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Absorption, translocation, and fate of herbicides in *Orobanche cumana*–sunflower system

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Abstract

The absorption and translocation of [^{14}C]pronamide, [^{14}C]glyphosate, and [^{14}C]imazapyr and the metabolism of [^{14}C]imazapyr, were studied in the *Orobanche cumana*–sunflower system. [^{14}C]Pronamide was applied as a sunflower seed treatment by coating or soaking. Herbicide absorption was affected by method of seed treatment, but not the subsequent pattern of herbicide distribution within the sunflower plant. Herbicide absorption by the seed was 9.8 and 3.4%, respectively. The translocation of the radioactive herbicide from the treated seed to the rest of the plant was greater for the coating than for the soaking treatment, regardless of the presence or absence of parasitic plants. The leaves were the sunflower component in which larger amount of [^{14}C]pronamide was detected, reaching up to 3.24 and 2.45% for the coating application and the non-infested and infested sunflower, respectively. Translocation of the herbicide from the root system of the host plant to the parasitic plant was 0.61 and 0.21% for the coating and soaking treatments, respectively, in infested sunflower. [^{14}C]Glyphosate and [^{14}C]imazapyr were applied as a post-emergence treatment to sunflower, whether it was parasitized by *O. cumana* or not. Glyphosate translocation within the host plant did not differ between *O. cumana*-infested and non-infested plants and most of the radioactivity remained in the treated leaf. Translocation of [^{14}C]glyphosate to *O. cumana* reached 5.5% at 3 DAT. The absorption of [^{14}C]imazapyr by the treated leaf was greater than [^{14}C]glyphosate absorption at 1 DAT. Similarly, higher levels of imazapyr were accumulated by *O. cumana* from the treated leaf. The metabolism study of [^{14}C]imazapyr showed a constant presence of imazapyr in the treated leaf at 16%, but in *O. cumana* the parent compound was 51.4% of the total recovered radioactivity 6 DAT. The deposit of imazapyr in *O. cumana* and the low metabolism in this species may explain the control of *O. cumana* by this herbicide.

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1. Introduction

Orobanche cumana Wallr. is an obligate, specific parasitic weed in the root system of the sunflower (*Helianthus annuus* L.), that causes yield reduction in sunflower in Southern and Southeastern Europe, Asia, and North Africa [1,2]. Parasitism alters physiological and biochemical processes in the host plant such as photosynthesis, respiration, water absorption, and amino acid and sugar biosynthesis [3]. These effects, together with competition for nutrients and water by *O. cumana*, cause yield losses, depending on the intensity of infestion, the host growth stage, and the environmental conditions [4].

There are different strategies for the management of *O. cumana* in sunflower. Thus, in recent years, sunflower seed treatment (HAS) with pronamide has been developed with promising results [5]. In addition, pre-emergence treatment with herbicides belonging to the imidazolinone, sulfonylurea, and substituted amide families and post-emergence application of glyphosate or imazapyr at a very low rate, are effective treatments to control parasitic weed in sunflower. However, the efficacy of those treatments depends on the crop growth stage, soil type, and environmental conditions [6–8].

Pronamide (3,5-dichloro-*N*-(1,1-dimethylpropanyl) benzamide) belongs to the substituted amide group. It inhibits mitosis in susceptible species and is a selective herbicide for the control of a wide range of grasses and certain broadleaf weeds [9]. Glyphosate (*N*-(phosphonomethyl)-glycine) is a non-selective herbicide, that inhibits shikimic acid pathway, and affects the aromatic amino acid biosynthesis phenylalanine, tyrosine, and tryptophan [10]. Imazapyr (2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid) inhibits the enzyme acetolactate synthase (ALS) [11,12].

Several studies on the absorption, translocation, and metabolism of these herbicides have been reported in different species [13–15]. However, no information is available on the absorption, translocation, and metabolism of these herbicides in the *O. cumana*–sunflower system. Such studies could provide an understanding of the use of a different herbicide for the control of this parasitic weed. Therefore, the objectives of the present research were: (1) to characterize pronamide, glyphosate, and imazapyr absorption and translocation, (2) to study metabolism of imazapyr in host sunflower plants, whether or not they are affected by *O. cumana* parasitism, and (3) to study the effect of application methods and timing on translocation of pronamide, glyphosate, and imazapyr.

2. Materials and methods

2.1. Seed material and growing conditions

Seeds of sunflower (*Helianthus annuus* L. var. Vyp) were used. *O. cumana* seeds were harvested from infested sunflower fields located in Córdoba (Southern Spain).

Seeds were germinated in petri dishes in a germination chamber at 21 °C ± 1. Sunflower seedlings were transplanted to the center of 20 cm × 20 cm plastic pots containing peat soil (sand:loam mix 1:1) and kept in a growth chamber. The growing conditions were 25/20 °C light/dark and relative humidity of 70%. The light intensity was 220 $\mu E\,m^{-2}\,s^{-1}$ for 14 h. Infested sunflower plants were obtained by inoculating seedlings with parasitic plant seeds (50 mg/pot) at transplanting. Plants were watered as needed and Hoagland solution was added once a week [16].

2.2. Radioactive herbicides

[^{14}C]Pronamide (Rohm and Haas) labelled on the benzamide ring (spe. act. 31.66 $\mu Ci\,mg^{-1}$) was used. Radioactive herbicide was diluted in methanol to provide a herbicide solution of 0.1 $\mu Ci\,\mu l^{-1}$. [^{14}C]Glyphosate (Monsanto) labelled on the methyl group (spe. act. 11.6 $\mu Ci\,mg^{-1}$) was used, and it was diluted with isopropylamine salt. Non-labelled glyphosate and surfactant MON 8081 were added to the solution to obtain a final concentration of 0.05 $\mu Ci\,\mu l^{-1}$. [^{14}C]Imazapyr (BASF AG) labelled on the pyridine ring (spe. act. 44.25 $\mu Ci\,mg^{-1}$) was diluted in distilled water with 0.1% v/v of the surfactant Tween 20 to obtain a concentration of 0.1 $\mu Ci\,\mu l^{-1}$.

2.3. Sunflower seed treatment with [^{14}C]pronamide

Seed coating consisted of stirring 10 g of sunflower seeds in 2:1 (v/v) dressing substance (Peridiam, Rhône-Poulenc) and distilled water for 15 min. Then, seeds were allowed to dry for 24 h at room temperature [17]. The radioactive solution was added to the sunflower seed at a proportion of 1 g of seeds per 1 ml of herbicide solution. The mixture was mixed for 3 min and the seeds were then dried at room temperature for 24 h. Whereas,

seed soaking was performed by immersing six seeds for 5 min in 5 ml of the radioactive solution of [^{14}C]pronamide as previously described.

Coating and soaking treated seeds were divided into the seed coat and embryo 24 h after herbicide application; then, both parts were dried for 48 h at 60 °C and combusted in a biological oxidizer (Packard, Model 307). The $^{14}CO_2$ was collected in 10 ml of Carbosorb E and 5 ml of Permafluor E$^+$ and the radioactivity was determined by using a liquid scintillation counter (LSS) (Beckman, 6000 TA) [18]. Infested and non-infested sunflower seedlings were developed as previously described. Seedlings were excised in the hypocotyle and radicle and the distribution of [^{14}C] was evaluated by LSS. In addition, [^{14}C] concentration in leaves, root of the host, seed coat, and *O. cumana* plants was determined by LSS in infested and non-infested sunflower plants maintained under growing conditions for eight weeks until they reached a vegetative growth of 15–17 leaves [18,19].

2.4. [^{14}C]Glyphosate and [^{14}C]imazapyr absorption and translocation

Sunflower seeds were germinated in infested and non-infested *O. cumana* pots as described before. Sunflower seedlings with 6–8 leaves were extracted from the pots. Host plants with six *O. cumana* parasitic plants at the visible sprout stage of 0.5 cm attached to the roots were chosen for the experiment. Sunflower roots with the attached parasite were washed to remove soil particles and plants were transferred to pots filled with perlite moistened with Hoagland solution. Then, pots were returned to the growth chamber for a week and watered as needed. Growing conditions were as described earlier. Subsequently, 0.05 μCi of labelled glyphosate or 0.1 μCi of [^{14}C]imazapyr solution/plant was applied in a single drop to the surface of the fourth leaf of *O. cumana*-infested and non-infested sunflower plants. In addition, similar amount of radioactivity was applied on non-infested sunflower plants. Plants were harvested at 1, 3, 6, and 12 days after treatment (DAT). To estimate the non-absorbed radioactivity, the treated leaf was washed with 20 ml of chloroform for 2 min and, once the chloroform had evaporated, 2 ml of ethanol and 10 ml of scintillation fluid were added, and the radioactivity was quantified by LSS, as described before [20]. Plants were harvested to treated leaf, other leaves, roots of the host, and *O. cumana* plants. Plant parts were frozen at −20 °C. Then, tissues were oxidized and radioactivity was determined as described earlier [19].

2.5. [^{14}C]Imazapyr metabolism

Imazapyr metabolism was studied in the parasite–host system. Plants were grown and treated with [^{14}C]imazapyr as described before. Plants were harvested at 1, 3, and 6 days after herbicide treatment and excised in the following components: treated leaf, other leaves, roots of the host, and parasitic plants of *O. cumana*. Plant tissues were frozen in liquid N_2 and stored at −20 °C until used. The tissues were ground with 3 ml of methanol:water (9:1, v/v). Then, samples were centrifuged three times at 20,000*g* for 10 min at 4 °C. The supernatants were evaporated until dryness and re-suspended in 500 μL of 90% methanol, and analyzed by HPLC. The suspensions were injected to a HPLC equipped with a reverse-phase column (C_{18}) connected to in-line a Beckman 171 radioactive detector. The solvent system consisted of 0.1% phosphoric acid in water and acetonitrile (ACN). The binary gradient consisted of 15–30% ACN in 5 min, 30–35% ACN in 10 min, and 35–85% ACN in 15 min [14,21]. The parent compound of imazapyr was identified by the respective retention time with the standard.

The experiment design was a randomized complete block with four replications and study was repeated twice. Absorption, translocation, and metabolism data were subjected to an analysis of variance. LSD multiple test was used to test statistical significance ($p \leqslant 0.05$).

3. Results and discussion

3.1. Absorption and translocation of [^{14}C]pronamide, [^{14}C]glyphosate, and [^{14}C]imazapyr

The absorption of [^{14}C]pronamide by sunflower seed 24 h after herbicide application was greater when the herbicide was applied by coating than by soaking and the herbicide was mainly located in the seed coat (Table 1). A similar pattern of [^{14}C]pronamide absorption was observed when the sunflower seedling was analyzed, being the hypocotyle, the seedling component in which the radioactivity was mainly detected (Table 1). This effect may be attributed to the presence of coating substance (Peridiam), which ensures a greater adherence of the herbicide when it is

Table 1
[^{14}C]Pronamide absorption and translocation as affected by application methods

Plant parts	Seed herbicide application method	
	Coating	Soaking
	Percentage of applied radioactivity	
Seed		
Seed coat	9.55 ± 0.59^{a}	3.30 ± 1.07^{b}
Embryo	0.25 ± 0.03^{a}	0.12 ± 0.03^{b}
Total absorbed	9.80 ± 0.61^{a}	3.42 ± 1.07^{b}
Seedling		
Hypocotyle	5.92 ± 1.01^{a}	0.85 ± 0.20^{b}
Radicle	0.11 ± 0.02^{a}	0.05 ± 0.07^{b}
Seed coat	3.32 ± 1.03^{a}	0.80 ± 0.31^{b}
Total absorbed	9.35 ± 0.24^{a}	1.70 ± 0.10^{b}

Data are mean of four replications of two different experiments ± standard error. Means of coating and soaking herbicide treatment of sunflower plant parts followed by the same letter are not significantly different according to a LSD test ($p \leqslant 0.05$).

applied by coating than when done by soaking comparing method [22].

[^{14}C]Pronamide uptake by the seeds was affect by application method. The translocation of radioactivity from the treated seed to the rest of the plant was greater for the coating than for the soaking treatments, regardless of the presence or absence of parasitic plants. Most of the absorbed radioactivity was detected in the leaves in both applications for non-infested and infested sunflowers (Table 2). The translocation of the herbicide from the root system of the host plant to the parasitic plant was 0.61 and 0.21% of applied radioactivity for the coating and soaking treatments in infested sunflower, respectively, and represented about 18 and 19% of herbicide total absorbed (Table 2). The relatively larger amount of [^{14}C]pronamide present in *O. cumana* with coating than with soaking may explain the greater control of the parasitic species under field conditions [5]. These results are in agreement with those obtained in applying imazethapyr in pea seeds by soaking, where the absorption and translocation of herbicide was greater when applied by coating than by soaking [19].

The absorption of [^{14}C]glyphosate by sunflower plants increased with time, reaching the maximum amount 6 DAT for infested and non-infested sunflower, respectively (Table 3). The radioactivity accumulation was much greater in leaves than in the underground parts (root and parasite). *O. cumana* absorbed a higher radioactivity with a [^{14}C]glyphosate post-emergence treatment than with a [^{14}C]pronamide coating treatment (Tables 2 and 3). *O. cumana* plants growing on the host acted as a sink for the herbicide, depleting it from the root system. Thus, the translocation of the herbicide in the root was always significantly superior when non-infested sunflower plants were analyzed at any time, being 0.9 and 4.5% for infested and non-infested sunflower plants at 12 DAT, respectively (Table 3). According to other authors, glyphosate has a poor or null metabolisation in the first week after application [23], which suggests that the results obtained in the different parts of the *O. cumana*–sunflower system corresponded to the parent compound.

Table 2
Distribution of [^{14}C]pronamide in sunflower plants and *O. cumana* plants eight weeks after herbicide treatment as affected by seed coating and soaking methods

Plant parts	Seed treatment method			
	Coating		Soaking	
	Infested	Non-infested	Infested	Non-infested
	Percentage of applied radioactivity			
Leaves	2.45 ± 0.45^{a}	3.24 ± 0.82^{a}	0.64 ± 0.02^{a}	0.70 ± 0.13^{a}
Root	0.20 ± 0.04^{b}	1.10 ± 0.28^{a}	0.15 ± 0.01^{b}	0.42 ± 0.09^{a}
Seed coat	0.21 ± 0.07^{a}	0.32 ± 0.40^{a}	0.10 ± 0.01^{a}	0.11 ± 0.01^{a}
O. cumana	0.61 ± 0.12	–	0.21 ± 0.04	–
Total absorbed	3.47 ± 0.28^{a}	4.66 ± 0.72^{a}	1.10 ± 0.02^{a}	1.23 ± 0.20^{a}

Data are mean of four replications of two different experiments ± standard error. Means of coating and soaking herbicide treatment of sunflower plant parts, infested and non-infested, followed by the same letter are not significantly different according to a LSD test ($p \leqslant 0.05$).

Table 3
Distribution of [^{14}C]glyphosate in sunflower and *O. cumana* plants as affected by days after herbicide application and *O. cumana* infestation (I, infested; NI, non-infested)

Plant parts	Days after treatment							
	1		3		6		12	
	I	NI	I	NI	I	NI	I	NI
	Percentage of applied radioactivity							
Treated leaf	38.0 ± 1.7[a]	37.0 ± 0.8[a]	37.2 ± 3.0[a]	37.6 ± 2.3[a]	41.4 ± 2.4[a]	43.1 ± 0.8[a]	38.5 ± 2.8[a]	39.3 ± 3.4[a]
Other leaves	5.3 ± 1.3[a]	4.8 ± 0.5[a]	7.1 ± 1.4[a]	6.6 ± 0.7[a]	7.8 ± 1.1[a]	5.9 ± 1.7[a]	7.8 ± 2.2[a]	7.1 ± 1.1[a]
Root	2.1 ± 0.2[a]	5.5 ± 0.5[b]	1.8 ± 0.3[a]	4.8 ± 1.2[b]	1.4 ± 0.4[a]	4.2 ± 0.2[b]	0.9 ± 0.2[a]	4.5 ± 0.1[b]
O. cumana	3.8 ± 0.5	–	5.5 ± 0.5	–	4.5 ± 0.2	–	4.4 ± 0.1	–
Total absorbed	49.2 ± 2.6[a]	47.3 ± 1.4[a]	51.6 ± 2.3[a]	49.0 ± 4.7[a]	55.1 ± 2.9[a]	53.2 ± 1.3[a]	51.6 ± 1.6[a]	50.9 ± 1.8[a]

Data are mean of four replications of two different experiments ± standard error. Means of infested and non infested sunflower plant parts for each day followed by the same letter are not significantly different according to a LSD test ($p \leqslant 0.05$).

Table 4
Distribution of [^{14}C]imazapyr in sunflower and *O. cumana* plants as affected by days after treatment and *O. cumana* infestation (I, infested; NI, non infested)

Plant parts	Days after treatment							
	1		3		6		12	
	I	NI	I	NI	I	NI	I	NI
	Percentage of applied radioactivity							
Treated leaf	53.6 ± 3.9[a]	59.3 ± 0.5[a]	52.4 ± 2.7[a]	51.3 ± 1.0[a]	31.7 ± 1.3[a]	40.2 ± 1.5[b]	22.1 ± 1.3[a]	38.8 ± 1.6[b]
Other leaves	5.4 ± 0.9[a]	8.9 ± 2.5[a]	20.1 ± 4.5[a]	28.4 ± 2.9[a]	34.3 ± 3.4[a]	39.7 ± 1.0[b]	33.0 ± 5.4[a]	39.1 ± 4.5[a]
Root	2.1 ± 0.6[a]	4.9 ± 1.0[b]	5.1 ± 0.5[a]	8.1 ± 0.5[b]	5.4 ± 1.3[a]	5.5 ± 1.5[a]	5.9 ± 0.4[a]	6.1 ± 1.7[a]
O. cumana	4.7 ± 1.0	–	13.4 ± 3.9	–	20.6 ± 2.9	–	25.7 ± 7.5	–
Total absorbed	65.8 ± 5.7[a]	70.1 ± 4.7[a]	91.0 ± 1.1[a]	87.8 ± 4.2[a]	92.0 ± 3.1[a]	85.4 ± 3.7[a]	86.7 ± 4.4[a]	84.0 ± 1.2[a]

Data are mean of four replications of two different experiments ± standard error. Means of infested and non infested sunflower plant parts for each day followed by the same letter are not significantly different according to a LSD test ($p \leqslant 0.05$).

The absorption and translocation of [^{14}C]imazapyr did not vary between infested and non-infested sunflower and for aerial parts (treated leaf and other leaves) when sampling date was 3 DAT or earlier. Radioactivity was over 50% in the treated leaf in both cases (infested and non-infested sunflower) 1 and 3 DAT (Table 4). However, translocation of [^{14}C]imazapyr was affected by the presence of *O. cumana* parasitism when sampling date was 6 DAT or later. Thus, the translocation of the herbicide out of the sunflower-treated leaf was greater in non-infested sunflower plants than in infested plants after 1 DAT, being the greatest difference 12 DAT with 22.1 and 38.8% of total radioactivity which was detected in the treated leaf in infested and non-infested sunflower plants, respectively. *O. cumana* plants growing on the host plants were stronger sink for imazapyr than when glyphosate or pronamide (Table 4). These results are similar with those obtained with imazethapyr (imidazolinone herbicide) applied post-emergence to the *Orobanche crenata–Pisum sativum* (pea) system, confirming the accumulation target in *Orobanche* spp. acting as a strong sink for this herbicide family [22].

3.2. [^{14}C]Imazapyr metabolism

HPLC showed two different compounds, one of them corresponded to imazapyr (Rt 12.5 min), and an unknown metabolite (Rt 3.5 min), which may correspond to nicotinic acid. Similar results were obtained with imazapyr in *Euphorbia esula* [14,21]. The percentage of non-metabolized herbicide was constant until 6 DAT in the treated leaf, which represented 16% of the total recovered radioactivity, and it was translocated to different parts of the plant (leaves and root) (Table 5).

The translocation of the imazapyr showed that at 6 DAT over 20.6% of [^{14}C] was accumulated in *Orobanche* (Table 4), corresponding 51.4% to the toxic compound imazapyr (Table 5). The sunflower parts analyzed showed low levels of toxic imazapyr, due to the rapid metabolization of this molecule to non-toxic compounds. This funding has been confirmed by other researchers [24] and may explain the tolerance of the sunflower to imazapyr. The selectivity may be due to the plant ability to metabolize the herbicide to a low mobility compound, preventing the imazapyr from reaching the target site growing area. In contrast, *O. cumana* was not able to significantly metabolize the herbicide to non-toxic compounds at a high rate and this may explain the control of *Orobanche* by imazapyr.

The absorption and translocation of imazapyr were higher than for glyphosate in sunflower–*O. cumana* system. A better control of weeds by imazapyr compared to glyphosate is due to mainly a better translocation of imazapyr and similar results have been reported earlier in other weeds [20]. However, even though imazapyr was absorbed and translocated better than glyphosate, the efficiency of both herbicides in controlling *O. cumana* was similar under field conditions [7,8].

In general, herbicide distribution in the sunflower–*O. cumana* complex showed the same pattern regardless of the application method. Thus, the maximum amounts of radioactivity were always obtained in the aerial parts. Herbicide accumulation in the parasitic species was comparatively much higher in the post-emergence treatments than in the sunflower seed treatment, being more evident for imazapyr than for glyphosate. The low level of detoxification by metabolism of imazapyr exhibited by *O. cumana* may explain the control of this parasitic plant by the herbicide [8].

Table 5
Percentage of radioactive [^{14}C]imazapyr recovered in sunflower plants infested with *O. cumana* 1, 3, and 6 days after herbicide application

Plant parts	Days after treatment		
	1	3	6
	Percentage of applied radioactivity		
Treated leaf	16.3 ± 0.8^{a}	16.2 ± 0.3^{a}	16.5 ± 2.2^{a}
Other leaves	0.1 ± 0.1^{c}	22.5 ± 2.0^{a}	13.1 ± 0.9^{b}
Root	0.1 ± 0.1^{c}	6.0 ± 0.2^{b}	8.8 ± 0.4^{a}
O. cumana	0.1 ± 0.1^{b}	0.2 ± 0.2^{b}	51.4 ± 11.0^{a}

Data are mean of three replicates of two different experiments ± standard error. Means within a row followed by the same letter are not significantly different according to a LSD test ($p \leqslant 0.05$).

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