominance and recessivity. An explanation for the lack of recessive resistance traits in weeds could therefore be that few studies were specifically designed to quantitatively evaluate the dominance level, ie the relative positions of dose-response curves of the SS, RS and RR plants over a range of herbicide concentrations that result in normal growth of SS plants through to no growth for the RR plants.¹²

In this study, we have quantitatively examined the dominance of two different herbicide resistances commonly found in weeds,² chlorsulfuron resistance and imazapyr resistance^{13,14} in the model cruciferous plant species *Arabidopsis thaliana* (L) Heynh (mouse-ear cress), for six ALS-inhibiting herbicides. The herbicides chlorsulfuron (sulfonylurea group) and imazapyr (imidazolinone group) both inhibit the branched-chain amino acid biosynthetic enzyme acetolactate synthase (ALS).^{15,16} Although *A thaliana* is not an important weed, its widespread use as a genetic model system makes it an ideal vehicle for testing hypotheses about resistance ratios and dominance levels. Moreover, the resistance ratios measured in *A thaliana* have been

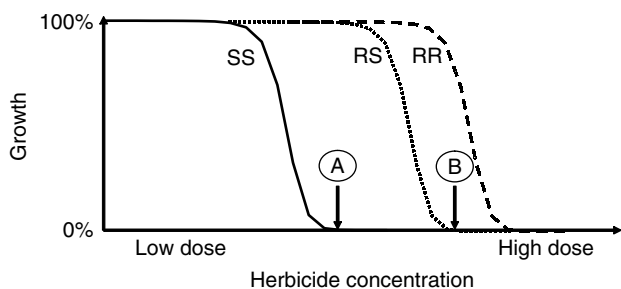


Figure 1. Estimation of the dominance of herbicide resistance. SS, RS and RR are the dose-response curves for homozygous susceptible plants, heterozygous resistant-susceptible plants and homozygous resistant plants, respectively. The herbicide concentrations at which SS plants and RS plants are completely inhibited are denoted by arrows A and B, respectively.

found to correlate with those found in some weed species with field-evolved resistance.¹⁷

2 MATERIALS AND METHODS

2.1 Plant material and chemicals

Two susceptible (SS) and two resistant (RR) homozygous lines of *Arabidopsis thaliana* were used in this study. The two commonly used Columbia (Col) and Landsberg (Ler) inbred lines were chosen as the susceptible references. We used *A thaliana* chlorsulfuron-resistant (*csr1-1* or GH50) and imazapyr-resistant (*csr1-2* or GH90) mutants isolated from ethyl methanesulfonate (EMS) mutagenized populations of the wild-type susceptible Col line.^{13,18} Resistance in the *csr1-1* mutant is endowed by a point mutation resulting in a Pro to Ser substitution at position 197, while resistance in the *csr1-2* mutant is endowed by a point mutation resulting in a Ser to Asn substitution at position 653.^{14,19,20} All *A thaliana* lines were provided by the Nottingham Stock Centre.

To obtain heterozygous individuals (RS) for the *csr1-1* and *csr1-2* resistance alleles, buds of NW77 male sterile SS plants derived from the Ler inbred line were pollinated by hand with either the *csr1-1* RR line or the *csr1-2* RR line. Crossing schemes are illustrated in Fig 2. Control individuals (SS) were generated by

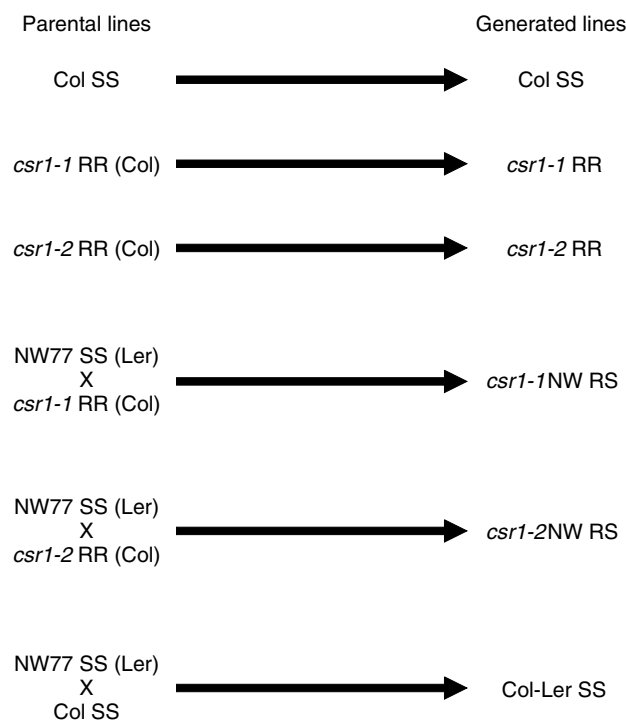


Figure 2. Crossing schemes. To obtain heterozygous individuals RS for the *csr1-1* and *csr1-2* resistance alleles, buds of Ler NW77 sterile SS plants (anthers and petals absent) were cross-pollinated by hand with either the *csr1-1* RR line or the *csr1-2* RR line. Control individuals SS were also generated by hand-pollination of Ler NW77 plants with the Col SS line. Individuals generated by cross-pollination with the *csr1-1* RR, *csr1-2* RR and Col SS lines are designated as *csr1-1*NW, *csr1-2*NW and Col-Ler lines, respectively. The name of the line from which the *csr1-1*, *csr1-2* and NW77 lines have been isolated is indicated in brackets.

Table 1. Herbicides applied on *Arabidopsis thaliana* for establishing dose-response and dominance levels of the *csr1-1* and *csr1-2* resistance alleles

Group ^a	Herbicide	Commercial formulation	AI content (g litre ⁻¹)
SU	Chlorsulfuron ^b	Glean	750
	Iodosulfuron ^c	—	100
	Nicosulfuron ^d	Milagro	40
IMI	Imazapyr ^e	Arsenal	250
	Imazaquin ^e	Scepter	700
TP	Metosulam ^f	Eclipse	100

^a SU, Sulfonylurea; IMI, Imidazolinone; TP, Triazolopyrimidine.

^b DuPont de Nemours (Paris, France).

^c Bayer Crop Sciences (Lyon, France).

^d Syngenta Agro (Saint-Cyr, France).

^e BASF Agro (Tassin la Demi Lune, France).

^f Dow AgroSciences (Sophia Antipolis, France).

hand-pollination of Ler NW77 plants with the Col SS line. Individuals generated by cross-pollination with the *csr1-1* RR, *csr1-2* RR and Col SS lines were designated as *csr1-1*NW, *csr1-2*NW and Col-Ler lines, respectively. Six lines were therefore used to assess the dominance level of the *csr1-1* and *csr1-2* resistance alleles (Fig 2): 2 SS lines (Col and Col-Ler), 2 RR lines (*csr1-1* and *csr1-2*), and 2 RS lines (*csr1-1*NW and *csr1-2*NW). The Col SS line was used as reference for the *csr1-1* RR and *csr1-2* RR lines as they share the same genetic background, while the Col-Ler SS line was used as reference for the *csr1-1*NW RS and *csr1-2*NW RS lines as they have a Col-Ler cross origin.

The six ALS-inhibiting herbicides used in this study (Table 1) were three sulfonylureas (SU), two imidazolinones (IMI) and one triazolopyrimidine (TP). All herbicides other than iodosulfuron were used as commercially available single-herbicide formulations. Iodosulfuron as a single-herbicide formulation was obtained directly from the manufacturer.

2.2 Bioassays and test of growth resistance to herbicides

Seeds were surface-sterilized by immersion in a solution of 85% (v/v) ethanol and 1% (v/v) commercial bleach (MIC, Chimie Plus) for 5 min followed by two rinses with 100% ethanol. Herbicides were 0.2-µm filter-sterilized and added to a medium complemented with micro- and macro-nutrients to optimize germination¹³ and solidified by adding 0.7% Sigma agar before autoclaving. The medium contained no supplemental sucrose.

We constructed a geometric series of herbicide concentrations with the aim of encompassing the range of zero to normal growth. For each herbicide, ten herbicide doses were applied to each of the six lines, equivalent to rates of 0.0045, 0.018, 0.073, 0.293, 1.172, 4.688, 18.75, 75, 300, and 1200 mg AI ha⁻¹, assuming 300 litres ha⁻¹. In that way, assuming that no major bias was induced by the various chemical formulations, the respective toxicity response of the

six lines for the six ALS inhibitor herbicides could be directly assessed.

About 50 surface-sterilized seeds were sown onto the surface of Petri dishes containing the sterile agar medium (15 ml). The dishes were arranged vertically in plastic racks and placed in a growth chamber under artificial light to provide a 16-h photoperiod. The temperature was maintained between 20 and 22 °C. To avoid micro-environmental effects, the racks of Petri dishes were regularly rotated during the growing period. For each line, rate and herbicide, the experiment was conducted once with two replicates, ie 100 seeds apportioned among two Petri dishes. Two weeks after treatment, root length was individually measured for 10 randomly chosen seedlings in each replicate. Twenty root lengths were therefore measured for each line, dose and herbicide combination.

2.3 Statistical analysis

For each line, dose and herbicide, root lengths were expressed as a percentage of their respective untreated controls to standardize comparisons between the six lines. For each line and herbicide, a replicate effect within each dose was then tested using the nested analysis of variance model: $y = \text{replicate}(\text{dose}) + \text{error}$. As no replicate effects were detected for line and herbicide (data not shown), data from the two replicates were pooled for each line, dose and herbicide combination, giving 20 root length measurements for subsequent analysis.

For each line, a non-linear regression was used to describe the response to ALS-inhibiting herbicides. We used the equation given by Kudsk and Streibig²¹ below and fitted the dose-response curve using Systat[®] (SPSS Inc., Chicago, USA):²²

$$W_{ij} = C + (D - C) / (1 + (x_{ij} / ED_{50i})^{b_i})$$

where W_{ij} denotes the root length at the j th dose of herbicide i ; D and C denote the upper and the lower limits of root length at zero and the highest doses of herbicide i ; ED_{50i} gives the dose of herbicide i to reduce root length by 50% between the upper and lower limits; and b_i is proportional to the slope of the curve around ED_{50i} . A high b_i value would be observed whenever a genetically homogeneous material gave a clear-cut response to herbicide dose, ie reduced environmental noise conditions. Comparisons of the four regression parameters between the Col SS line and either the *csr1-1* RR or the *csr1-2* RR lines (group 1) were conducted by examining the overlap between their respective 95% Wald's confidence intervals. When none of the regressions parameters differed between the Col SS line and either the *csr1-1* RR or *csr1-2* RR lines, dose response curves were considered identical. The same procedure was conducted to compare the Col-Ler SS line with either the *csr1-1*NW RS or the *csr1-2*NW RS lines (group 2).

The dominance level D_{HC} assessing the relative herbicide concentration required to give a similar effect on root length was then measured as:⁵

$$D_{HC} = (ED_{RS} - ED_{SS}) / (ED_{RR} - ED_{SS})$$

Following convention, the resistance allele is dominant in presence of herbicide when $D_{HC} = 1$, semi-dominant when $D_{HC} = 0.5$, and recessive when D_{HC} approaches 0.

3 RESULTS

For all herbicides other than imazapyr, the range of application rates was sufficient to correctly establish the dose-response curve for each line. For imazapyr, a new geometric series of herbicide concentrations (0.073, 0.293, 1.172, 4.688, 18.75, 75, 300, 1200, 4800 and 19 200 mg AI ha⁻¹) was applied to the six lines.

For all lines and herbicides, the parameters C and D were identical for root lengths (data not shown). For group 1, the parameter b (a measure of the sensitivity of the response to herbicide and environmental noise when identical genetic material is compared) did not differ between the Col SS line and either the *csr1-1* RR or the *csr1-2* RR lines (Table 2). The dose-response curves were thus parallel for the Col SS line and either the *csr1-1* RR or *csr1-2* RR lines. In the same manner, the dose-response curves were parallel for the Col-Ler SS line and either the *csr1-1* NW RS or the *csr1-2* NW RS lines (Table 2). As illustrated by the Col SS and *csr1-2* NW RS lines responses to chlorsulfuron and iodosulfuron (Table 2), the slope of heterozygous lines could differ significantly from that of the Col SS line. This discrepancy between groups 1 and 2 justifies the necessity for the *csr1-1* NW and *csr1-2* NW RS lines to be compared to their appropriate SS reference, ie the Col-Ler cross.

The parallel slopes within each group mean that the resistance ratio and the dominance level are identical over a range of herbicide dose effects. Hence, we focused on ED_{50} , ie a reduction of the root length by 50%, to estimate the resistance ratio and the dominance levels associated with the *csr1-1* and *csr1-2* resistance alleles. Resistance factors R/S were thus defined from the ED_{50} values as ED_{RR}/ED_{SS} .

For each resistance allele and each herbicide, the resistance ratios and their associated dominance level are given in Table 3. The *csr1-1* allele conferred no resistance to imazapyr or imazaquin, and cross-resistance to chlorsulfuron, iodosulfuron, nicosulfuron and metosulam. Such a pattern of cross-resistance is similar to previous observations,¹⁷ except for nicosulfuron (resistance ratio R/S < 5). The resistance ratios of the *csr1-1* resistance allele ranged from 6 (nicosulfuron) to 882 (chlorsulfuron). For the chlorsulfuron and iodosulfuron herbicides, the dose-response curves of the *csr1-1* RR and *csr1-1* NW RS lines with their corresponding reference Col SS and

Table 2. Means of the parameters ED_{50} and b for each line and each herbicide used in the study^a

	Group 1			Group 2		
	Col SS	<i>csr1-1</i> RR	<i>csr1-2</i> RR	Col-Ler SS	<i>csr1-1</i> NW RS	<i>csr1-2</i> NW RS
Chlorsulfuron	b 0.69 [0.49–0.90]	1.81 [0.03–3.59]	0.86 [0.55–1.16]	1.08 [0.70–1.47]	2.80 [0.39–5.21]	4.12 [1.37–6.88]
	ED_{50} 0.23 [0.13–0.34]	202.87 [80.28–325.47]	0.29 [0.16–0.42]	0.57 [0.37–0.78]	224.11 [177.78–270.44]	0.70 [0.55–0.86]
Iodosulfuron	b 0.65 [0.42–0.88]	1.00 [0.68–1.31]	0.95 [0.65–1.19]	1.49 [0.85–2.14]	0.90 [0.61–1.19]	1.20 [0.91–1.48]
	ED_{50} 0.11 [0.05–0.17]	45.83 [29.63–62.04]	0.57 [0.35–0.78]	1.59 [1.07–2.10]	11.93 [7.30–16.55]	0.99 [0.77–1.21]
Nicosulfuron	b 0.88 [0.52–1.24]	0.91 [0.21–1.61]	2.00 [0.14–3.86]	2.01 [0.25–3.77]	1.50 [0.07–2.93]	1.95 [0.61–3.29]
	ED_{50} 10.01 [4.65–15.38]	59.80 [22.68–96.92]	191.12 [83.62–304.62]	16.81 [10.50–23.12]	73.28 [24.07–122.45]	26.94 [16.23–37.65]
Imazapyr	b 2.07 [0.35–3.79]	1.36 [0.02–2.70]	3.34 [1.17–4.51]	0.57 [0.31–0.84]	1.64 [0.08–3.20]	3.16 [0.73–5.59]
	ED_{50} 6.88 [3.50–10.27]	5.20 [1.05–9.36]	3470.75 [401.39–6540.16]	1.96 [0.27–3.66]	8.16 [1.85–14.47]	910.57 [310.29–1510.85]
Imazaquin	b 1.42 [0.69–2.13]	1.06 [0.70–1.42]	1.25 [0.49–2.01]	1.52 [0.73–2.31]	0.60 [0.46–0.75]	2.03 [1.00–3.07]
	ED_{50} 1.99 [1.19–2.80]	5.37 [0.04–10.70]	93.84 [43.00–144.68]	1.34 [0.86–1.82]	0.64 [0.30–0.98]	29.17 [20.16–38.19]
Metosulam	b 1.15 [0.47–1.84]	2.07 [0.35–3.79]	2.10 [0.38–3.82]	2.00 [1.22–2.78]	2.54 [0.49–4.60]	1.17 [0.77–1.57]
	ED_{50} 2.20 [0.90–3.50]	616.27 [258.77–973.77]	3.07 [1.62–4.52]	2.95 [2.23–3.68]	219.49 [156.37–282.60]	1.28 [0.89–1.67]

^a Values in brackets indicate 95% Wald's confidence limits. ED_{50} is expressed in mg AI ha⁻¹. Group 1 refers to the genotypes sharing the same Col genetic background, while group 2 have a Col-Ler genetic background.

Table 3. Resistance ratios and dominance levels of the *Arabidopsis thaliana* *csr1-1* and *csr1-2* resistance alleles for six ALS-inhibiting herbicides. R/S: resistance ratio; D_{HC} : dominance level. ED_{50} in Table 2 was used to compare RS and Col-Ler SS lines and to calculate R/S and D_{HC} . Values in brackets indicate 95% confidence limits based on the 95% Wald's confidence limits of the ED_{50} values given in Table 2: right value (upper 95% confidence limits value of ED_{RR} /lower 95% confidence limits value of ED_{SS}); left value (lower 95% confidence limits value of ED_{RR} /upper 95% confidence limits value of ED_{SS})

	<i>csr1-1</i>			<i>csr1-2</i>		
	R/S	Comparison RS-SS	D	R/S	Comparison RS-SS	D
Chlorsulfuron	882.0 [236.1–2503.6]	RS > SS	1.10	1.3 [0.5–3.2]	— ^a	— ^a
Iodosulfuron	416.6 [174.3–1240.8]	RS > SS	0.23	5.2 [2.1–15.6]	RS = SS	0
Nicosulfuron	6.0 [1.5–20.8]	RS = SS	0	19.1 [5.4–65.5]	RS = SS	0
Imazapyr	0.8 [0.1–2.7]	— ^a	— ^a	504.5 [39.1–1868.6]	RS > SS	0.26
Imazaquin	2.7 [0–9]	— ^a	— ^a	47.2 [15.4–121.6]	RS > SS	0.30
Metosulam	280.1 [73.9–1082]	RS > SS	0.35	1.4 [0.5–5]	— ^a	— ^a

^a R allele does not confer any resistance advantage so that D_{HC} cannot be calculated.

Col-Ler SS lines are illustrated in Fig 3 where the differences between the patterns clearly demonstrates that the dominance index changes according to the herbicide used. The *csr1-1* resistance allele is completely dominant in the presence of chlorsulfuron (Fig 3), while it is largely recessive in the presence of iodosulfuron (Fig 3) or metosulam.

The *csr1-2* resistance allele conferred no resistance to chlorsulfuron or metosulam, and cross-resistance to imazapyr, imazaquin, nicosulfuron and iodosulfuron. Such a pattern was previously observed,¹⁷ except for iodosulfuron for which the *csr1-2* allele was found to confer no significant increased resistance compared to the wild type SS reference (resistance ratio R/S = 1). The resistance ratios of the *csr1-2* resistance allele ranged from 5 (iodosulfuron) to 554 (imazapyr). For iodosulfuron and nicosulfuron, the dose-response curve of the *csr1-2*NW RS line did not differ from that of the Col-Ler SS line (Table 2). The *csr1-2* resistance allele in the presence of iodosulfuron or nicosulfuron is therefore recessive. In the presence of imazapyr or imazaquin, values of D_{HC} were 0.26 and 0.30, respectively. The *csr1-2* resistance allele is largely recessive in the presence of imazapyr or imazaquin, ie the root length for the *csr1-2* RS line more closely matched the root length of the SS line than that of the *csr1-2* RR line.

4 DISCUSSION AND CONCLUSIONS

Assessing the dominance level of a resistance allele in the presence of a herbicide is of primary importance to predict the potential for resistance evolution in weeds, especially in outcrossing species. Using two resistances to ALS-inhibiting herbicides, the herbicide class with the greatest number of resistant weeds worldwide,² we found that the dominance level in the presence of herbicide can indeed vary from dominance to recessivity, depending on the resistance allele, the herbicide tested and the applied dose. Overall, the *csr1-1* resistance allele in *A thaliana* is dominant in the presence of chlorsulfuron, and recessive or nearly recessive for the other ALS-inhibiting herbicides. This

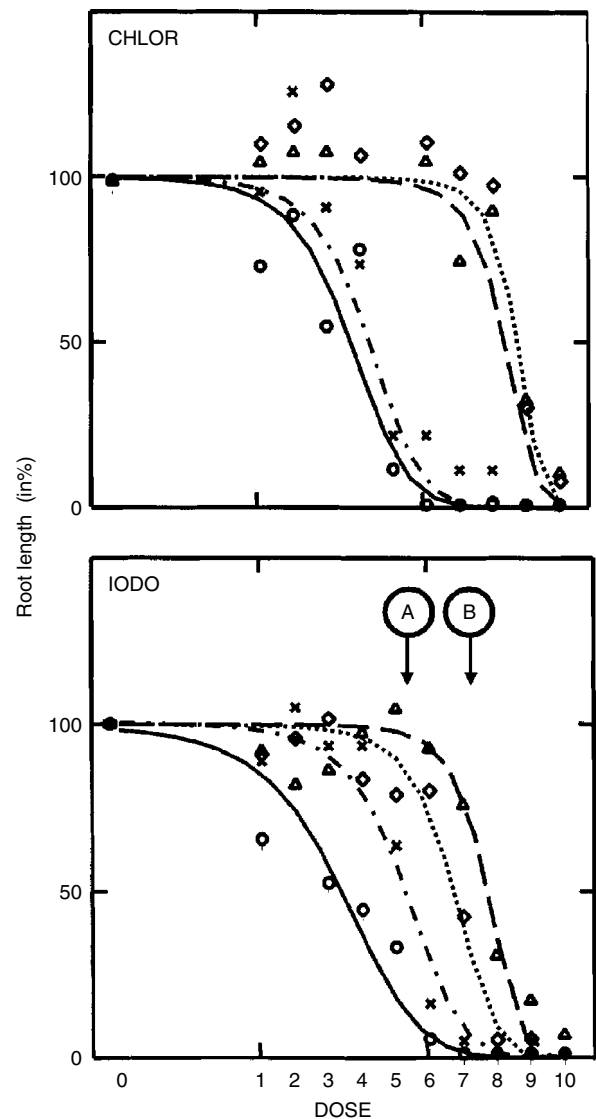


Figure 3. Illustration of the effect of two ALS-inhibiting herbicides on the root length of (O) Col SS, (x) Col-Ler SS, (Δ) *csr1-1* RR and (◇) *csr1-1*NW RS lines. Response curves: (—) Col SS; (- - - -) Col-Ler SS; (- - - -) *csr1-1* RR; (· · · · ·) *csr1-1*NW RS. CHLOR: chlorsulfuron; IODO: iodosulfuron. Doses 1 to 10 correspond to herbicide concentrations 0.0045 to 1200 mg AI ha⁻¹ (see Section 2.2). The lowest iodosulfuron concentrations at which SS plants and RS plants were completely inhibited are denoted by arrows A and B, respectively.

result is consistent with previous observations in *Beta vulgaris* L where the dominance level was evaluated quantitatively.¹² In this study, a chlorsulfuron resistant allele was found to be dominant in the presence of chlorsulfuron, and nearly recessive in the presence of primisulfuron, thifensulfuron or chlorimuron with dominance levels ranging from 0.26 to 0.36. By contrast, in *Cichorium intybus* L, a quantitative analysis of a chlorsulfuron-resistant line indicated that the resistance allele was nearly recessive with a dominance level of 0.17.²³ This discrepancy in dominance level in the presence of chlorsulfuron between *A thaliana* and *C intybus* could result from different point mutations within the ALS gene conferring resistance. Indeed, several mutations in the ALS gene other than the two used here are also known to confer resistance to chlorsulfuron, but to varying degrees.²⁴ Sequencing the ALS gene of the chlorsulfuron resistant line of *C intybus* would indicate if the point mutation conferring resistance differs from the *csr1-1* mutation in *A thaliana* (Pro to Ser substitution at the 197th amino acid). Here, the *csr1-2* resistance allele in *A thaliana* was also found to be recessive or nearly recessive, depending on the herbicide.

These results contrast with observations based on the use of a single herbicide dose which classify almost all resistances as partially to totally dominant traits.^{6,11} To date, the only report of recessive herbicide resistance is that of *Setaria viridis* (L) Beauv (green foxtail) to trifluralin.²⁵ Because of the potential and observed discrepancy between dominance estimates based on a single *versus* several herbicide doses, our results highlight the need to quantitatively evaluate the dominance level rather than to assess the degree of dominance of a herbicide-resistant trait by using only the lowest discriminating dose of the herbicide. For example, the *csr1-1* resistance allele would be recorded as dominant for iodosulfuron if tested at dose A (Fig 3) or recessive if tested at dose B. With a quantitative evaluation of dominance levels, it may be possible to look for opportunities to delay the evolution of herbicide resistance by using appropriate field doses which would delay the diffusion of RS plants, especially for recessive resistance traits.

Although resistance ratios in *A thaliana* remain consistent across two studies based either on root length (this study) or shoot biomass (especially for the *csr1-2* resistance),¹⁷ the estimates of the dominance level based on morphological traits may be an imperfect predictor of the degree of dominance at the fitness level. Indeed, the *csr1-1* RR plants are known to express a seed production penalty of 37% compared with RS plants, ie the fitness cost is recessive.²⁶ If chlorsulfuron is applied to a population, both *csr1-1* RR and RS plants would survive the treatment due to the complete dominance of the *csr1-1* resistance allele in the presence of herbicide. After the treatment, however, RS plants would have a higher fitness than RR plants, because RS plants do not suffer the seed production penalty.²⁶ As a consequence, it could be

expected that the *csr1-1* resistant allele would be over-dominant in the presence of chlorsulfuron. For the other ALS-inhibiting herbicides, a semi-dominance or a partial recessivity observed at the root level in presence of herbicide for the *csr1-1* resistance could be balanced by a higher fitness associated to the RS plants. As no cost was observed at the fitness level for the *csr1-2* resistance allele,²⁶ the dominance levels observed for roots are expected to correctly predict those observed for fitness. Studying the effect of the herbicide on the whole life cycle of the SS, RS and RR plants is clearly required if we have to accurately assess the dominance level in the presence of the herbicide, ie the D_{WT} estimate.⁵ This important point has been largely ignored by most studies and discussions of herbicide resistance, and has resulted in an oversimplification and false assumptions about the evolution of herbicide resistance.

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