Absorption and Translocation of Carbon 14-glyphosate Applied to Olive Tree Suckers

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Abstract. Field and laboratory studies were conducted in Córdoba, Spain, on olive (*Olea europaea* L.) trees to determine the absorption and translocation of ¹⁴C-glyphosate applied to suckers at different growth stages and different times of the growing season. Absorption of ¹⁴C-glyphosate by olive sucker leaves was very low, ≈3% to 5% of the total ¹⁴C recovered. Absorption and accumulation of ¹⁴C-glyphosate decreased if suckers were more developed when herbicides were applied. This explains the greater susceptibility to herbicides of smaller olive shoots compared to larger ones. Translocation of ¹⁴C-glyphosate in the early season application (May) was predominantly acropetal, whereas it was basipetal in the last application (September). Therefore, herbicide applied early in the season has a lower risk of translocating to other parts of the tree. The concentration of ¹⁴C-glyphosate in different parts of the olive tree varied with time. From 10 to 30 days after herbicide treatment, its concentration decreased by 41% in the woody basal parts and by 33% in the fruit. In the same period, it accumulated in the leaves, increasing in concentration by 30%.

The olive is a very important crop in the Mediterranean basin, with over 8 million ha planted (Luchetti, 1989). Olive production has improved considerably recently because of new technologies such as double-density plantings (Pastor Muñoz-Cobo, 1978), nontillage, herbicides (Pastor Muñoz-Cobo, 1988; Pastor Muñoz-Cobo et al., 1982), production at an earlier tree age (Caballero, 1981), mechanical harvesting (Civantos, 1984), drip irrigation, and chemical thinning of table olives (Suárez et al., 1986). Generally, these techniques increase yield and decrease costs.

The olive tree naturally produces a large number of basal shoots, or suckers, during the growing season. These shoots, if allowed to grow, will transform the tree into an unproductive bush (Morettini, 1972; Ortega-Nieto, 1969). Olive suckers are traditionally removed manually at the end of the growing season using a sharp cutting tool. A person can cut, pile, and burn the suckers of 100 trees in 8 h. The mechanization of this operation is difficult because of the irregular shape of the basal part of the tree. Since suckers compete for growth with the rest of the tree, they behave like weeds and are undesirable (Atkinson et al., 1978; Stinchcombe and Stott, 1980).

Valera-Gil and García-Torres (1993) studied sucker growth and control with *N*-phosphonomethyl glycine (glyphosate) + (4-chloro-2 methylphenoxy) acetic acid (MCPA). The efficiency of this herbicide combination varied according to sucker height at application time; suckers were very susceptible at early growth stages (e.g., between 10 to 20 cm) compared to later stages (e.g., 40 to 60 cm). Thus, to obtain >90% sucker control, a dose ≥0.7 + 0.7 and 1 + 1 kg·ha⁻¹ glyphosate + MCPA was required for suckers of 10 to 20 cm and 40 to 60 cm, respectively. Repeated applications of glyphosate + MCPA at 1 + 1 kg·ha⁻¹ effectively controlled

suckers and increased olive tree growth.

Glyphosate is a nonselective, highly translocated herbicide that is absorbed by leaves and stems and accumulates in the actively growing parts of the plants (Caseley and Coupland, 1985). Translocation, however, can be considerably reduced when combined with 2,4-D-like herbicides such as MCPA (O'Donovan and O'Sullivan, 1982). No information exists on the absorption and movement of glyphosate in olive trees. Therefore, the aim of this study was to examine the translocation of glyphosate applied to suckers and determine its accumulation in several parts of the olive tree to understand its mode of action as a potential sucker-control chemical.

Materials and Methods

Field studies were conducted at the Córdoba Agricultural Research Center in 1989 and 1990 on 'Picual' olive suckers previously selected for uniformity. Carbon 14-methyl glyphosate with a specific activity of 1.97 μCi/mmol (1 Ci = 37 GBq) was used. The herbicide (4.57 mg) was diluted and converted to the isopropylamine salt by adding 2494 μl of water, 150 μl commercial glyphosate, 2 μl Mon-8081 surfactant, and 4 μl isopropylamine made up to 2.65 ml of solution equivalent to 1 kg of glyphosate in 635 ml of water. Ten microliters of radioactive herbicide (0.4 μCi) was applied to two opposite leaflets of each sucker using microsyringes. Herbicide was applied to the fourth pair of leaves >1 mm long by placing a drop on the central part of the adaxial surface of each leaflet. Suckers receiving radioactive herbicide were then protected with plastic bags for 24 h to enhance absorption. The growth stages of olive trees at the herbicide application times were determined according to Colbrant and Fabre (1975). The experimental design consisted of four randomized complete blocks unless otherwise stated. Each treatment comprised one tree. Data were subjected to analysis of variance, previously transformed by $\sqrt{x}$ or $\sqrt{100 - x}$ if expressed by percentages.

Effect of sucker height. Suckers of ≈20, 60, and 110 cm were selected on four trees. Radioactive herbicide was applied to the suckers on 16 July 1989, coinciding with the pit-hardening growth

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stage. Treated suckers were cut 10 days after herbicide application and divided into treated leaves, apex, upper leaves, lower leaves, upper stem, and lower stem. Each treated leaf was shaken for 5 min in 10 ml of distilled water to remove the unabsorbed ^{14}C . The plant material was oven-dried at 60°C for 4 days, weighed, and cut into small pieces. Lignified samples (stems) were also cut into small pieces <3 mm using a bapitaurus mixer. Radioactivity was determined on a 0.4-g subsample.

Samples were homogenized in a 20-ml solution of 9 dioxane : 1 water (v/v) for 10 min. The suspension was then centrifuged at 7000 rpm for 45 min and 3 ml of the supernatant was added to 15 ml of a commercial cocktail (Ready-Safe; Beckman, Spain) to determine radioactivity. Data were corrected for background, quenching, and dilutions. Counts per minutes were converted to disintegrations per minute (dpm) using the channel ratio method. Data were expressed as percentage of total ^{14}C applied (0.2 μCi) or as dpm per mg of dry tissue.

Effect of application time. The radioactive herbicide was applied to one sucker per tree at three phenological stages: fruit set, pit hardening, and green harvest date, coinciding with 20, 80, and 110 days after bloom (10 May 1989), respectively. The maximum and minimum temperature and the relative air humidity were also recorded daily.

Herbicide translocation from the treated sucker. Five trees and 10 suckers per tree were selected for uniformity. Ten microliters (0.2 μCi) of radioactive herbicide was applied, as previously described, to pairs of leaves of each selected sucker. Thus, 20 μl (0.4 μCi) per sucker and 200 μl (4 μCi) per tree were applied. Treatments were applied on 29 Sept. 1990, at the olive growth stage of color change, to suckers 55 cm long. Each treatment (sampling date) was replicated three times.

Plant parts were sampled 10, 20, and 30 days after radioactive herbicide application. Each sample consisted of 10 shoot apexes from various parts of the tree ≈ 1.5 m above the soil, 500 g of fruit, and eight to ten 50-g wood samples from a) the lower tree trunk

area where most of the suckers arose and b) at 25 cm from the base of the treated sucker. At the last sampling, another piece of wood from the lower basal stem below the treated sucker was taken. All plant material sampled was processed as previously described.

A glyphosate metabolism study was not conducted because studies with many plant species have shown that little or no glyphosate metabolism occurs within 1 to 2 weeks after treatment (Gotttrup et al., 1976; Sandberg et al., 1980; Shultz and Burnside, 1980; Wyrill and Burnside, 1976).

Results and discussions

Effect of sucker height. The average recovery of ^{14}C was 74.2% of the total applied. About 3% of the ^{14}C recovered was absorbed. Herbicide remaining on the treated leaf surface was 96.7%, 96.8%, and 97.2% of the total recovered in suckers 20, 60, and 110 cm long, respectively (Table 1).

The absorption process of glyphosate takes place mostly by passive diffusion (Caseley and Coupland, 1985). Therefore, the epidermal structure plays an important role. Because the olive leaf cuticle grows thicker as the leaf expands (León and Bukovac, 1978), olive plants may absorb less chemical than other glyphosate-susceptible plant species such as barley (*Hordeum vulgare* L.), which can absorb as much as 27% of the total applied herbicide (O'Donovan and O'Sullivan, 1982). Similarly, leaves from longer, older suckers have thicker cuticles than those from shorter, younger suckers, decreasing the absorption of ^{14}C -glyphosate. However, the amount of ^{14}C -glyphosate translocated out of the treated leaf was similar regardless of sucker height (Table 1).

The ^{14}C concentration in the treated leaves was much higher than in any other parts of the sucker and decreased with height (Table 2). Generally, radioactivity in sucker fractions decreased as sucker heights increased. This can be explained as a dilution process, because the amount of radioactive herbicide was similar regardless of sucker size, and translocation from the sucker was

Table 1. Absorption and translocation of ^{14}C -glyphosate as affected by sucker height.

Sucker ht (cm)	Total ¹⁴ C-glyphosate recovered (dpm ^z × 10 ³)	¹⁴ C-glyphosate recovery		
		Treated leaves		Other plant parts (% of total)
		Absorbed (%) ^y	Not absorbed (%)	
20 (2.6) ^x	646 a ^w	2 a	96.7 a	1.2 a
60 (3.4)	652 a	1.8 a	96.8 a	1.3 a
110 (4.1)	662 a	1.3 a	97.2 a	1.3 a

^zdpm = Disintegrations per minute.

^yPercentage of total recovery.

^xSE in parentheses.

^wFor each column, means followed by the same letter are nonsignificant at $P = 0.05$ by Duncan's multiple range test.

Table 2. Distribution of ^{14}C -glyphosate in the sucker (dpm^z/mg) as affected by sucker height.

Analyzed fraction	Sucker ht (cm)		
	20	60	110
Apex	15.3 ^y a,m	15 a,n	9.5 b,n
Upper leaves	7.8 a,m	2.9 a,m	2.2 a,mn
Lower leaves	6.1 a,m	2.1 ab,m	1.1 b,mn
Upper stem	16.7 ab,m	19 a,n	7.3 b,mn
Lower stem	9.8 a,m	1.6 b,m	0.4 b,m
Treated leaves	190.8 a,n	116.6 b,o	70.9 c,o

^zdpm = Disintegrations per minute.

^yMeans followed by the same letter within rows (a–c) or columns (m–o) are nonsignificant at $P = 0.05$ by Duncan's multiple range test.

apparently not affected by its size. Conversely, this can also explain why younger suckers are better controlled than older ones.

Effect of application time. In this study, the average recovery of ^{14}C was 77.8% of the total applied. Herbicide absorption was higher for the early (30 May) than for the mid-season (30 July) or late applications (20 Sept.) (Table 3). These results can be partly explained by the higher relative humidity and lower temperature and the thinner leaf cuticle at the time of the first application compared to later applications. Both factors and the thinner cuticle at earlier growth stages favor absorption as suggested by the previous study. Carbon 14 absorption by the olive leaves is very low compared to other species like *Cirsium arvense* L., which absorbed 85% of the herbicide applied when the relative humidity was high (Gottrup et al., 1976). Absorption would also be reduced by the low stomatal density in the adaxial leaf (León and Bukovac 1978) and the waxy cuticle of the olive leaf.

The ^{14}C translocated from the treated leaf was $\approx 30\%$ higher when applied in May than in July or September (Table 3), resulting in 2.4%, 1.6%, and 1.7% of the total herbicide recovered for the three successive applications, respectively. Glyphosate movement occurs mostly through the phloem (Caseley and Coupland, 1985) and, since photosynthesis activity is higher in spring than in the middle or late summer (Rallo and Suárez, 1989), this may account for these results.

The distribution of ^{14}C -glyphosate in the treated sucker varied with time (Table 4). Generally, the herbicide concentration at the apex was higher than any other part, other than the treated leaves, and decreased as suckers developed. Radioactivity at the apex was $\approx 50\%$ and 20% lower for the 30 July and 20 Sept. applications than for the May application. Similarly, herbicide concentration in the upper leaves and upper stem also tended to decrease with sucker growth. In contrast, radioactivity in the lower leaves was similar regardless of application time, while in the lower stem it increased as the sucker grew older. These results suggest that glyphosate movement is mainly acropetal when applied to actively growing suckers (early application), while it is more basipetal in older suckers (late applications). Therefore, sucker control from early herbicide applications is more efficient and has less risk of phytotoxicity to other parts of the olive tree than from late applications. This agrees with Atkinson et al., (1978) and Staalduine (1979), since both observed that spring applications of glyphosate to apple and pear suckers did not cause phytotoxicity symptoms in the trees, while fall applications did.

Herbicide translocation from the treated sucker. Carbon 14 concentration in the various parts of the olive tree varied with time (Table 5). The radioactivity in the trunk 10 days after herbicide application was ≈ 2.5 , three, and four times higher than in the leaves, stems, and fruit, respectively. Furthermore, ^{14}C in the trunk

Table 3. Absorption and translocation of ^{14}C -glyphosate as affected by application time.

Application date	Total ¹⁴ C-glyphosate recovered (dpm ^z × 10 ³)	¹⁴ C-glyphosate recovery		
		Treated leaves		Other plant parts (% of total)
		Absorbed (%) ^y	Not absorbed (%)	
30 May	606 a ^x	3.1 a	94.4 a	2.4 b
30 July	761 c	2 b	96.4 a	1.6 a
20 Sept.	686 b	1.5 b	96.9 a	1.7 ab

^zdpm = Disintegrations per minute.

^yPercentage of the total recovery.

^xFor each column, means followed by the same letter are nonsignificant at $P = 0.05$ by Duncan's multiple range test.

Table 4. Distribution of ^{14}C -glyphosate in the sucker (dpm^z/mg) as affected by application time.

Analyzed fraction	Application time		
	30 May	30 July	20 Sept.
Apex	32.2 ^y a,m	15.8 b,m	6.7 b,m
Upper leaves	8.3 a,m	5.2 a,m	5.2 a,m
Lower leaves	4.1 a,m	3.7 a,m	4.3 a,m
Upper stem	8.8 a,m	4.6 a,m	7.1 a,m
Lower stem	2.4 a,m	2.8 a,m	5.8 b,m
Treated leaves	287.1 a,n	188.1 a,n	182.2 a,n

^zdpm = Disintegrations per minute.

^yMeans followed by the same letter within rows (a–b) or columns (m–n) are nonsignificant at $P = 0.05$ by Duncan's multiple range test.

Table 5. Distribution of sucker applied ^{14}C -glyphosate (dpm^z/mg) in several olive parts.

Sampling (dat) ^y	Olive part				
	Leaves	Stems	Trunk area	Fruit	Total concn
10	1.74 ^w a,m	1.4 a,m	4.2 a,n	0.9 a,m	8.24
20	1.6 a,n	0.8 a,m	1.4 b,n	0.4 a,m	4.2
30	4 b,o	1.2 a,m	2.5 b,n	0.3 a,m	8

^zdpm = Disintegrations per minute.

^ydat = Days after treatment.

^wNear treated sucker.

^xMeans followed by the same letter within columns (a–b) or rows (m–o) are nonsignificant at $P = 0.05$ by Duncan's multiple range test.

decreased significantly with time. Therefore, glyphosate seems to be moving through this part of the tree. The ^{14}C concentration in the leaves increased >200% 10 to 30 days after application, while radioactivity in the fruit did not significantly change in the same period of time. Therefore, the leaf : fruit radioactivity ratio increased with time. For example, radioactivity in the fruit was similar and four and 13 times lower than in the leaves 10, 20, and 30 days after herbicide application, respectively.

The increase of radioactivity of leaves with time could be due to the photosynthate sink effect of young leaves (Klein and Weinbaum, 1984). In contrast, the comparatively low herbicide accumulation in the fruit and its tendency to decrease with time may have been due to the advanced stage of fruit growth (color change and green harvest) when herbicide was applied. At this phenological stage, the fruit receive comparatively lower amounts of photosynthates (Rallo and Suárez, 1989).

Conclusions

Generally, leaf absorption of glyphosate applied to actively growing suckers was limited to only 1% to 4% of the total herbicide applied. Absorption tended to decrease as sucker height increased and with later growth stages at herbicide application time. This explains the greater effectiveness of glyphosate on short suckers or at early growth stages, as Valera-Gil and García-Torres (1993) showed in field studies.

Glyphosate translocation in treated suckers is more acropetal in young than in old suckers. This explains the high sucker control and the reduced risk of phytotoxicity in other parts of the olive tree from early applications. Movement of ^{14}C -glyphosate out of the treated suckers was detected in several parts of the olive trees, but only in extremely small amounts. It accumulated mainly in the trunk area and leaves, with very little found in the fruit.

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