

Control of common ragweed in ALS herbicide-resistant sunflower hybrids (*Helianthus annuus*)



Tamas Kömives¹, Peter Reisinger², Andras Bittsanszky¹

¹Plant Protection Institut, Centre for Agricultural Research, Hungarian Academy of Sciences, 1022 Budapest Herman Ottó u. 15, Hungary; komives.tamas@gmail.com;

²Faculty of Food and Agricultural Sciences, University of West-Hungary, Var 2, 9200 Mosonmagyaróvár, Hungary

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Summary

Common ragweed is the number one weed in Hungary: it covers ca. 5% of the arable land, causing huge losses in row crops. In addition, because of the high allergenicity of its pollen, common ragweed is a huge burden on the health care system of the country. In 2011 and 2012 field studies were carried out in order to evaluate the common ragweed control efficacy of two acetolactate-synthase inhibitor postemergence herbicides (imazamox and tribenuron methyl) in sunflower hybrids NK Neoma and PR63E82, respectively, that carry the resistance gene against such herbicides. Common ragweed control by these herbicides was excellent: they suppressed the growth of the weed plant until the canopy closure of the crop plant (8-leaf stage). Common ragweed plants germinating after this date were unable to compete with the crop: although they survived, they remained small (ca. 75% reduction in height), produced ca. 90% less male flowers (source of the allergenic pollen), and caused no significant reduction in the crop yield. In areas where sunflower germination was poor, however, a second, mechanical common ragweed control measure was necessary to keep the weed density below damaging levels.

Introduction

The main cause of allergy and pollen asthma in North America and Central Europe is pollen from ragweed (*Ambrosia* spp.) a widespread genus in the Asteraceae (Cecchi et al., 2006). In Europe short or common ragweed (*A. artemisiifolia*) is prevalent (Grangeot et al., 2006). In Hungary, common ragweed infestation is heaviest in sunflower (*Helianthus annuus*), the third most important crop of the country (also an Asteraceae plant, thus, a botanical relative of common ragweed). In August-September common ragweed produces the overwhelming majority of allergenic pollen in the air ever in urban areas (Cecchi et al., 2006) (Cecchi et al., 2007). Increasing importance of bioenergy production together with recent advances in improving the dietary value of sunflower oil (Binkoski et al., 2005) (Edgerton, 2009) will certainly increase the production area of sunflower in the future. Thus, there is an urgent need for new tools to improve the control of common ragweed in this crop.

It is important to note that about seventy percent of the global sunflower harvest is produced in the European Union, Russia and Ukraine, where ragweed is spreading rapidly (Streit, 2012).

Recently, several new sunflower hybrids were registered in Hungary, including PR63E82 (Pioneer, Johnston, IA, U.S.A.) and NK Neoma (Syngenta, Basel, Switzerland), that are resistant to the acetolactate synthase-inhibiting herbicides tribenuron methyl and imazamox (Figure 1), respectively. These herbicides are known to control broadleaf weeds, such as common ragweed, efficiently (Merotto et al., 2009) the multilocus (tm). It is important to note that the new sunflower hybrids were developed by traditional plant breeding methods. Thus, they are not considered as genetically modified (GM) crops (Green and Owen 2011), and as a result, they can be produced in Hungary (and in other EU countries) where GM plants are not allowed.

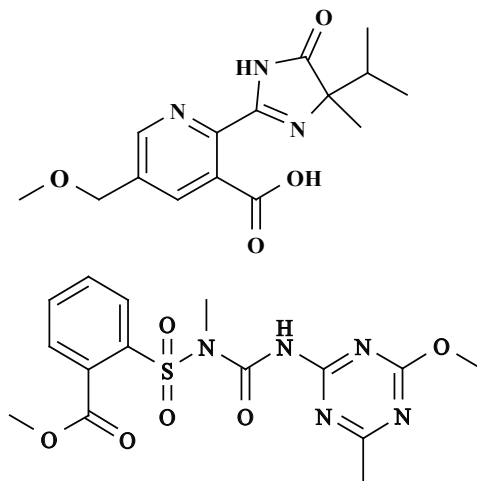


Figure 1. Chemical structures of imazamox and tribenuron methyl

Herbicide-tolerant crops were introduced into weed management practice about two decades ago. Most widely the RoundupReady™ (developed in 1994) and the LibertyLink™ (developed in 1992) GM-based technologies are used: these are founded on the application of the herbicides glyphosate (N-[phosphonomethyl]glycine) and glufosinate (2-amino-4-[hydroxymethylphosphinyl]butanoic acid), respectively (Green and Owen, 2011) (Benbrook, 2012).

The first commercial imidazolinone tolerance trait (Clearfield™) in sunflowers (Sala et al., 2012) 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 (ahas1/ahas1 was developed from imidazolinone-tolerant wild sunflowers that were discovered in the USA in 1996 (Al-Khatib et al., 1998). Tolerance to sulfonyleurea herbicides was obtained using induced mutagenesis (Streit, 2012) and led to the development of the ExpressSun™ technology (Bulos et al., 2013) SURES, and CLPlus are three herbicide tolerance traits in sunflower (*Helianthus annuus* L..

Materials and Methods

Field experiments

Sunflower hybrids PR63E82 and NK Neoma (21 ha each) were seeded in a commercial farm in Győr-Kismegyer, Hungary, in Mollic Fluvisol soil (3.75% organic matter, pH 7.6) containing large seed banks of weeds and common ragweed in particular. The crops were sown between April 20 and 25 at planting density 55,000 plants ha⁻¹. Imazamox (Pulsar 40 SL, 40 g a.i. L⁻¹, BASF AG, Ludwigshafen, Germany) and tribenuron methyl (Express 50 SX, 50 g a.i. L⁻¹, DuPont, Wilmington, DE, U.S.A.) herbicides were applied post-emergence at the 4-6-leaf growth stage between May 21 and 25 at the recommended rates of 1.2 L ha⁻¹ and 45 g ha⁻¹, respectively, using a Berthoud sprayer (Berthoud, Belleville, France) with an application rate of 220 L/ha, a spray pressure of 300 KPa and Visiflo TP8005 nozzles (Teejet Technologies, Wheaton, IL, U.S.A.). In the field ten 2x2 m sampling plots were randomly assigned. Half of the sampling areas were covered during the application of the herbicides: these plots served as untreated controls. Weeds at the sampling sites were surveyed as described previously (Reisinger et al., 2005).

During the weed surveys herbicide damage to the crop plants, if any, has also been recorded.

Meteorological data: During the registration of pollen counts, wind speed and wind direction were determined using a Weather Station WS-3600 instrument (Conrad Electronic SE, Hirschau, Germany).

Sunflower yields: Sunflower yields were recorded by the fields' owner.

Results

Weed surveys carried out before the application of the herbicides showed high weed densities in all fields (Table 1). Both tribenuron methyl and imazamox were highly efficient in controlling weeds in sunflower: plots planted with the herbicide-resistant sunflower remained free of common ragweed until the end of June (Table 1). Tribenuron methyl provided less control of barnyardgrass (*Echinochloa crus-galli*) and proso millet (*Panicum miliaceum*). In July and August a small number of common ragweed plants emerged (weed cover < 1%): they were ca. 75% shorter and produced more than 90% less male flowers than the untreated controls (Table 1). Established common ragweed plants were found only in untreated sampling sites and in areas where sunflower crop plants poorly germinated.

Average yields of the new hybrids (Table 1) were slightly but not significantly higher than that of the average local sunflower hybrids used in the region ($2.22 \pm 0.42 \text{ t ha}^{-1}$, provided by six growers).

Table 1: 2011. Data collected on August 31, sunflower harvest date September 17. Ragweed data in the herbicide-treated plots were determined from the few surviving plants.

Hybrid / Herbicide	Total weed cover (%)	Ragweed cover (%)	Number of male flowers per plant	Ragweed height (cm)	Sunflower yield (t/ha)
NK Neoma / Imazamox	1.2 ± 0.8	0.0	216 ± 80	27.4 ± 8.7	2.6
PR63E82 / Tribenuron methyl	4.6 ± 2.4	0.0	260 ± 118	26.0 ± 6.8	2.3
Untreated	97.0 ± 2.1	41.0 ± 14.6	3968 ± 1278	99.1 ± 19.3	n.d.

Table 2: 2012. Data collected on August 31, sunflower harvest date September 11. Ragweed data in the herbicide-treated plots were determined from the few surviving plants.

Hybrid / Herbicide	Total weed cover (%)	Ragweed cover (%)	Number of male flowers per plant	Ragweed height (cm)*	Sunflower yield (t/ha)
NK Neoma / Imazamox	1.0 ± 1.0	0.0	276 ± 113	28.4 ± 5.9	2.6
PR63E82 / Tribenuron methyl	3.7 ± 2.4	0.0	333 ± 182	27.0 ± 5.9	2.3
Untreated	97.4 ± 2.3	39.4 ± 14.6	4488 ± 753	101.8 ± 16.8	n.d.

Discussion

As regards to control of common ragweed both herbicides gave excellent results. It should be noted that various perennial and annual grasses may be poorly controlled by some sulfonylurea herbicides (Sikkema et al. 2007): in our study tribenuron methyl provided less control of barnyardgrass (*Echinochloa crus-galli*) and proso millet (*Panicum miliaceum*). Pollen production by common ragweed is at its maximum from late August to early September (Cecchi et al. 2006). Our weed surveys carried out repeatedly at the end of August showed that the few common ragweed plants (weed cover < 1%) emerging in July and August could not efficiently compete for light, water, and nutrients in established sunflower stands. Reduced common ragweed density, plant height and number of pollen-producing flowers practically halted release of common ragweed pollens from stands of herbicide-resistant sunflowers (Table 1).

In conclusion, our study clearly indicates that the new technology based on the use of sunflower hybrids resistant to ALS-inhibiting herbicides is a highly efficient tool to control common ragweed in sunflower fields and, as a result, to reduce concentrations of its allergenic pollen in the air. Major factors for the success of common ragweed control when using this technology will be 1) the management of resistance due to recurrent use of ALS-inhibiting herbicides (Délye et al., 2009) herbicide-resistant alleles; container-title: "Weed Research", page: "326-336", volume: "49", issue: "3", abstract: "Lolium species (ryegrasses, 2) the control of volunteer sunflowers (Yu et al., 2010) the effect of resistance mutations on AHAS functionality and plant growth has been investigated for only a very few mutations. This research investigates the effect of various AHAS resistance mutations in *Lolium rigidum* on AHAS functionality and plant growth. The enzyme kinetics of AHAS from five purified *L. rigidum* populations, each homozygous for the resistance mutations Pro-197-Ala, Pro-197-Arg, Pro-197-Gln, Pro-197-Ser or Trp-574-Leu, were characterized and the pleiotropic effect of three mutations on plant growth was assessed via relative growth rate analysis. All these resistance mutations endowed a herbicide-resistant AHAS and most resulted in higher extractable AHAS activity, with no-to-minor changes in AHAS kinetics. The Pro-197-Arg mutation slightly (but significantly) expressing ALS-resistance genes when emerging in following crops (Breccia et al., 2013), and 3) the accuracy of herbicide application, since under extreme weather conditions the new sunflower varieties may suffer from herbicide damage.

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