Effect of Glyphosate on the Sprouting of Purple Nutsedge (*Cyperus rotundus* L.) Tubers[1]

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Abstract

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Studies with individual plant were conducted to determine the effect of glyphosate rate, time for translocatioin, and shade on the sprouting ability of tubers attached to treated plants. Rates of 4 and 8 kg/ha glyphosate inhibited tuber sprouting, 72h was required for most translocation at 8 kg/ha whereas 120h was sufficient at 4 kg/ha Shading reduced the tuber number and sprouting ability of purple nutsedge, but did not affect glyphosate translocation to the tubers.

(Additional key words: tuber sprouting, translocation, dosage, shade)

嘉磷塞對香附子地下塊莖發芽活性之影響

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摘　　要：本研究探討嘉磷塞施用後在香附子地下塊莖之移轉情形及遮光對其移轉之影響。嘉磷塞以 4 及 8 kg/ha之劑量施用30天，分別減少25及50％之塊莖發芽率。以 2 kg/ha 量施用120小時後，才可抑制25％之塊莖發芽，但在4 kg/ha劑量下，則只需36小時，至於施用 8 kg /ha 72 小時後可達50％上之抑制率，而以TTC法測定塊莖活性的結果與其發芽率相符合，但在測定被處理植株塊莖之糖，澱粉及全氮含量時，發現前二者會被嘉磷塞所抑制，但全氮含量則無影響。至於遮光處理本身即會抑制塊莖的生成及其發芽活性，但對嘉磷塞的運轉則似乎無顯著的抑制效果。

(其他關鍵語：發芽活性，移轉，劑量，遮光)

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INTRODUCTION

Purple nutsedge (*Cyperus rotundus* L.) is one of the most notorious weeds in many of the major crops in the warm regions of the world, because of its adaptation to a wide variety of soil types and environmental conditions and rapid propagation (4,7). Reproduction of this plant is mainly by a complex underground system of rhizomes and tubers(3). Vegetative reproduction is both rapid and vigorous. Under favorable conditions, a single tuber can produce 146 tubers and basal bulbs within 3.5 months(3). In recent years purple nutsedge has become a greater problem due to the development and use of herbicides that control and cultivation. Although tubers of purple nutsedge commonly begin sprouting soon after planting, some may sprout for the first time 1 yr after planting(4). The tubers commonly sprout several times, and dormant tubers make chemical control difficult and eradication nearly impossible. Otherwise, the presence of four to five buds per tuber and sufficient stored food to sprout these buds contribuis to the resistance of purple nutsedge to control measures(4).

Although purple nutsedge exhibits some tolerance to glyphosate at normal use rates, control of purple nutsedge in the field is sometimes greater with glyphosate than others(6). Glyphosate [N-(phosphonomethyl) glycine] is a systemic, foliar-applied herbicide which has shown the greatest potential for suppressing resprouting of tubers attached to treated plants. But it has not been obtained to eradicate from infested field even with repeated applications. In general, successful control of perennial weeds with foliar-applied herbicides depends on the rapid absorption and the basipetal translocation of the biologically active compound into the underground storage organs insufficient quantities to kill entire plant before metabolism can degrade the compound (4,6,8). The amount of glyphosate absorbed and translocated in nutsedge plants varies considerably. Variation in the rate and duration of absorption and translocation can be influenced by many factors, such as temperature, soil moisture and light intensity(5). Environmental conditions can also affect herbicide activity by altering source to sink relationship and thus translocation(5). Researchers have reported that most weeds responded faster to glyphosate treatment under higher light intensities(1,5). It has been postulated that toxicity of this herbicide may be caused by a light-induced secondary substance such as a toxic photosynthetic intermediate or a free radical formed by an interaction between the herbicide and light(1,5,6). So the lock of translocation of herbicide within purple nutseedge rhizome-tuber chains may be the major reason for the poor control(8).

Based on the potential that glyphosate has shown for reducing sprouting of tubers of purple nutsedge, and a better understanding of the physiological role of glyphosate may lead to reduce application rates or improve efficacy on purple nutsedge difficult to control, we condncted a greenhouse study to determine the efficacy of glyphosate on the sprouting ability of tubers attached to treated plants. The following aspects were studied: (1) effect of rate of application of glyphosate on the regrowth of purple nutsedge, (2) time needed for glyphosate translocation to tubers, (3) effect of shade on glyphosate translocation.

MATERIALS AND METHODS

All trials were conducted outside the greenhouse in the summer of 1985 and 1986 at Taiwan Agricultural Chemicals and Toxic substances Research Institute (TACTRI), in Taichung, Taiwan. The average temperatures were 30-35C day and 20 -25C night with a daylength of approximately 10-12h. Under these conditions the purple nutsedge (*Cyperus rotundus*) began sprouting about 3 days after planting. A completely randomized design was used with three replications per treatment and 2 pots per replication. Data were subjected to an analysis of variance and treatment means compared using Duncan's multiple range test.

Effect of glyphosate rate on the regrowth of purple nutsedge

This experiment was conducted to determine the phytotoxicity of several concentrations of the commerical formulation of 41% glyphosate on purple nutsedge growth. Purple nutsedge tubers were collected from fields at TACTRI, and tubers that weighed 200 to 250 mg each were selected for use in the pot experiment. Single tubers, one per pot, were planted 2 cm deep in 15cm-diam, 15cm-depth plastic pots containing paddy soil. Soil had a pH of 6.8 and contained 1.5 % organic matter were collected form adjacent research-plot areas was fumigated with methyl bromide at 112kg/ha to kill existing weed seeds and tubers. Pots were watered as needed to insure optimum moisture condition and fertilizer was applied ($N:P_2Q_5:KCl=0.5:0.25:0.25$g/pot). The parent tuber might produce new plants with an average of over thirth tubers within 30 days after planting. Plants were treated with the comerical formulation of 41% glyphosate, at 0, 2, 4 and 8 kg/ha. And it was applied with flat fan nozzles to 30-day-old plants in 800l/ha of water at 30 psi. The plants and underground parts were harvested and washed free of soil 15 and 30 days after appllcation.

They were separated into shoots, rhizomes, and tubers. The separated parts of each plant were weighed, and grounded for determining total nitrogen(9), sugar and starch content(10). Otherwise, the tubers were counted and replanted into pots containing the same paddy soil. Pots were watered as necessary to encourage tuber sproting. The number of sprouted tubers in each pots was recorded at daily intervals for two months. After sprouting, tubers were removed from pots and discarded.

Since 50 to 75% of the firm tubers collected from treated plants failed to resprout after 2 months, the viability of these tubers was further determined using 2,3,5-triphenyl-2H-tetrazoliumchloride (TTC)[4].

Effect of time for translocation

The above procedure was used to plant the tubers, and apply glyphosate to 30-day-old plants. In this trial time periods of 6, 12, 24, 36, 72, and 120 h for translocation were used. Rates of 0, 2, 4 and 8 kg/ha of 41% glyphosate were applied. After treatment, shoots were removed to stop further translocation. The plants were harvested as described above and also investigated tubers viability and plants composition.

Effect of shade on translocation

The effect of darkness reduced the ability of glyphosate to inhibit tuber sproution was determined by maintaining treated plants in darkness for varied periods of time. Plants ware grown for 30 days in pots and placed under 1 × 1 × 2 cm cages covered with plastic shade cloth. Average photon flux density was 400 uE. m^{-2}. s^{-1} at plant level for 10 h each day as non-shaded treatment. And the relative shades of 50 and 100% were produced by the shade cloth. After plants were shade 0, 4 and 8 kg/ha 41% glyphosate were applied. Each shade treatment consisted of two herbicide-treated pots and two control pots and was replicated three times for a total of six pots per treatment. At 7, 14 and 28 days after application, the plants were harvested as described above and also investigated tubers viability.

Tubers treated with tetrazolium chloride

The TTC is reduced to formazan by the respiration of living tissue. A red pigmented formazan solution can be produced that will absorb light and can be measured by a colorimeter. The TTC solution was prepared by dissolving 0.1g TTC in 100ml distilled water. The tubers were cut into 0.5mm sections and were placed in a test tube containing 5ml of the TTC solution. After 8h incubation in the dark at 25C, fifteen ml of 95% ethanol was then added to the test tubes and evaporated to 2 ml. Five ml ethanol was added to the evaporated solution, and light absorbance the solution was determined in a colorimeter at a wave length of 530nm. The sections of tubers turn pink when reduced by the enzyne dehydrogenase, indicating that respiration is taking place and live tissue is present.

RESULTS AND DISCUSSION

Effect of glyphosate rate on regrowth of purple nutsedge

Glyphosate at 4 and 8 kg/ha caused a significant reduction in total shoot fresh weight as well as in rhizome number and tuber number at 15 and 30 days after application (Table 1). It also inhibited tuber sprouting at either 4 or 8 hg/ha (Table 2). The 4 kg/ha rate reduced sprouting 25% at 30 days after application whereas the 8 kg/ha rate almost inhibited tuber sprouting to 50% or more. Our results indicated that 4-8 kg/ha of glyphosate effectively inhibited the sprouting of purple nutsedge tubers attached to treated plants. Under hot and dry conditions, purple nutsedge grew prosperously and higher glyphosate rates might be required [7,8].

Table 1. Effect of different rates of glyphosate on regrowth of 30-day-old purple nutsedge at 15 and 30 days after application (DAA)

Rate	Total shoot fresh weight		Rhizome number		Tuber number		
(kg/ha)	15	30	15	30	15	30	DAA
	----(g/pot)---		---------(no./pot)		---------		
0	72a	84a	161a	174a	129a	139a	
2	69a	73a	144b	152b	118b	129b	
4	52b	54b	122c	120c	107c	115c	
8	38c	38c	92d	90d	95d	89d	

* Numbers in the same column followed by the same letter are not statistically different at 0.05 level by Duncan's multiple range test.

Table 2. Effect of different rates of glyphosate on tuber sprouting of 30-day-old purple nutsedge at 15 and 30 days after application

Rate	Tuber sprouted(%)		
(kg/ha)	15	30	DAA
0	95a	95a	
2	89a	87a	
4	81a	71b	
8	59c	40c	

* Numbers in the same column followed by the same letter are not statistically different at 0.05 level by Dunca's multiple range test.

Effect of time for translocation

Maximum sprouting inhibition at the 2 kg/ha rate occurred with 120 h for translocation (Table 3). Doubling the rate reduced the time needed for maximum inhibition only 36 h. At 8 kg/ha, glyphosate could inhibit tuber sprouting to 50% more with 72 h for translocation. Therefore, more glyphosate was absorbed or translocated as the rate increased. And the time needed for translocation of phytotoxic amounts of glyphosate into the tubers was rate-dependent[5]. Higher application rates led to more translocation than low rates. Our results also indicated that glyphosate translocated to the tubers from treated plants at least 36-72 h necessary [2]. When tubers cut from treated plants were placed in solution of TTC, these solutions absorbed less light than solutions from tubers of unte rated plants (Table 4).

This indicated that the biological activity of these tubers was low when compared to untreated tubers. Whereas over 80% of the tubers from untreated plants appeared viable (50 to 100% absorbance range), only about 42-70%, 40-50% and 0-34 % tubers from treated plants with 2, 4 and 8 kg/ha glyphosate appeared viable, respectively. Generally, the longer the time after application, the less viable the tuber activity observed for each rate in treated plants. And the amount of glyphosate translocation was dependent on the time after application. Although buds of tubers might be killed by glyphosate, the presence or absence of viable buds probably contributed little to the formation of formazan. This assumption was based on observation that equal amounts of formazan were produced in solutions coutaining apical buds (buds present) and basal halves (buds absent) of unsprouted tubers. Furthermore, close examination of tubers indicated that essentially all tissues within the endodermis of healthy tubers were brightly staines. Similar tissues from most tubers of treated plants were either lightly stained or not at all. The reduced light absorbance of the solutions from the majority of the unsprouted tubers from treatred plants, interpreted as reduced viability, suggested that these tubers would not sprout [4]. Sugar and starch contents in purple nutsedge tubers were also significantly reduced by higher dosage and longer time after glyphosate application (Fig. 1). But it had no difference in total nitrogen content among the tubers of treated plants with glyphosate. It appeared only carbohydrates were ihibited to translocate to tubers by glyphosate application[1].

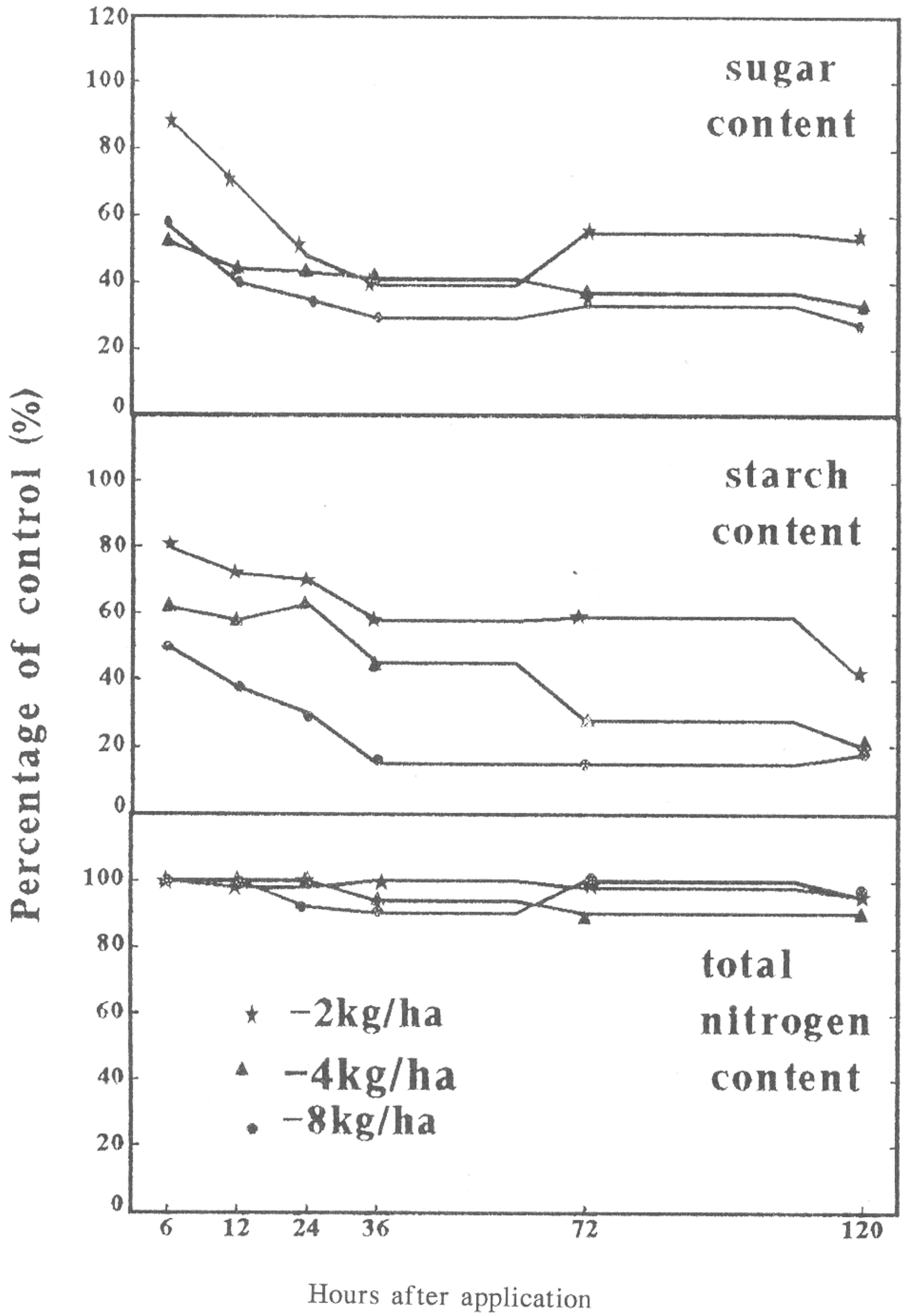

Fig. 1. Sugar, starch and total nitrogen contents in purple nutsedge tubers after glyphosate application.

Table 3. Percentage of tubers sprouted 30 days after replanting for various time after application to 30-day-old purple nutsedge

Rate (kg/ha)	Tuber sprouted(%)					
	6	12	24	36	72	120h
0	100a	95a	93a	99a	95a	95a
2	99a	95a	91a	97a	84b	71b
4	97a	90a	83b	77b	74c	55c
8	88b	75b	60c	53c	46d	30d

* Numbers in the same column followed by the same letter are not statistically different at 0.05 level by Duncan's muitiple range test.

Table 4. Light absorbance of extracts of purple nutsedge tubers from untreatedplants and plants treated with different rate of glyphosate following exposure to triphenyltetrazolium chloride

Rate (kg/has)	time after application (h)	Light absorbance ranges		
		0-30%	30-50%	50-100%
			%	
0	6	5	12	83
	12	5	12	83
	24	2	15	85
	36	1	12	87
	72	0	13	87
	120	0	18	82
2	6	10	20	70
	12	10	21	69
	24	10	29	71
	36	9	26	65
	72	19	33	48
	120	19	39	42
4	6	25	25	50
	12	22	26	52
	24	22	35	43
	36	22	34	44
	72	24	32	44
	120	24	36	40
8	6	30	36	34
	12	39	34	27
	24	47	31	22
	36	65	30	5
	72	70	30	0
	120	66	34	0

Effect of shade on translocation.

The tuber number of purple nutsedge was decreased marked by shading (Table 5). It showed 40% reduction under full shading environment for 28 days. But shading treated plants did not reduce the translocation of glyphosateto the tubers (Table 6). Shade had also effect on the sprouting ability of tubers of the control plants. More tubers sprouted from treated plants placed in full sunlight than those from plants placed in the shade[5]. Using the TTC method to test tuber viability, it might indicate the same results as well as tuber sprouting ability (Table 7). It seemed the influence of glyphosate application on tuber viability was enchanced by shade to the plants. Glyphosate inhibited tuber sprouting even when treated plants were shaded.

Appearently, sufficient glyphosate translocation occurred even under cond itions of reducing light. These studies showed that glyphosate killed purple nutsedge foliage and the tubers attached to treated plants. Therefore, regrow th after glyphosate application under field conditions was due to dormant tub ers which sprout after treatment. So subsequent applications may be required to kill plants which emerge after glyphosate application. Future research on purple nutsedge should seek to induce all tubers to sprout uniformly. Then a single foliar treatment of glyphosate should eradicate this weed.

Table 5. Effect of shade on tuber number of purple nutsedge.

Shade	Tubers number			
(%)	7	14	28	days after shading
0	48a	56a	74a	
50	40a	51a	69a	
100	35b	36b	42b	

* Numbers in the same column followed by the same letter are not statistically different at 0.05 level by Duncan's muitiple range test.

Table 6. Effect of shading plants treated with different rates of glyphosate on tuber sprouting of purple nutsedge

Shade	Rate (kg/ha)									
	0			4			8			
(%)	7	14	28	7	14	28	7	14	28	DAS**
0	97a	95a	97a	70a	65a	53a	35a	29a	27a	
50	91a	90a	92a	65a	60a	48a	30b	21b	20b	
100	63b	57b	59b	30b	21b	18b	15c	13c	11c	

* Numbers in the same column followed by the same letters are not statistically different at 0.05 level by Duncan's multiple range test.

* * DAS : days after shading.

Table 7. Light absorbance of extracts of purple nutsedge tuber from treated with different rates of glyphosate and shade following exposure to triphltetrazolium chloride

Rate	Shade	Light absorbance									
		0-30%			30-50%			50-100%			
(kg/ha)	(%)	7	14	28	7	14	28	7	14	28	DAS
					%						
0	0	11	22	53	29	39	26	60	39	21	
	50	19	37	63	31	30	20	50	33	17	
	100	37	82	92	37	18	8	26	0	6	
4	0	38	75	98	21	21	2	41	4	0	
	50	32	88	97	38	13	3	30	0	0	
	100	36	87	98	36	13	2	28	0	0	
8	0	43	82	97	32	14	3	25	4	0	
	50	45	81	94	35	16	5	20	3	1	
	100	48	84	93	28	12	7	24	4	0	

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