SEASONAL EFFECT ON ^{14}C-GLYPHOSATE TRANSLOCATION PATTERN IN DIFFERENT PLANT PARTS OF *CYPERUS ROTUNDUS*

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SUMMARY

The ^{14}C-glyphosate uptake and translocation pattern in different plant parts of *Cyperus rotundus* in different seasons were determined by feedig herbicide to a single spot on the fourth leaf from the top of thirty days old plants. Results showed that much of the activity (80-90%) of ^{14}C-glyphosate remained in fed leaf and in mother tuber (2-24%) and least was recorded in daughter tuber (0.8-3.8%) in both the seasons (February and June). Translocation of the herbicide was more during February than June growing season.

Key words: **^{14}C-glyphosate, season, translocation, uptake**

INTRODUCTION

Glyphosate is a very effective systemic non selective herbicide used to control problematic perennial weeds like *Cyperus rotundus, Cynodan dactylon, Lantana camera* and other. Systemic pesticide applied to foliage must penetrate the cuticle before they become biologically active. Various adjuvants included in formulations are known to increase their efficiency by rapid uptake (Khoche and Bukovac 1993, Santier and Chamel 1996). Smith *et al.* (1996) had explained that herbicides and surfactant at plant surface interact with each other and the increased uptake of the herbicides has many dimensions than merely lowering of surface tension. Current view of the influence of surfactant is related to solubalization of lipophilic active ingredient of herbicide, wax dissolution of cuticle, penetration and membrane permeability (Kirkwood 1993). Jordan (1997) reported that most of ^{14}C-glyphosate translocated out of the treated leaf into other parts of the *Cynodan dactylon* plant at 32°C than at 22°C at 40% RH but such a significant difference was not observed at 100% RH. Further 5-7 times more trnaslocation of ^{14}C-glyphosate was observed in *Cynodan dactylon* at 100% RH than at 40% RH. Chase and Appleby (1979) observed that *Cyperus rotundus* was more susceptible to glyphosate during rainy season than during dry season. Three times more ^{14}C-glyphosate was translocated into underground plant parts at 90% RH and -11 bars of water potential. The ^{14}C-glyphosate uptake and translocations was reduced in quack grass in water stressed plants compared to non-stressed plants, whereas, ^{14}C-photoassimilated concentration in rhizomes of water stressed quack grass was similar to that of non-stressed plants (Klevorn and Wyse 1984). The effect of added methylated seed oil to labeled herbicide and the penetration was studied in isolated cuticle of tomato, pepper (fruit cuticle) and rubber (leaf). They showed that penetration of labeled surfactant was more in methyl oleate form than glycerol trioleate from. Lipophilic herbicide (Fenoxyprop ethyl and quizalfop ethyl) penetration was more with methyl oleate than glycerol trioleate in tomato and rubber but it was low in pepper cuticle compared to herbicide alone. Similar to *Lantana camara*, the *Cyperus rotundus* is also a noxious weed having predominant underground propagating plant part

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(tubers). Several studies suggested that much of the foliar applied herbicide remains in the fed leaf and very little quantity reaches to the under ground tubers (Carr *et al.* 1986, Anonymous 1998) .

MATERIALS AND METHODS

Single *Cyperus rotundus* tuber was planted in each battery pot and irrigated all along the experimental period. Thirty days old *Cyperus rotundus* healthy plants were selected from pots and treated with ^{14}C-glyphosate alone (T_1), ^{14}C-glyphosate + surfactant (Triton x 100) (T_2), chloroform followed by (fb) glyphosate + surfactant (T_3) and chloroform fb glyphosate (T_4).

Preparation of radioactive isotope

Methyl ^{14}C-glyphosate having specific activity of 34 millicurie/mm was mixed with 1 ml of 2000 ppm glyphosate. From this stock 10 µl of cold (unlabeled 2000 ppm) glyphosate was added with and without surfactant. The surfactatnt used was Triton x 100 at the concentration of 0.1% (droplet weight basis w/v). 0.5 µl single droplet ^{14}C-glyphosate of T_1-T_4 was placed on 4th leaf from top of each plant and allowed to penetrate and translocate for 24 hours. Cotton bud dipped in chloroform was swabbed twice to the spot before the application of ^{14}C-glyphosate (T_1 and T_2) droplet. The fed spot was marked with a permanent marker and left undisturbed for 24 hours. After treatment period the weed plants were harvested and separated into different plant parts viz. treated leaf (fed leaf), mother tuber, daughter tuber, mother tuber shoot and daughter tuber shoot. Treated leaf was further separated as residue on the fed spot cuticle (by repeated washing with distilled water) and the rest of the fed leaf without fed spot were separated.

Isolation of cuticles

Floating leaves in concentrated hydrochloric acid can break pectin and cellulose bridges between the cuticle and other mesophyl tissues. Fully expanded undamaged leaves were taken, gently washed with water and leaf bits prepared using scissors. 3-4 leaf bits were kept in 100 ml beaker containing a mixture of 20 ml concentrated HCl and $ZnCl_2$ in such a way that the adaxial surface of the leaf bits have contact with acid mixture. The beakers with leaf bits were kept undistrubed for 8-10 hours. After 10 hours of the incubation period acid was discarded slowly and a stream of water was released through walls of the beaker till the beaker get filled up to 3/4 of its capacity. To observe the radioactivity in these plant parts liquid scintillation counter (LSC) was used. Observation of all the samples were recorded as counts per minute (CPM). Using this information relative percentage of radioactivity in various plant parts was calculated. The total activity recovered by different plant parts reflected the entry of ^{14}C-glyphosate and per cent activity in different plant parts reflected the translocation pattern. Similar experiment was conducted in two seasons (June and February).

RESULTS AND DISCUSSION

In both the seasons, total ^{14}C-glyphosate activity recoverd was relatively low with chloroform pretreatment compared to without chloroform pretreatment. However, significant difference between with and without chloroform pretreatment was observed during February season. Addition of surfactant did not resulted in enhanced entry of ^{14}C-glyphosate in both the seasons (Table 1).

Table 1. Effect of season, surfactant and chloroform pretreatment on uptake of ^{14}C-glyphosate (cpm/plant)

Treatments	June	February
T_1: Glyphosate	74560 (4.8)	20028 (4.3)
T_2: Glyphosate+Triton X100	89625 (4.9)	24516 (4.4)
T_3: Chloroform fb Glyphosate+Triton X100	87857 (4.9)	12964 (4.1)
T_4: Chloroform fb Glyphosate	44493 (4.6)	12454 (4.0)
CD (P=0.05)	(NS)	(0.26)

(Number in parenthesis are log transformed values)

The radioactivity present in the droplet prepared for uptake and translocation study during June was considerably higher than February, both in glyphosate with and without Triton x 100 (Table 2). Due to this reason fresh radio isotope was procured during June. Further, high activity was fed with same glyphosate concentration as few plant parts had low activity during February. Percent activities over total activity recovered in different plant parts were than compared.

Table 2. ^{14}C-glyphosate activity (cpm per droplet) fed to foliage in two seasons

Solution	June	February
Glyphosate	73376*	23729
Glyphosate + Triton X 100	65010	19271

*Activity in cpm/0.5 µl of droplet

Effect of seasons, surfactant and chloroform pretreatment on translocation of ^{14}C-glyphosate

In general, a larger amount of spot applied ^{14}C-glyphosate remained in fed leaf itself, only a small concentration of ^{14}C-glyphosate activity was recovered in mother tuber and the least activity was observed in daughter tuber. The percent radio activity recovered from these plant parts are 86% , 12.3% and 1.5% in fed leaf, mother tuber and daughter tuber respectively (Table 3).

Addition of Triton x 100 increased ^{14}C-glyphosate translocation to mother tuber and daughter tuber during February than June. In February the activity increased to 19.0 % and 1.3 % in mother tuber and daughter tuber with Triton x 100 compared to 9.2 % and 0.8 % with glyphosate alone respectively. During June the translocation was enhanced in mother tuber only. However, significant enhancement in glyphosate translocation in mother tuber was observed with chloroform pretreatment during February compared to without chloroform pretreatment. Further, such significant differences were not observed during June.

Seasonal effect on ^{14}C-glyphosate uptake and translocation within fed leaf

In general, highest activity was recorded in leaf portion away from fed spot and above cuticle as residue compared to inside cuticle and below cuticle of fed spot in all treatments. Similar trends were observed during

Table 3. Seasonal effect on ^{14}C-glyphosate translocation pattern (per cent over total) in different plant parts of *Cyperus rotundus*

Treatment	Mother tuber		Daughter tuber		Fed leaf	
	June	February	June	February	June	February
T_1	2.0	9.2	3.80	0.80	94.3	87.3
	(8.13)	(17.56)	(12.52)	(4.80)	(76.19)	(69.30)
T_2	6.8	19.0	1.30	1.30	93.5	79.3
	(15)	(21.30)	(8.58)	(6.50)	(72.23)	(62.94)
T_3	7.1	24.4	0.83	1.7	95.3	72.5
	(15.34)	(29.53)	(4.80)	(1.49)	(77.48)	(58.37)
T_4	6.4	23.8	0.48	1.9	95.4	72.8
	(14.54)	(29.13)	(3.14)	(7.70)	(77.12)	(59.21)
Mean	5.6	19.1	1.6	1.4	94.6	78.0
	(13.6)	(25.9)	(7.23)	(6.8)	(76.5)	(62.3)
	12.3		1.5		86.3	
	(20.58)		(7.03)		(68.28)	

Figures in parenthesis indicates angular transformed values
CD (P=0.05) For plant parts = 3.71; For season x plant parts = 5.22; For seasons x plant parts x treatments = 10.47

both the seasons. However, during February, significantly lower activity was observed in residue portion compared to June, exception being glyphosate alone (T_2). Further, the total activity in fed leaf was significantly lower in all treatments during February compared to June (Table 4). The ^{14}C-glyphosate in residue was drastically lower in February compared to June while the activity away from fed spot with Triton x 100 was more in February compared to June. Interestingly, chloroform pre treatment enhanced ^{14}C-glyphosate entry into cuticle and residue compared to without Triton treatment during February. Significant effect was not observed in other treatments. This suggests that, addition of Triton x 100 to glyphosate alters the translocation pattern within the fed leaf itself depending on the season.

Influence of season, surfactant and chloroform pre-treatment on translocation of ^{14}C-glyphosate to mother and daughter tuber

It was observed that during February more entry and translocation of glyphosate in mother shoot occurred compared to June. Such a diference was not seen in other plant parts viz. mother tuber, daughter tuber, daughter shoot respectively. Addition of Triton x 100 was sufficient enough to bring the enhanced translocation in both the seasons compared to chloroform pretreatment. The ^{14}C-glyphosate-distribution pattern showed that much of the activity will remain in fed leaf mother tuber and in daughter tuber. Within mother tuber much of activity went to shoots than the mother tuber (Table 5) and within the fed leaf highest activity was observed in leaf portion other than the fed spot followed by residue on the cuticle, activity in cuticle and the activity below cuticle.

The radio active glyphosate data suggests that during February more entry and translocation of glyphosate occur compared to June. Addition of Triton x 100 was sufficient enough to bring the enhanced translocation in both the seasons compared to chloroform pretreatment. Season to season variation in percent translocation was observed in different plant parts. More translocation was observed during February than June growing season. The ^{14}C-glyphosate distribution pattern showed that much of the activity will remain in fed leaf, mother tuber and in daughter tuber compared to other plant parts.

Table 4. Seasonal effect on cuticle penetration and translocation pattern of ^{14}C-glyphosate in fed leaf, 24 hours after feeding.

Treatment	Above cuticle		Cuticle		Below cuticle		Away from fed spot	
	June	Feb	June	Feb	June	Feb	June	Feb
T_1 Glyphosate	10.2±0.2 (18.29)	31±6.9 (33.69)	4±1.5 (10.0)	0.5±1.5 (4.22)	0.5±1.7 (4.22)	0.5±0.3 (2.4)	82±2.5 (65.51)	52±5.6 (46.72)
T_2 Glyphosate + Triton x 100	66.9±4.4 (55.12)	15±5.0 (20.50)	4.6±0.4 (12.49)	4.1±0.37 (11.1)	4.0±1.8 (10.3)	1.6±1.1 (5.0)	18±4.3 (25.0)	58.6±8.2 (50.29)
T3 Chloroform + glyphosate + Triton x 100	34.4±4.2 (35.86)	9.1±4.0 (15.31)	6.1±1.0 (4.31)	10.5±9.3 (18.01)	3.8±0.3 (11.29)	3.9±1.8 (10.1)	5.1±3.9 (45.83)	49±6.8 (44.26)
T_4 Chloroform + glyphosate	51.9±10 (45.8)	0.5±0.4 (2.5)	3.6±0.3 (10.0)	3.3±1.3 (10.0)	1.9±0.3 (7.9)	1.0±0.7 (4.2)	38±7.2 (37.2)	68±4.3 (56.5)

(Figures in parenthesis indicates angular trnasformed values CD= P=0.05) (10.47)

Table 5. Effect of growing season, surfactant and chloroform pre treatment on translocation pattern of ^{14}C-glyphosate in mother and daughter tuber and shoots growing from these tubers

Treatment	Mother tuber		Mother shoot		Daughter tuber		Daughter shoot	
	June	Feb	June	Feb	June	Feb	June	Feb
T_1 Glyphosate	0.4±0.1	0.2±0.2	1.6±0.1	9.0±1.8	0.2±0.07	0.3±0.2	1.1±0.6	0.5±0.4
	(3.2)	(1.1)	(7.4)	(18.0)	(2.9)	(1.9)	(4.6)	(1.8)
T_2 Glyphosate +	2.2±0.4	1.0±0.5	4.6±0.4	18.0±5.0	1.3±0.4	1.0±0.3	0±0	0.3±0.3
triton x 100	(8.55)	(4.1)	(12.4)	(24)	(6.3)	(5.5)	(0)	(1.9)
T_3 Chloroform +	1.0±0.2	7.1±0.7	6.1±1.0	17.3±7.9	0.8±0.0	1.3±0.5	0.03±0.03	0.4±0.4
gly +triton x 100	(5.6)	(5.6)	(4.2)	(20.5)	(5.1)	(6.2)	(0.6)	(1.6)
T4: Chloroform	2.8±0.5	0.6±0.6	3.6±0.31	23.2±3.7	0.4±0.1	0.7±0.4	0.06±0.03	1.2±1.2
+ gly phosate	(9.5)	(2.0)	(10.9)	(28.5)	(3.7)	(3.4)	(1.0)	(2.9)

(Values in parenthesis indicates angular transformation); CD (P=0.05) (10.47).

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