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CHEMICAL CONTROL OF BROOMRAPE (*Orobanche cernua* Loefl.) IN SUNFLOWER (*Helianthus annuus* L.) RESISTANT TO IMAZETHAPYR HERBICIDE

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SUMMARY

A wild *Helianthus annuus* L. population collected in Kansas, U.S.A., showed about 25% of its plants fully resistant to the herbicide imazethapyr applied in post-emergence at doses between 26.6 and 53.2 g.a.i./ha. Some plants survived treatments of 851.2 g.a.i./ha, i.e., 32 times the normal doses used in soybean. Artificially infected plants of this population with the parasitic weed species *Orobanche cernua* Loefl. (including *O. cumana* Wallr.) showed 100% of the plants susceptible. Artificially infected plants treated with imazethapyr in post-emergence on 40-day old plants killed most of the parasite nodules growing on the sunflower roots. The control of broomrape was 100% if the count only included the number of emerged broomrape shoots per plant. These results open the possibility of a new method for the control of *Orobanche cernua* in sunflower based on the use of resistance to this natural herbicide and the post-emergence treatment with imazethapyr.

Key words: **Sunflower, herbicide resistance, *Orobanche*, parasitic weed, chemical control, imazethapyr**

INTRODUCTION

The genus *Orobanche* compiles some important parasitic weeds which cause severe damage and serious reductions of yield in different crops and countries. The species *O. cernua* Loefl. (including *O. cumana* Wallr.), is responsible of severe infections in cultivated sunflower in many countries. So far, the only control measures included very long rotation periods and the use of resistant cultivars.

The genetic resistance to the sunflower's broomrape has allowed the production of the oilcrop in many countries with areas heavily infected with this parasitic weed. Nevertheless, the continuous build up of new pathotypes has forced the sunflower breeding programs to search continuously for new genes for resistance and backcrossing them into best sunflower populations and inbred lines, (Pustovoit, 1967 and 1976; Vrânceanu *et al.*, 1981; Cubero, 1986).

Among the different strategies to gain control over different *Orobanche* species, the use of selective herbicides has proven to be successful in faba bean and other species (Kasasian, 1973 a, b; Garcia-Torres and Lopez-Granados, 1991a, b; Jacobson and Eldar, 1992). However, there is not a good chemical control method for *Orobanche* in sunflower so far.

Some herbicides from the imidazolinones have proven to control or reduce broomrape infections in some crops including sunflower (Lolas, 1994; Garcia-Torres, 1994). Among them, imazethapyr, a herbicide used in soybean (Pursuit in USA and Overtop in Europe) has shown relatively good broomrape control in some legumes, both in treatments made pre- and post-emergence (Garcia-Torres and Lopez-Granados,1991a; Saber *et al.*, 1994). Contrary to legume crops, this herbicide causes toxicity in cultivated sunflower when applied post-emergence. Treatments with imazethapyr pre-emergence in sunflower have allowed an incomplete broomrape control (from 35% to 100%), but some plant toxicity was observed at the higher doses of the herbicide, *i.e.*, 80 g.a.i./ha, (Garcia-Torres *et al.*, 1994). Another imidazolinone, imazapyr, has shown some use for the control of broomrape in sunflower (Garcia-Torres *et al.*, 1995).

The potential for a herbicide applied post-emergence being translocated through the host and damaging the parasite at an early stage on the roots was proposed by Whitney (1973) when he had shown that 2,4 D applied to faba bean was accumulated in the *O. crenata* tubercle to a level 14 times that in the host root. Even with this differential concentration, damage to the host was severe. In a routine herbicide evaluation, Kasasian (1973 a, b) found some tolerance to glyphosate in faba bean which allowed the use of this herbicide for selective control of *O. crenata*.

Recently, a wild sunflower population of *Helianthus annuus* L., resistant to imazethapyr has been found in Kansas, U.S.A., in Mr. Doug Frech's farm which had been under soybean monocultive for many years (Don Lilleboe, 1997). If the nature of this resistance allows the translocation of the herbicide from the leaves to the roots before its detoxification inside the plant, it could represent a new method for the control of *Orobanche* in sunflower. This method would be based on the combined use of the imazethapyr resistant sunflower cultivars along with post-emergence treatment with imazethapyr. Furthermore, this could also be an effective weed control method in sunflower.

In this work the potential post-emergence use of imazethapyr for the control of *O. cernua* in sunflower has been studied.

MATERIAL AND METHODS

A wild *H. annuus* population (K890) was collected in 1997 in a soybean monocultive field in Kansas (U.S.A.) by one of the authors, in the same location where Alkhatib found a wild *H. annuus* population resistant to imazethapyr (Figure 1a.)

As the wild sunflower seed germinate with great difficulty, a special protocol was followed to obtain enough germinating seeds from K890 for the present study.

The seeds were surface sterilized by soaking in ethylalcohol (70%) for 30 seconds, then transferred to a calcium hypoclorite solution (5%) for 3 minutes. After water-washing the seeds, they were kept for 24 hours in sterilized distilled water. The achene's pericarp (hull) and seed coat were removed and the naked seeds were planted in petri dishes with filter paper saturated with a 60 ppm solution of Etephon 48% (Ethrel 48) for three days. As soon as the seeds started the radicle growth, they were placed in pots. Different pot sizes were used for different parts of the study. A standard soil mixture was used as substrate. It has a 4:5:5 ratio of natural-sterilized vertisol soils, sand and peat, respectively.

The first trial was made to establish if the wild sunflower population K890 had plants resistant to imazethapyr (Overtop 35 from CYANAMID), when sprayed in post-emergence. The percentage of resistant plants in the wild population had to be found if the population was segregating for the herbicide resistance.

Six doses of herbicide were tested: *i.e.*, 26.6, 53.2, 106.4, 212.8, 425.6 and 851.2 g.a.i./ha. They were sprayed on 30 K890, and 30 cultivated sunflower plants per treatment. The treatments were applied when the plants had 5 pairs of leaves and were allowed to grow in a greenhouse. The survival rate and level of herbicide resistance was recorded three weeks after treatment.

The second trial was carried out to find the effect of the herbicide on *O. cernua* growing on the roots of the sunflower population K890 when the herbicide was applied.

Seedlings of K890 were placed in 330 cc pots filled with the above mentioned soil mixture each artificially infected with 25 mg of 99% viable *O. cernua* seeds. The plants were grown in a growth chamber with 16-daylight hours at 24°C/20°C day/night temperatures, respectively. Fourty-day old plants had about 5 pairs of leaves when the herbicide treatments were applied.

Before the herbicide treatments, 46 K890 plants were removed from the pots and the number of *O. cernua* "nodules" per plant were recorded. This set would be referred to as Check 1.

Two herbicide rates were sprayed onto two different sets of 200 K890 plants. i.e., 26.6 and 53.2 g.a.i./ha of imazethapyr. A set of 52 K 890 plants were not treated and were kept as Check 2.

Twenty-five days after the herbicide treatment, the surviving treated plants of the two treatments and the Check 2 plants were removed and the *O. cernua* "nodules" on the roots were counted.

The third trial was made in order to find the effect of post-emergence imazethapyr treatment on the number of *O. cernua* shoots showing out of the soil. Sunflower K890 plants growing in 5 liters pots infected with 25 mg of *O. cernua* seeds were grown in a greenhouse up to the 5 pairs of leaves stage. One set of 100 plants was sprayed with imazethapyr (53.2 g.a.i./ha). Only plants without herbicide damage were kept. The second set, the control, consisted of 30 plants and was not treated. The number of emerged broomrape shots was counted every 10 days.

RESULTS AND DISCUSSION

In the first trial, one of the sets of treatments (106.4 g.a.i./ha), was lost due to an accident. The other sets were kept after the treatments until the plants were killed or survived.

Plants of the wild sunflower population K890 treated with different imazethapyr doses showed that the population has plants susceptible to the herbicide that were killed by the herbicide as well as resistant plants that were either not damaged at all or only showed some damage but the plants were not killed.

At very high doses of the herbicide (above 212.8 g.a.i./ha) all resistant plants had some damage. Nevertheless, with the doses of 26.6 and 53.2 g.a.i./ha between 26.6% and 33.3% of the plants did not have any damage and between 26.6% and 46.6% showed damage but survived the treatment (Table 1). At higher doses, the number of surviving plants went down as the doses increased. Nevertheless in all the treatments there were about 33% of the plants that were killed as quickly as the cultivated sensitive checks. All plants in the cultivated checks were killed even at the lower herbicide doses.

Table 1: Reaction of sunflower plants from cultivated and wild K890 population to different doses of the herbicide imazethapyr

Rate	Herbicide g.a.i./ha	Sunflower type	Number of plants	Resistant		Susceptible killed
				Unafected	Damaged	
X	26.6	WILD	30	10	14	6
2X	53.2	WILD	30	8	8	14
4X	106.4	WILD	-	-	-	-
8X	212.8	WILD	29	0	8	21
16X	425.6	WILD	30	0	4	26
32X	851.2	WILD	27	0	2	25
X	26.6	CULTIVATED	30	0	0	30
2X	53.2	CULTIVATED	30	0	0	30

4X set of plant was lost before reading

Genetic studies presented elsewhere suggest that herbicide resistance may be conditioned by two additive dominant genes.

In the second trial, it was shown that 100% of the K890 population plants were susceptible to *O. cernua* and showed nodules on the roots. This was expected as wild *H. annuus* populations are normally susceptible to broomrape (Table 2). The attaching rate (number of nodules per plant) averaged 14.3 nod./plant in the 46 plants analyzed before the herbicide treatment (Check 1).

Table 2: Effect of two doses of imazethapyr in broomrape control on a herbicide-resistant sunflower population (K890) under controled chamber conditions

Treatment g.a.i./ha	No. of plants	% of plants			Number of nodules/plant		
		Infected	With fresh nodules	With necroted nodules	Total	Fresh	Necrosed
Check 1	46	100%	100%	0%	14.3±2.1	14.3±2.1	0
Check 2	52	100%	100%	0%	31.7±2.2	31.7±2.2	0
26.6	40	100%	40%	100%	14.3±1.9	2.1±0.9	12.2±1.5
53.2	40	100%	20%	100%	12.5±2.6	0.7±0.4	11.8±2.3

Check 1 read on the day of herbicide treatment
Check 2 read 25 days after herbicide treatment

Figure 1: a) The wild sunflower population K890 in the Kansas's soybean field owned by Mr Doug Frech (right). The first author (left).
b) Healthy appearance of broomrape nodule growing on sunflower (K890) root. Scale 1:7
c) Dead broomrape nodule on sunflower (K890) root after imazethapyr treatment. Scale 1:7.

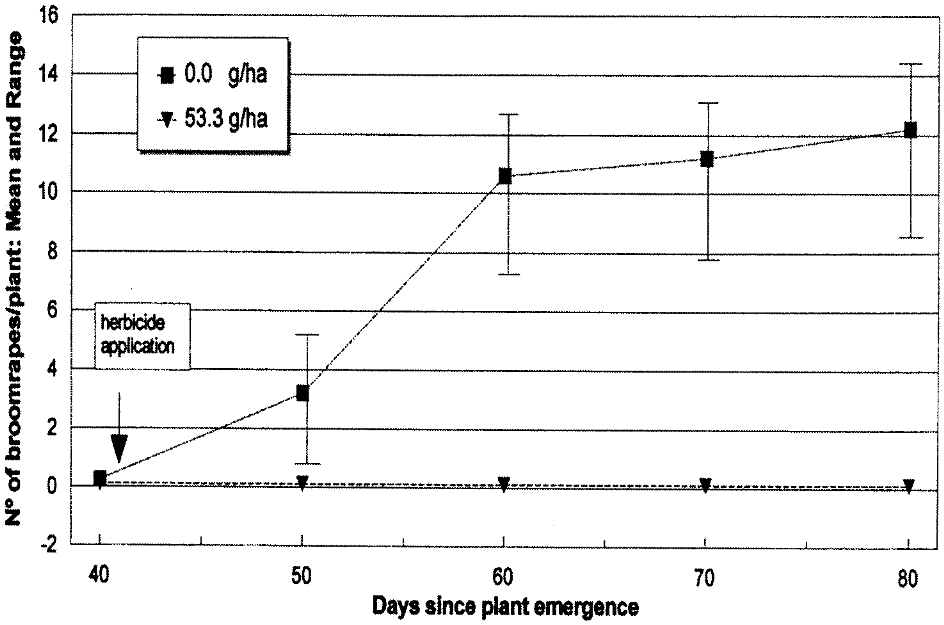

Figure 2: Effect of imazethapyr in broomrape control in K890 population under greenhouse conditions

Between the day 40 (day of treatment) and the day 65 of plant growth (end of experiment), *O. cernua* continued germinating and attaching to the sunflower roots. Thus the 52 untreated plants of Check 2 showed an average attaching rate of 31.7 nod./plant.

The plants treated with imazethapyr showed most of the nodules clearly necrosed (Figures 1b and 1c). Nevertheless, some normal appearing nodules also were observed on herbicide-treated plants. The clearly dead nodules averaged 12.2 and 11.8 nod./plant with the 26.6 and 53.2 g.a.i./ha treatments, respectively. The average numbers of normal-appearing nodules were 2.1 and 0.7 nod./plant with these treatments, respectively. Only 40% of the plants showed surviving nodules at 26.6 g.a.i./ha and 20% at 53.2 g.a.i./ha. All the treated plants had dead nodules.

It was suspected that some of the normal-appearing nodules could have been the result of a late attachment and that the nodules could start dying as soon as they would increase their demand for nutrients and water from the host plant. Thus, herbicide-treated plants showing some normal-appearing nodules were replanted. None of these pants showed emerged broomrape shoots 35 days after the transplanting.

These results suggest that *O. cernua* control was obtained both on the nodules already growing on the roots of sunflower at the moment of herbicide treatment, as well as with the new attachments after the treatment.

The third trial was conducted to see if the effect of the herbicide treatment would prevent the appearance of *Orobanche* shoots out of the soil during the entire sunflower cycle. Only herbicide-resistant plants were kept in the treated set.

It was shown that the treated plants did not show any emerged broomrape shoots during the sunflower cycle. On the other hand, the untreated plants commenced to show broomrape shoot in the pots about 50 day after planting. The average number of shoots per plant continued to increase during the entire cycle (Figure 2).

CONCLUSIONS

The wild sunflower population K890 from Kansas, U.S.A., has about 25% of the plants with full resistance to the herbicide imazethapyr applied at the doses between 26.6 and 53.2 g.a.i./ha.

Imazethapyr applied in post-emergence at the rate between 26.6 and 53.2 g.a.i./ha gives a high degree of control of *O. cernua* in herbicide-resistant sunflower plants of K890. This control could be between 93% and 100% of the root nodules (possibly 100%) and 100% in the control of the emerged broomrape shoots (method patent pending P 9801280).

The utilization of this natural herbicide resistance, once transfered to cultivated sunflower, along with the herbicide treatments in combination with the genetic resistance to the parasitic weed would probably be a very effective method to control *Orobanche* in sunflower and to prevent the development of new pathotypes of this parasitic weed.

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CONTROL QUÍMICO DEL JOPO *(Orobanche cernua* Loefl.) EN EL GIRASOL (*Helianthus annuus* L.) RESISTENTE AL HERBICIDA IMAZETAPIR

RESUMEN

Una población silvestre de *Helianthus annuus* L. recolectada en Kansas, U.S.A. tiene aproximadamente el 25% de sus plantas resistentes al herbicida imazetapir a dosis entre 26.6 y 53.2 g.m.a./ha. Algunas plantas sobrevivieron a tratamientos con 851.2 g.m.a./ha, es decir: 32 veces la dosis normal utilizada en soja. Plantas de esta población, artificialmente infectadas con jopo, *Orobanche cernua* Loefl. (incluyendo *O. cumana* Wallr.), mostraron susceptibilidad al jopo en el 100% de las plantas. Plantas de girasol de esta población infectada con jopo y tratadas con imazetapir en post-emergencia mostraron la mayoría de los núdulos del parasito muertos en las raices de girasol. El control era del 100% si solo se contabilizaban los jopos emergidos del terreno. Estos resultados abren la posibilidad de un nuevo método para el control de *O. cernua* en el girasol basado en el uso de esta resistencia natural al herbicida y el uso de imazetapir en post-emergencia.

CONTRÔLE CHIMIQUE DE L'Orobanche (*Orobanche cernua* Loefl.) CHEZ LE TOURNESOL (*Helianthus annuus* L.) RÉSISTANT À L'HERBICIDE IMAZETHAPYR

RÉSUMÉ

Une population d'*H. annuus* L. sauvage collectée au Kansas, USA, a présenté chez environ 25 % de ses plantes, une résistance à l'herbicide imazethapyr appliqué après la levée à des doses comprises entre 26.6 et 53.2 g.a.i./ha. Plusieurs plantes ont survécu aux traitements à 851.2 g.a.i. /ha., soit 32 fois la dose normale utilisée pour le soja. L'infection artificielle de plantes de cette population par l'espèce parasite *Orobanche cernua* Loeftl. (incluant *O. cumana* Wallr.), a donné 100% de plantes sensibles. Des plantes âgées de 40 jours, infectées artificiellement et traitées par l'imazethapir en post levée, présentent une destruction de la plupart des nodules du parasite se dévelop-pant sur les racines de tournesol. Le contrôle de l'Orobanche est de 100 %, si l'on compte seulement le nombre de rameaux d'Orobanche développés par plante. Ces résultats ouvrent la perspective d'une nouvelle méthode de contrôle d'*Orobanche cernua* chez le tournesol, fondée sur l'utilisation de cette résistance naturelle à un herbicide et du traitement par l'imazethapyr, en post levée.