Root biomass response to foliar application of imazapyr for two imidazolinone tolerant alleles of sunflower (*Helianthus annuus* L.)

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Imisun and CLPlus are two imidazolinone tolerance traits in sunflower (*Helianthus annuus* L.) determined by the expression of two alleles at the locus *Ahasl1*. Both traits differed in their tolerance level to imazapyr—a type of imidazolinone herbicide—when aboveground biomass is considered, but the concomitant herbicide effect over the root system has not been reported. The objective of this work was to quantify the root biomass response to increased doses of imazapyr in susceptible (*ahasl1/ahasl1*), Imisun (*Ahasl1-1/Ahasl1-1*) and CLPlus (*Ahasl1-3/Ahasl1-3*) homozygous sunflower genotypes. These materials were sprayed at the V2–V4 stage with increased doses of imazapyr (from 0 to 480 g active ingredient ha\(^{-1}\)) and 14 days after treatment root biomass of each plant was assessed. Genotype at the *Ahasl1* locus, dose of imazapyr and their interaction significantly contributed (*P < 0.001*) to explain the reduction in root biomass accumulation after herbicide application. Estimated dose of imazapyr required to reduce root biomass accumulation by fifty percent (GR\(_{50}\)) differed statistically for the three genotypes under study (*P < 0.001*). CLPlus genotypes showed the highest values of GR\(_{50}\), 300 times higher on average than the susceptible genotypes, and almost 8 times higher than Imisun materials, demonstrating that both alleles differ in their root biomass response to foliar application of increased doses of imazapyr.

**Key Words:** root growth, herbicide tolerance, *AHAS*, breeding, imidazolinones.

Introduction

Sunflower (*Helianthus annuus* L. var. *macrocarpus* Ckll.) is grown all over the world with three main purposes: beauty (ornamental sunflower), direct consumption of the seeds (confectionary sunflower) and oil production (oilseed sunflower). By far, the last of them is the most important objective in terms of acreage and production (Miller and Fick 1997). Sunflower oil has been traditionally viewed as a healthful vegetable oil and it is considered premium oil for salad, cooking and margarine production (Dorrell and Vick 1997) and also is being evaluated as a source of biodiesel (Bunta and Mario 2008). Weeds compete with sunflower for moisture, nutrients, and depending on species for light and space. Weed competition cause substantial yield losses in sunflower, with reports ranging from 20 to 70% (Bedmar et al. 1983, Brighenti et al. 2004, Chubb 1975, Fleck et al. 1989, Robinson 1973). Herbicides are the most desirable method for weed control, however the availability of selective herbicides for the sunflower crop is quite limited and, due to the high cost of herbicide registration, new molecules of herbicides are unlikely to be specifically developed for weed control in sunflower. For this reason, gene discovery and trait development for herbicide resistance in this crop, particularly imidazolinones and sulfonyleureas, was an active area of research during the past decade (Sala et al. 2012b).

Imidazolinone and sulfonyleurea herbicides have been demonstrated to have a broad spectrum of weed control activity, flexibility in timing of application, low usage rates, and low mammalian toxicity (Brown 1990, Tan et al. 2005). These herbicides inhibit the enzymatic activity of acetohydroxyacid synthase (AHAS, EC 4.1.3.18 also known as acetolactate synthase, ALS; Ray 1984, Shaner et al. 1984), the first enzyme in the pathway for the synthesis of the branched chain amino acids valine, leucine and isoleucine (Singh 1999).

The first commercial imidazolinone tolerance trait in sunflowers is known as ‘Imisun’ and it was developed from an imidazolinone-tolerant wild sunflower population crossed with cultivated sunflower (Al-Khatib et al. 1998). The inheritance of Imisun is additively controlled by two components, where one of them is a partially dominant allele, *Ahasl1-1* and the other, *Imr\(_{2}\)*, is a modifier or enhancer factor (Bruniard and Miller 2001, Miller and Al-Khatib 2002). To produce Imisun sunflower hybrids that express commercial tolerance levels to imidazolinone herbicides, both components need to be homozygous in the final variety. The second imidazolinone tolerance trait in sunflowers, known as ‘CLPlus’, is controlled by the expression of the partially dominant nuclear allele *Ahasl1-3* which was developed by seed mutagenesis and selection with an imidazolinone herbicide: imazapyr (Sala et al. 2008a). To achieve commercial
tolerance levels in CLPlus sunflower hybrids, only one homozygous component, namely Ahas1-3, is needed due to the high levels of imidazolinone tolerance conferred by this allele (Sala et al. 2008c). Sequencing results demonstrated that Ahas1-1 (also known as Imr1 or Arp, Bruniard and Miller 2001, Kolkman et al. 2004; respectively) harbors a C-to-T mutation in codon 205 (relative to Arabidopsis thaliana nomenclature) and Ahas1-3 has a G-to-A mutation in codon 122 (Sala et al. 2008b).

Crop injury in herbicide tolerant (HT) crops consists of several symptoms such as chlorosis, stunting, yellowing, decreased biomass production and yield loss. The crop injury phenotype can be attributed to the interaction between genotype and environment (GxE). The environmental component for herbicide tolerance is a sum of abiotic and biotic factors coupled with the effect of the type of herbicide and application parameters such as herbicide rates, surfactants and application timing (Frihau et al. 2005, Geier et al. 2004, Stougaard et al. 2004). The genotypic factor in a HT plant is the sum of the HT gene(s) effect plus the remaining genetic background, and the interaction between the two. For these reasons, comparison of HT gene performances can be carried out by means of dose response experiments under environmental controlled conditions and using the same genetic backgrounds. Comparisons of different HT genes or the additive effects of HT genes controlling the tolerance to a given herbicide are scarce in the literature. Using the described approach, Hanson et al. (2006) were able to conclude that biomass accumulation after imazamox treatment was similar among tolerant winter wheat cultivars carrying the resistance genes Ahas1B or Ahas1D. Tolerance in this type of cultivars was always higher than that shown by spring wheat cultivars carrying the same resistant genes. It was also observed that the spring wheat cultivars carrying two resistant genes had an additive level of tolerance to imazamox compared with single-gene resistant spring wheat (Hanson et al. 2006). Recently, the results of a quantitative imazapyr response assay in Imisun and CLPlus homozygous sunflower lines and hybrids were reported using the same approach. A122T substitution in the Ahas1 gene displayed the lowest level of inhibition of the AHAS enzyme extracts by imidazolinones, which resulted in the highest level of accumulation of above-ground biomass at all rates of herbicide application. A205V substitution, on the other hand, showed a higher inhibition of AHAS activity and a moderate level of above-ground biomass accumulation (Sala et al. 2012a).

Nevertheless, root biomass response to increased levels of foliar imidazolinone application was not reported yet. This is surprisingly since it has been shown, for example in susceptible plants of Arabidopsis thaliana, that one of the earliest responses to imidazolinone treatment is the inhibition of root growth, which occurs several hours after herbicide application (Manabe et al. 2007). In sunflower, assessment of root growth during germination demonstrated that the susceptible genotypes showed arrested root growth at all herbicide treatments and the Imisun tolerant genotype developed a complete root system even when exposed to the highest dose of herbicide (Vega et al. 2009). To the best of our knowledge the impact of foliar herbicide application over root growth for different HT genes has not been reported. For this reason, the objective of this work was to quantify the root biomass response to increased doses of imazapyr in susceptible, Imisun and CLPlus homozygous sunflower genotypes.

Materials and Methods

Plant material

Three different genotypes for the Ahas1 locus were assessed in three different genetic backgrounds: a commercial restorer line (R20), a maintainer inbred line (BTK47) and the F1 hybrid cmsgBTK47/R20, which totalize nine genetic materials (Table 1). Susceptible genotypes (ahasl/ahasl) included the original lines BTK47, R20 and their F1 hybrid (cmsgBTK47/R20). CLPlus tolerant genotypes (Ahas1-3/Ahas1-3) included GM40, R720 and their F1 hybrid (H3 = cmsgGM40/R720). GM40 is the original mutant line from BTK47 which carries the Ahas1-3 mutation in a homozygous state (Sala et al. 2008b). R720 is a BC1 F2 restorer line obtained by converting R20 to the CLPlus trait using GM40 as a donor line. Imisun tolerant genotypes (Ahas1-1/Ahas1-1) included IB9, IR7 and their F1 hybrid H2 (= cmsgIB9/IR7). IB9 traces back to BTK47 and IR7 to R20.

| Sunflower line or hybrid | Reproductive group | Pedigree or Origin | Ahas1 Genotype | IMI Tolerance | Name of the trait |
|-------------------------|--------------------|--------------------|----------------|--------------|-----------------|
| BTK47                   | Maintainer         | —                  | ahas1/ahasl1   | Susceptible  | —               |
| R20                     | Restorer           | —                  | ahas1/ahasl1   | Susceptible  | —               |
| H1                      | Hybrid             | BTK47/R20          | ahas1/ahasl1   | Susceptible  | —               |
| IB9                     | Maintainer         | IB9/IR7            | Ahas1-1/ahasl1 | Tolerant     | Imisun homozygous |
| IR7                     | Restorer           | —                  | Ahas1-1/ahasl1-1 | Tolerant     | Imisun homozygous |
| H2                      | Hybrid             | BTK 47 mutant      | Ahas1-3/ahasl1-3 | Tolerant     | CLPlus homozygous |
| GM40                    | Maintainer         | BTK 40 conversion  | Ahas1-3/ahasl1-3 | Tolerant     | CLPlus homozygous |
| R720                    | Restorer           | R20                | Ahas1-3/ahasl1-3 | Tolerant     | CLPlus homozygous |
| H3                      | Hybrid             | GM40/R720          | Ahas1-3/ahasl1-3 | Tolerant     | CLPlus homozygous |
Dose response experiments

Seeds of each genotype were sown in Petri dishes and, after germination, seedlings were transplanted into potting media consisting of equal parts of vermiculite, soil and sand in 10 cm diameter pots. Plants were grown in a greenhouse under natural light conditions supplemented with 400 W sodium halide lamps to provide a 16 h photoperiod. Day/night temperatures were 25 and 20°C, respectively. At the V2–V4 stage (Schneider and Miller 1981) 10 plants of each genotype were randomly assigned to each treatment consisting of seven doses of imazapyr (0, 40, 80, 160, 240, 320, 400, 480 grams of active ingredient per hectare — g a.i. ha\(^{-1}\) — which corresponded to 0x, 0.5x, 1x, 2x, 3x, 4x, 5x and 6x field rates, respectively). The experiment was arranged as a randomized block design with a full factorial (sunflower line x treatment) arrangement of treatments in 10 replications.

Plants were maintained for 14 days after imazapyr treatment at which time the root biomass were recorded. To do this, each plant was extracted from its pot and the substrate was carefully washed out from the roots. Roots were dried at 60°C for 48 h for root dry weight determination. Dry biomass data were converted to percentages of the untreated control plants within each line to allow direct comparisons between groups and subjected to ANOVA using the mixed model procedure of SAS (Littell et al. 1996, SAS Institute 2004), with degrees of freedom calculated by Satterthwaite’s approximation method (Satterthwaite 1946). Genotype at the Ahasl1 locus was considered fixed in the model, while genetic background and imazapyr doses were considered random variables. Means were separated using Fisher’s protected least significant difference (LSD) test at the 1% and 5% level of probability.

Statistical analysis of dose-response curves followed the procedure outlined by Seefeldt et al. (1995). Data were fit to a log-logistic model given by:

\[ y = 100/[1 + (x/GR_{50})^{b}] \]

Where \( y \) = root biomass (expressed as the percent of the untreated control), \( x \) = imazapyr dose (g a.i. ha\(^{-1}\)), \( b \) is a rate parameter (slope) related to the response to increasing imazapyr dose and GR\(_{50}\) is the imazapyr dose that caused a 50% of reduction in root biomass accumulation. Regressions were performed on all data using nonlinear least square regression procedure (PROC NLIN, SAS Institute 2004). Adequacy of model fit was determined by significance of the model approximate F-statistic and the coefficients of determination. Comparisons of the regression parameters among the three genotypes for the Ahasl1 locus were conducted by a nested analysis of variance using the model: \( y = \text{genotype for the Ahasl1 locus} + \text{genetic background (genotype for the Ahasl1 locus)} + \text{error} \). Means were separated using Fisher’s protected least significant difference (LSD) test at the 1% and 5% level of probability.

Results

Genotype at the Ahasl1 locus, imazapyr doses, their first order interaction and the three-factor interaction with the genetic background significantly contributed (\( P < 0.001 \)) to the variation in root biomass accumulation 14 DAT. On the other hand, analysis of variance indicated no significant (\( P < 0.05 \)) effect of genetic background and its two-factor interaction with genotype at the Ahasl1 locus and imazapyr doses. Observed significant interactions suggest that differences among HT genes for their response to increased doses of imazapyr.

Plants of the susceptible inbred lines and hybrids died at any application rate of imazapyr tested showing a complete burning of the shoot apex and necrosis of the root system. Root biomass in these genetic materials decreased from 18.8 to 6.4% of the untreated control plants as the imazapyr rate increased from 40 to 480 g a.i. ha\(^{-1}\) (Table 2). The genetic materials carrying the Ahasl1-1 allele in homozygous state showed different levels of yellowing, stunting, leaf abnormalities and necrosis according to the applied dose of herbicide. In correspondence to these phytotoxicity symptoms, root biomass significantly decreased from 59.4 to 16% of the untreated control plants when plants were challenged with increased doses of imazapyr (Table 2). In contrast, homozygous genotypes for the Ahasl1-3 allele did not show any injury in the aboveground organs, but their root biomass also decreased from 84.6 to 51.0% of the untreated controls when they were challenged with increased doses of imazapyr, from 40 to 480 g a.i. ha\(^{-1}\). Both tolerant genotypes, Imisun and CLPlus, significantly differed in their root biomass responses to imazapyr and these differences were expressed from the lowest to the highest doses (Table 2 and Fig. 1).

The log-logistic model accurately described root biomass response after imazapyr application for susceptible and

### Table 2

Average root biomass accumulation (percentage over untreated control plants) 14 days after treatment with imazapyr on three sunflower lines or hybrids for each of three genotypes at the Ahasl1 locus of sunflower

| Doses | Ahasl1 genotypes | Ahasl1 genotypes | Ahasl1 genotypes |
|-------|------------------|------------------|------------------|
|       | \textit{Susceptible} | \textit{Imisun} | \textit{CLPlus} | LSD among |
| \textit{ahasl1/ahasl1} | \textit{Ahasl1-1/ Ahasl1-1} | \textit{Ahasl1-3/ Ahasl1-3} | \textit{Ahasl1} |
| 0 | 100 a* | 100 a | 100 a | 5.6 |
| 40 | 18.8 ± 2.2 b | 59.4 ± 12.2 b | 84.6 ± 3.4 b | 5.9 |
| 80 | 11.2 ± 4.0 c | 50.2 ± 8.9 b | 75.7 ± 7.3 c | 5.6 |
| 160 | 10.1 ± 3.1 c | 40.9 ± 5.6 c | 73.0 ± 8.5 c | 5.5 |
| 240 | 8.7 ± 1.9 c | 36.0 ± 5.9 c | 63.9 ± 5.5 d | 5.4 |
| 320 | 6.6 ± 0.3 c | 22.6 ± 3.5 d | 61.8 ± 5.4 de | 5.3 |
| 400 | 6.5 ± 0.3 c | 18.8 ± 3.7 d | 56.9 ± 5.6 e | 5.1 |
| 480 | 6.4 ± 0.3 c | 16.0 ± 4.6 d | 51.0 ± 1.2 f | 5.0 |

* mean values with the same letter do not differ among doses.
tolerant sunflower materials (Fig. 2). Estimates of the doses of imazapyr needed to reduce the root biomass accumulation by the half (GR$_{50}$) and tolerant (T)/susceptible (S) ratio estimated by nonlinear regression for root biomass accumulation of three genotypes for the Ahasl1 locus in response to increasing doses of imazapyr

| Type of material | GR$_{50}$ (g.a.i ha$^{-1}$) | GR$_{50}$ ratio (T/S) |
|-----------------|-----------------------------|----------------------|
| CLPlus          | 603.0 ± 59.0 a*             | 314.1                |
| Imisun          | 78.6 ± 9.6 b                | 40.9                 |
| Susceptible     | 1.9 ± 0.7 c                 | 1                    |
| LSD             | 115.3                       |                      |

* mean values with the same letter do not differ among genotypes.

(Table 3). On the other hand, GR$_{50}$ estimate for the Imisun genotypes was 78.6 ± 9.6 g.a.i. ha$^{-1}$, a dose which corresponds to a 1x application rate under field conditions.

**Discussion**

It was shown in many species that the biosynthesis of the branched chain amino acids primarily occurs in young tissues, a fact that is sustained by the ubiquitous accumulation of AHAS mRNAs in fast growing organs (Degrande et al. 2000, Singh and Matthews 1994). For example, the organs of young chicory plants (Cichoryum intybus) that displayed the highest AHAS activity and AHAS mRNA content are the roots and the youngest leaf. Both roots and young leaves are known to have important sink strength toward carbohydrate produced in the old leaves. Young tissues of the root and the youngest leaf are not autotrophic toward carbohydrate and mostly depend on photosynthate influx provided by older leaves (Turgeon 1989).

Imidazolinone is absorbed through both foliage and root tissues (Tu et al. 2001). After entering a plant, imidazolinone is transported through the xylem and phloem to meristematic tissues where it binds to AHAS and inhibits its activity. Inhibition of AHAS leads to global elevation of free amino acids level and imbalances in their relative proportions (Höfgen et al. 1995); a relatively frequent outcome resulting from inhibition of an enzyme involved in amino acid biosynthesis pathways (Kim et al. 2002). In fact, time course analysis of transcriptome profiles in imidazolinone-sensitive (wildtype) and imidazolinone-resistant genotypes of Arabidopsis thaliana has demonstrated that in wildtype plants, the genes which responded earliest to imazapyr treatment were detoxification-related genes. Later stages of the imazapyr response involved regulation of genes participating in biosynthesis of amino acids, secondary metabolites and tRNA. In contrast, the transcriptome of resistant plants did not exhibit significant changes following imazapyr-
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treatment. Thus, all of the changes caused by imazapry treatment in susceptible plants, including global transcriptome expression, growth inhibition and eventual plant death are all caused by the inhibition of AHAS function (Manabe et al. 2007).

The results obtained in this study showed that root growth of susceptible plants of sunflower is inhibited by low doses of imazapry. In fact, a dose of imazapry of 1.92 g a.i. ha$^{-1}$ reduced by 50% the biomass of the roots 14 DAT, a value that is almost the same of that reported for the inhibition of the aboveground organs with the same herbicide (GR$_{50}$ = 1.9; Sala et al. 2012a). This indicates that the inhibition of AHAS activity in susceptible plants should be similar in both roots and young leaves.

Interestingly, the genetic background effect and its first order interactions with herbicide doses and genotype at the Ahasl1 locus, did not contribute significantly to the observed variability in root biomass responses; an observation also reported for the aboveground biomass accumulation after herbicide treatment in wheat (Willenborg et al. 2008) and sunflower (Sala et al. 2012a). However, the alleles that confer tolerance to imidazolinones in sunflower, Ahasl1-1 and Ahasl1-3, showed significantly different root biomass responses to increased doses of imazapry. In fact, the estimated value for GR$_{50}$ was almost 8 times higher for plants carrying the Ahasl1-3 allele than those carrying the Ahasl1-1 allele. The accumulation of root biomass after two weeks of herbicide application for both genotypes was highly associated with the already reported in vitro AHAS inhibition kinetics with imazapry (Sala et al. 2012a). In fact, plants carrying the Ahasl1-3 allele in homozygous condition displayed the lowest level of inhibition of the AHAS enzyme extracts which would result in the higher level of accumulation of root biomass at all rates of herbicide application. Plants homozygous for the Ahasl1-1 allele, on the other hand, showed a higher inhibition of AHAS activity and hence, a moderate level of root biomass accumulation after IMI application.

Interestingly, GR$_{50}$ estimated value for the root biomass response to imazapry in CLPlus genotypes (603 ± 59) was almost the same as the corresponding GR$_{50}$ value for shoot biomass response already reported (658.4, Sala et al. 2012a) indicating that this genotype shows the same pattern of response to increased levels of imidazolinones for shoots and roots. However, for the Imisun genotypes the pattern of response for the shoot is three times higher than for the root (GR$_{50}$ = 78.57 g a.i. ha$^{-1}$ for root biomass and 233 g a.i. ha$^{-1}$ for shoot biomass) indicating that biomass accumulation in the roots for this genotype is more sensitive to imidazolinone application than biomass accumulation in the shoots. Since Imisun genotypes need an enhancer factor to achieve high levels of tolerance apart from the target tolerance conferred by Ahasl1-1 (Bruniard and Miller 2001), it is tempting to speculate that this factor is expressed basically in the shoots, but this issue deserves to be fully investigated previous to reach to any conclusion. Nevertheless, this result indicates that the level of biomass accumulation after herbicide application in the aboveground parts of a HT plant may not be associated with the corresponding level of biomass accumulation in the roots and that both variables should be empirically assessed when comparing two HT traits of a given crop.

The results obtained clearly show that CLPlus genotypes are more tolerant to imidazolinones than Imisun genotypes at the root level when evaluated under non-stress conditions. In Arabidopsis, it has been shown that non target genes involved in the response to imidazolinone in wild type plants (for example, glutathione transferase (GST), cytochrome P450, ATP-binding cassette (ABC) transporter, multidrug and toxin extrusion (MATE) and alternative oxidase (AOX) protein families) also function in other abiotic stress responses (Manabe et al. 2007). This raises the possibility that abiotic stress and imidazolinone treatment may have additive effects that result in plant death or severe injury at lower concentrations of imidazolinone application. It is likely that the combined effect of imidazolinone application and environmental stresses under field conditions might result in even greater differences between CLPlus and Imisun sunflowers.

Literature Cited

Al-Khatib, K., J.R. Baumgartner, D.E. Peterson and R.S. Currie (1998) Imazethapyr resistance in common sunflower (Helianthus annuus). Weed Sci. 46: 403–407.

Bednar, F., M.I. Leaden and J.J. Eyherabide (1983) Efectos de la competencia de las maizales con el girasol (Helianthus annuus L.). Malezas 11: 51–61.

Brighenti, A.M., C. Castro, R.S. Oliveira Jr., C.A. Scapim, E. Voll and D.L.P. Gazziero (2004) Periodos de interferência de plantas daninhas na cultura do girasol. Planta Daninha 22: 251–257.

Brown, H.M. (1990) Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. Pesticide Sci. 29: 263–281.

Bruniard, J.M. and J.F. Miller (2001) Inheritance of imidazolinone herbicide resistance in sunflower. Helia 24: 11–16.

Bunta, G. and B. Mario (2008) The first results regarding the breeding of some sunflower hybrids for biodiesel. Analele Univ. din Oradea, Fascicula: Protectia Mediului, XIII, pp. 33–38.

Chubb, W.O. (1975) Weed competition in sunflower. Manitoba Agron. Conf. (Winnipeg, Man.) Tech. Pap., pp. 119–132.

Degrande, D., E. Dewaele and S. Rambour (2000) The AHAS gene of Cichorium intybus is expressed in fast growing and inflorescional organs. Physiol. Plantarum 110: 224–231.

Dorrell, D.G. and B.A. Vick (1997) Properties and processing of oil-seed sunflower. In: Schneider, A.A. (ed.) Sunflower Technology and Production, American Society of Agronomy/Crop Science Society of America/Soil Science Society of America, Wisconsin, pp. 709–746.

Fleck, N.G., J.J.O. Pinto and I.P. Mengarda (1989) Interferencia de plantas daninhas na cultura do girassol. Competição no tempo. Pesq. Agropec., Bras., 24: 1139–1147.

Frihauf, J.C., S.D. Miller and C.M. Alford (2005) Imazamox rates, timings, and adjuvants affect imidazolinone-tolerant winter wheat cultivars. Weed Technol. 19: 599–607.

Geier, P.W., P.W. Stahlman, A.D. White, S.D. Miller, C.M. Alfords and D.J. Lyon (2004) Imazamox for winter annual grass control in...
imidazolinone-tolerant winter wheat. Weed Technol. 18: 924–930.

Hanson, B.D., D.L. Shaner, P. Westra and S.J. Nissen (2006) Response of hard red wheat lines to imazamox as affected by number and location of resistance genes, parental background, and growth habit. Crop Sci. 46: 1206–1211.

Höfgen, R., B. Laber, I. Schütte, A.K. Kloums, W. Strieber and H.D. Pohlzn (1995) Repression of acetolactate synthase activity through antisense inhibition (molecular and biochemical analysis of transgenic potato (Solanum tuberosum L. cv Désirée) plants. Plant Physiol. 107: 469–477.

Kim, J., M. Lee, R. Chalam, M. Neal Martin, T. Leustek and W. Boerjan (2002) Constitutive overexpression of cystathionine γ-synthase in Arabidopsis leads to accumulation of soluble methionine and S-methylmethionine. Plant Physiol. 128: 95–107.

Kolkmann, J.M., M.B. Slabaugh, J.M. Bruniard, S. Berry, B.S. Bushman, C. Olungu, N. Maes, G. Abratti, A. Zambelli, J.F. Millet et al. (2004) Acetohydroxycy acid synthase mutations conferring resistance to imidazolinone or sulfonylurea herbicides in sunflower. Theor. Appl. Genet. 109: 1147–1159.

Littell, R.C., G.A. Milliken, W.W. Stroup and R.D. Wolfinger (1996) Common mixed models. In: SAS System for Mixed Models, SAS Inst., Cary, NC, pp. 31–86.

Manabe, Y., N. Tinker, A. Clville and B. Miki (2007) CSR1, the sole target of imidazolinone herbicide in Arabidopsis thaliana. Plant Cell Physiol. 9: 1340–1358.

Miller, J.F. and G.N. Fick (1997) The genetics of sunflower. Sunflower technology and production. American Society of Agronomy, Madison, Wis. (USA).

Miller, J.F. and K. Al-Khatib (2002) Registration of imidazolinone herbicide-resistant sunflower maintainer (HA425) and fertility restorer (RHA 426 and RHA 427) germplasms. Crop Sci. 42: 988–989.

Ray, T.B. (1984) Site of action of chlorsulfuron. Inhibition of valine and isoleucine biosynthesis in plants. Plant Physiol. 75: 827–831.

Robinson, R.G. (1973) The sunflower crop in Minnesota. Minnesota Agric Ext Bull, p. 299.

Sala, C.A., M. Bulos, A.M. Echarte, S. Whitt, G. Budziszewski, W. Howie, B. Singh and B. Weston (2008a) Development of CLHA-Plus: a novel herbicide tolerance trait in sunflower conferring superior imidazolinone tolerance and ease of breeding. Proc XVII Int Sunflower Conf, Córdoba, Spain, pp. 489–494.

Sala, C.A., M. Bulos and A.M. Echarte (2008b) Genetic analysis of an induced mutation conferring imidazolinone resistance in sunflower. Crop Sci. 48: 1817–1822.

Sala, C.A., M. Bulos, A.M. Echarte, S.R. Whitt and R. Ascenzi (2008c) Molecular and biochemical characterization of an induced mutation conferring imidazolinone resistance in sunflower. Theor. Appl. Genet. 108: 115–112.

Sala, C.A., M. Bulos, E. Altieri and B. Weston (2012a) Response to imazapyr and dominance relationships of two imidazolinone-tolerant alleles at the Ahasl1 locus of sunflower. Theor. Appl. Genet. 124: 385–396.

Sala, C.A., M. Bulos, E. Altieri and M.L. Ramos (2012b) Sunflower: improving crop productivity and abiotic stress tolerance. In: Tuteja, N., S. Gill, A.F. Tubercio and R. Tuteja (eds.) Improving Crop Resistance to Abiotic Stress, Vol. 2, Chapter 47, Wiley-Blackwell Wiley-VCH Verlag GmbH & Co., Germany, pp. 1205–1249.

Satterthwaite, F.E. (1946) An approximate distribution of estimates of variance components. Biom. Bull. 2: 110–114.

Statistical Analysis Systems (2004) SAS User’s Guide. Version 8.2. SAS, Cary, NC.

Schneider, A.A. and J.F. Miller (1981) Description of sunflower growth stages. Crop Sci. 21: 901–903.

Seefeldt, S.S., J.E. Jensen and E.P. Fuerst (1995) Log-logistic analysis of herbicide dose response relationships. Weed Technol. 9: 218–227.

Shaner, D.L., P.C. Anderson and M.A. Stidham (1984) Imidazolinones: Potent inhibitors of acetohydroxy acid synthase. Plant Physiol. 76: 545–546.

Singh, B.K. and B.F. Matthews (1994) Molecular regulation of amino acid biosynthesis in plants. Amino Acids 7: 165–174.

Singh, B.K. (1999) Biosynthesis of valine, leucine and isoleucine. In: Singh, B.K. (ed.) Plant Amino Acids, Marcel Dekker Inc., New York, pp. 227–247.

Stougaard, R.N., C.A. Mallory-Smith and J.A. Mickelson (2004) Downy brome (Bromus tectorum) response to imazamox rate and application timing in herbicide-resistant winter wheat. Weed Technol. 18: 1043–1048.

Tan, S., R.R. Evans, M.L. Dahmer, B.K. Singh and D.L. Shaner (2005) Imidazolinone-tolerant crops: history, current status and future. Pest Management Sci. 61: 246–257.

Tranel, P.J. and T.R. Wright (2002) Resistance of weeds to AHAS inhibiting herbicides: what have we learned? Weed Sci. 50: 700–712.

Tu, M., C. Hurd and J.M. Randall (2001) 17. Imazapyr. Weed Control Methods Handbook, The Nature Conservancy, http://tncweeds.ucdavis.edu.

Turgeon, R. (1989) The sink-source transition in leaves. Ann. Rev. Plant Physiol. Plant Mol. Biol. 40: 119–138.

Vega, T., G. Breccia, G. Nestares, M.L. Mayor and L. Picardi (2009) Soilless bioassays for early screening for resistance to imazapyr in sunflower (Helianthus annuus L.). Pest Management Sci. 65: 991–995.

Willenborg, C.J., A.L. Brulé-Babel, L.F. Frissen and R.C. Van Acker (2008) Response of heterozygous and homozygous imidazolinone-resistant spring wheat genotypes to imazamox. Crop Sci. 48: 2107–2114.