

HERBICIDAL ACTIVITY AND TRANSLOCATION
OF GLYPHOSATE IN CYPERUS ROTUNDUS L.

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ABSTRACT

Purple nutsedge (Cyperus rotundus L.) plants grown in the greenhouse were treated with 4 kg/ha glyphosate [N-(phosphonomethyl)glycine] or 2.2 kg/ha paraquat (1,1'-dimethyl-4,4'-bipyridinium ion). Glyphosate greatly reduced fresh weight of leaves, number of sprouts per original tuber, and number of sprouts per new tuber. Paraquat reduced fresh weight of leaves about as well as glyphosate, but was not as effective in reducing germination of tubers.

Glyphosate at 2 and 4 kg/ha was compared in the field to paraquat, dicamba (3,6-dichloro-o-anisic acid), and MSMA (monosodium methane-arsenate) for purple nutsedge control in repeated applications over 8 months. After two applications glyphosate had greatly reduced the number of plants. The other herbicides were not as effective in reducing number of shoots. At 5 months, after the field was rotovated and herbicides reapplied, glyphosate and MSMA gave better control of purple nutsedge plants than the other herbicides. Glyphosate and MSMA also reduced germination of tubers from treated plants. After five applications, glyphosate and MSMA reduced germination by 70%, and dicamba reduced germination by 43%. Paraquat did not reduce germination.

Since glyphosate gave good control of purple nutsedge, further studies were conducted to determine the most effective rate of glyphosate, and the most susceptible age of purple nutsedge at application. Purple nutsedge was treated with glyphosate at 2, 4, 6, 12, or 24 weeks after field preparation. Glyphosate was reapplied at 2, 4, 6, or 12 week intervals, respectively, until no shoots emerged.

Plants were counted in treated plots every 2 weeks, and tubers were dug every 4 weeks. These tubers were germinated to test viability by placing them in Petri dishes and incubating with 100 ppm N-6 benzyl adenine.

All purple nutsedge plants treated at 12 weeks old were killed by glyphosate at 2 and 4 kg/ha, as evidenced by no regrowth of shoots, and almost no germination of tubers. Control of purple nutsedge in plots treated at 2 to 6 weeks old was less effective and several applications of glyphosate were needed to achieve good control levels. Application of glyphosate at 24 weeks killed purple nutsedge foliage, but new growth emerged immediately. Generally, application of glyphosate every 2 weeks reduced plant numbers more rapidly than every 4 or 6 weeks. Rates of 1, 2, and 4 kg/ha were equally effective in reducing number of plants after several applications, but 2 and 4 kg/ha were more effective with fewer applications. Two kg/ha gave as good control as 4 kg/ha. Viability of tubers from plants treated at 2 to 6 weeks old was higher than of tubers from plants treated at 12 weeks old. Applications of glyphosate at 24 weeks did not reduce viability of tubers.

The field was rotovated 10 months after the initial preparation, and the purple nutsedge allowed to regrow. Regrowth was rapid in all plots. However, all plots treated with glyphosate except those treated at 24 weeks, produced less regrowth than the controls.

Succeeding experiments carried out in the greenhouse further examined the effects of age and stage of purple nutsedge growth on its control with glyphosate. Tubers from purple nutsedge plants grown in

the greenhouse for 2 to 10 weeks did not germinate after foliar application of glyphosate. Some tubers from plants 12 and 24 weeks old survived glyphosate application.

^{14}C -glyphosate was used to study translocation of glyphosate in 1- to 6-week-old purple nutsedge plants. Translocation of ^{14}C -glyphosate from treated leaves increased from 5% of the amount applied at 1 day to 19% at 4 days after application. Specific activity of ^{14}C in tubers was greater than in leaves at all growth stages. With increasing plant age, specific activity decreased in both tubers and leaves. Also with increasing plant age, total ^{14}C translocated increased in tubers, and decreased slightly in leaves. Thin layer chromatography showed no evidence of glyphosate metabolism in purple nutsedge.

These results indicated that stage of growth is an important factor in obtaining control of purple nutsedge with glyphosate. Purple nutsedge in the field was most susceptible 12 weeks after the field was prepared. Purple nutsedge grown in the greenhouse was most susceptible at 2 to 10 weeks after planting. Evidently the stage of growth, or physiological age of the plant, is more important than chronological age. Once purple nutsedge plants have flowered, senescence sets in, and effectiveness of glyphosate declines.

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CHAPTER I

INTRODUCTION

Purple nutsedge (Cyperus rotundus L.) may be considered the world's worst weed (30). It is a serious pest in most crops in the warm regions (29). In recent years it has become a greater problem due to several factors: 1. development and use of herbicides that control most annual weeds; 2. a decrease in hand hoeing, deep plowing, and cultivation; 3. adoption of mechanized farming methods; 4. intensive production efforts needed to obtain higher crop yields (5, 25).

The persistence of purple nutsedge is due primarily to its anatomy and physiology. The plant consists of an interconnected system of shoots, basal bulbs (corms), tubers, and rhizomes (46, 73), all at various stages of growth and development. The tubers become dormant as they mature, and are virtually unaffected by most herbicides applied to leaves. Each tuber contains several buds, each of which can sprout (usually one at a time), forming a new shoot; these may germinate when the dormant tubers are at the end of chains or broken off from the rhizome and original shoot. Killing one shoot allows another bud to sprout. Thus the reproductive capacity of purple nutsedge is almost unlimited.

The control of purple nutsedge, then, depends on the ability to kill or keep the buds dormant on the tubers. Some soil-applied herbicides which have been fairly successful in purple nutsedge control have maintained dormancy of the tubers for varying lengths of time, but have not killed the tubers (24). A better approach is to apply a herbicide to the leaves, which will be translocated to and kill the

underground organs. A new herbicide, glyphosate [N-(phosphonomethyl) glycine], seems to meet the criteria for good purple nutsedge control. Several workers have reported that glyphosate controls purple nutsedge (40, 66, 74).

The objectives of the present study are: 1. to compare glyphosate to other herbicides presently used for purple nutsedge control; 2. to determine the feasibility of controlling purple nutsedge with glyphosate in the field; 3. to determine the extent of translocation and sites of accumulation of glyphosate at various growth stages in purple nutsedge, as related to control.

CHAPTER II

REVIEW OF LITERATURE

Biology of Purple Nutsedge

The purple nutsedge plant is made up of an interconnected system of shoots, basal bulbs, tubers, and rhizomes (22, 46). Upon germination, a rhizome grows upwards from the tuber to the soil surface, where it forms a corm, often referred to as a basal bulb. The leaves of the shoot arise from this basal bulb. When the shoot has reached maturity, new rhizomes emerge from the basal bulb and form either additional basal bulbs and shoots, or dormant tubers (46).

The tuber consists of several short internodes, with buds and scale leaves arising at each node. Tubers generally have three to ten buds, each of which can produce a rhizome, which either forms a tuber at its tip and then continues to grow, or a basal bulb at the soil surface, which forms a shoot. A dormant apical bud on a tuber will always form a basal bulb (46).

The tuber and basal bulb both serve as storehouses of food material, mainly starch (46, 73). Their formation is similar: cells in the meristematic region of the rhizome apex increase in girth and accumulate starch. The internodes do not elongate, thus forming a swelling that develops into the respective organ. In the case of tubers, the leaf primordia remain dormant; in basal bulbs, the leaf primordia produce shoot growth (73).

Young rhizomes are white and succulent, with an outer covering of scale leaves. Old mature rhizomes are brown and wiry, but remain completely functional (1, 31, 48, 73).

Tubers are distributed throughout the upper 90 cm of soil (46). However, by far the majority are near the surface. On 10 soil types in Alabama, 90% or more were found in the upper 15 cm of soil (53). In Puerto Rico, 60% of the tubers were found in the top 18 cm of soil (41). Upon germination, all tubers send up a rhizome to the surface which forms a basal bulb and plant. Subsequently, other buds may germinate, sending more rhizomes to the surface, forming more plants. The deeper the tuber is located in the ground, the fewer buds that sprout. This is probably a mechanism of the plant to insure that at least one rhizome reaches the surface and forms a plant (46).

New tubers generally begin to form about 3 weeks after initial shoot emergence (46, 52). In some areas it takes longer. In Georgia, first new tubers were formed 6 to 8 weeks after shoot emergence (22). These differences in time until tubers form may be due to environmental differences, or differences between biotypes.

The sequence of tuber formation has been observed by growing purple nutsedge in a glass box (41). The rhizome emerged from a tuber or basal bulb, grew for an unspecified length, then stopped growing longitudinally and began swelling just back of the rhizome apex; once the new tuber was formed, longitudinal growth resumed. In this manner long chains of tubers and basal bulbs are formed.

Flowers emerge about 5 to 8 weeks after initial shoot emergence. Flowering increases until about 12 weeks after shoot emergence, after which the shoots decline in vigor and number.

Production of underground structures increases through the 20th week, and possibly longer (22).

The tubers and basal bulbs are the primary reproductive organs of the purple nutsedge plant. In a test in Georgia, tubers planted at 30 cm spacing produced 3,090,000 plants and 4,420,000 tubers and basal bulbs per 0.4 ha (21).

Purple nutsedge seed is not viable under most conditions. Tests in the southern United States found no viable seeds (52, 73). Ranade and Burns in India obtained up to 80% germination of seeds in the laboratory, but less than 1.5% in the field. They concluded that the seed was not of major importance in propagation of purple nutsedge in the field (46).

Basal bulbs are similar morphologically to tubers; however, since they are directly connected to a shoot, they are much more susceptible to herbicide treatment than are tubers. Therefore, several workers have tried to determine the causal factor in basal bulb formation. Ranade and Burns suggested some relationship between light and basal bulb formation (46). Muzik and Cruzado also suggested this possibility (41). Hauser found basal bulbs at 15 cm and deeper in the soil, and thus discounted the light theory (22). Standifer et al. found a relationship between light and basal bulb formation but noted evidence of other endogenous aspects of basal bulb regulation (59). In a recent study, Chetram and Bendixen discovered that red light caused rhizomes to form basal bulbs and concluded that phytochrome was the controlling factor in basal bulb formation. They also found that application of cytokinins

to tubers and rhizomes replaced the red light requirement and induced basal bulb formation (12).

Purple nutsedge is a very prolific and hardy weed. Its great reproductive capacity is due, in part, to its ability to produce reproductive and storage organs in a short time after emergence. Purple nutsedge utilizes the C-4 photosynthesis pathway, thus making more efficient use of CO₂ with increases in light and temperature (8). Wills determined that optimum growth of purple nutsedge plants, as measured by shoot, basal bulb, tuber, and rhizome numbers and dry weight, occurs after 3 months at 32 C and 19 Klux. Higher temperature and light intensities did not increase production of plant parts (74).

The purple nutsedge plant demonstrates two types of apical dominance in the underground organs: apical dominance within the tuber, and within the chain. In isolated tubers, the apical bud usually sprouts first: other buds usually sprout in succession from the apical bud if the foliage of the previous shoot is killed, or if growing conditions permit more sprouting (46, 52). The apical dominance in the tuber chains is not as strong as in individual tubers; however, if planted in a chain, usually only the uppermost tuber will sprout; or if planted horizontally, usually only the terminal tubers will sprout (41, 52). This dormancy can be overcome by severing the rhizomes between tubers, or killing them with heat. Then all the tubers sprout equally (41).

Many tubers are dormant even when conditions favor sprouting. Several workers have tried to establish the cause of dormancy, and

others have tried to overcome it. Berger and Day reported that photoperiods of 10 hours or less induced flowering and tuber formation, stimulated salicylic acid formation in the leaves, and inhibited bud growth. Longer photoperiods inhibited flowering, reduced tuber production and salicylic acid formation, and enhanced bud growth. They concluded that salicylic acid may be a major cause of seasonal dormancy in tubers. However, they were not able to detect salicylic acid in the tubers (6).

Other workers were able to extract phenolic substances from purple nutsedge tubers that may be involved in dormancy. Friedman and Horowitz found phenolic substances that inhibited growth of barley seedlings (18). Jangaard et al. found phenolics, but suggested that their role in dormancy was minor. They also found abscisic acid in purple nutsedge leaves, and felt that it might have a role in dormancy (34). Teo et al. found phenolics and abscisic acid in purple nutsedge tubers. They suggested that phenolic compounds are the major cause of dormancy in tuber buds, and that abscisic acid is a minor factor (65).

Ueki used temperature and light variations to stimulate sprouting of dormant tubers. He found that the highest germination took place at 30 to 35 C. Light did not seem to affect germination (69). Jackson et al. reported that ethylene and ethephon stimulated purple nutsedge bud sprouting (33). Teo and Nishimoto reported that N-6 benzyl adenine (BA) and other synthetic cytokinins will overcome tuber dormancy. When treated with BA, all the buds on a tuber sprouted (64). They also found that inhibition

of bud sprouting, induced by the presence of abscisic acid, phenolics, or other inhibitors, could be overcome by addition of BA. They suggested that dormancy is controlled by a balance between sprout promoters and inhibitors; dormant tubers are deficient in cytokinin and do not sprout. Accumulation of cytokinin or disappearance of the inhibitors allows sprouting to occur (65).

Control of Purple Nutsedge

Control of purple nutsedge is possible under certain cultural and climatic conditions. Ranade and Burns reported that desiccation of the tubers killed them rapidly. They estimated that 8 days of exposure to sun or completely dry soil in hot weather would kill purple nutsedge tubers. This could be achieved by two or more plowings during the dry season in India. They also found that continuous removal of shoots will reduce tuber viability by depleting food reserves, but that was a very slow process and never achieved eradication (46). Smith and Fick reported that, since new tubers were formed in about 21 days from emergence of the shoot, any tillage operation that would break up the plant and rhizome system at this or shorter intervals would lead to eradication (52). They reasoned that if no new tubers were formed, the purple nutsedge plants would die when all buds on existing tubers had sprouted. In further work, they substantiated this contention: Smith and Mayton reported eradication of purple nutsedge with plowing or disking every 3 weeks or less over two growing seasons. If the time between tillings was increased to 4 weeks, purple nutsedge plant numbers increased the first year, but decreased the

second year (53). It appears that the 4 week period was long enough to allow some new tubers to form, and the tillings broke up the rhizome chains, allowing them to sprout. By the second year most of the buds on the original tubers have sprouted, and they die, thus causing the decrease in plant numbers the second year. They also reported that eradication was not achieved when low-lying, wet soil was tilled every 2 or 3 weeks. Since wet soil does not break up as well as dry soil, many plants were left intact, and tubers were not desiccated. Thus drying was an essential part of the eradication program (54).

Davis and Hawkins were able to eradicate purple nutsedge in a test in Arizona by weekly hoeings over three growing seasons. The area was quite dry, so a combination of desiccation and shoot removal contributed to the death of the plants (14). Day and Russell reported that drying was the only proven effective control of purple nutsedge on a large area basis in California (15).

Many herbicides have some activity on purple nutsedge. Among foliar-applied materials, the hormone type herbicides have been studied extensively. 2,4-D [(2,4-dichlorophenoxy)acetic acid] has given varying results. Hauser reported fair control of purple nutsedge when 0.23 kg/ha was applied at 1 to 2 weeks after emergence, and reapplied every 2 weeks. If treatment was initiated later, poorer control resulted (23). After 10 years of testing, Parker et al. concurred that repeated applications of 2,4-D will reduce stand, but will not give complete kill (42). Standifer, on the other hand, achieved nearly complete shoot eradication with

repeated applications of 2,4-D at 0.62 kg/ha over 2 years (58). Burr and Warren were able to increase penetration of 2,4-D into purple nutsedge plants with isoparaffinic oil as a carrier. Two applications reduced fresh weight of leaves, inhibited tuber and shoot production, and reduced number of viable tubers, but the degree of control achieved was not commercially acceptable. Ray and Wilcox reported no control with one application of 2,4-D at rates up to 2.2 kg/ha in a chemical fallow system after 1 year (47).

The variation in level of purple nutsedge control with 2,4-D indicates that it is not sufficiently active for most situations. Since 2,4-D is a hormone, its activity depends on the physiological condition and growth rate of the plants. When these conditions are not optimum, herbicidal activity is not satisfactory.

Amitrole (3-amino-s-triazole) has also given some control of purple nutsedge. Andersen found that amitrole is readily translocated to actively growing meristems in buds, shoots, and root tips. It was not present in dormant buds, storage parenchyma or other mature tissues (2). In field tests Hauser obtained good control of purple nutsedge with amitrole at 9 kg/ha when applied at 4 weeks after emergence. However, if application was delayed until 6 weeks after emergence, there was less effect on the purple nutsedge (23). Ray and Wilcox, in Florida, used several herbicides in a 1 year chemical fallow system to try to eradicate purple nutsedge. Amitrole gave no control at rates up to 9 kg/ha (47). In their 10 year study, Parker et al. reported that amitrole was unable

to give a sufficient level of control to be considered satisfactory for crop use (42).

As with 2,4-D, amitrole does not have sufficient activity on purple nutsedge to give consistent control. When all factors, such as age, physiological condition, and climatic factors are correct, fair control may be obtained. However, its limited activity has resulted in limited use of amitrole on purple nutsedge.

Dicamba (3,6-dichloro-o-anistic acid) is translocated to aerial parts of the purple nutsedge plant after application to leaves or roots. It accumulates in areas of meristematic activity above ground. Small amounts are detectable in underground organs. The most active translocation occurred during the vegetative growth stage before flowering (39). Ray and Wilcox found that dicamba moves readily between plants connected by rhizomes (48). However, it did not give adequate control under field conditions at rates up to 5.5 kg/ha (47). Sasser and Locascio also reported poor control with dicamba (51).

The organic arsenical herbicides have shown fair activity on purple nutsedge. Holt et al. found that arsenic, when applied as amine methyl arsenate to purple nutsedge plants, was translocated to basal bulbs and tubers. They suggested that lethality, after repeated applications, was due to depletion of food reserves, interruption of oxidative-phosphorylation, and exhaustion of bud supply due to increased sprouting (31). Doble et al. found that DSMA (disodium methanearsonate), when applied to a shoot, was translocated to other shoots in a chain. It accumulated in terminal

tubers, shoots, roots, and rhizomes. Intermediate and dormant tubers did not accumulate DSMA (17). Keeley and Thullen reported 80% reduction in purple nutsedge stand in the field after one application of MSMA (monosodium methanearsonate) at 3.36 kg/ha. DSMA was not as effective as MSMA (38). Repeated applications of MSMA in the field at 13.4 kg/ha over a year's time eradicated purple nutsedge shoots. Rates of 5.6 and 6.7 kg/ha also reduced stand in repeated applications. There was no difference after 1 year between reapplications at 2, 3, or 4 weeks continuously (19).

The increase in activity of MSMA up to 13 kg/ha is probably due to a saturation effect. As more is applied to the plants, more is translocated. At high rates, MSMA loses most selectivity. However, the organic arsenicals remain as some of the most active herbicides available for purple nutsedge control.

Nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether), at 2 kg/ha in water or herbicidal oil, suppressed purple nutsedge shoot growth by 60% or more when applied at night. Its activity was better during the warm, wet season than during the cool, dry season. Activity of nitrofen was less when applied during the day. This was probably due to increased absorption at night, less evaporation from leaf surfaces, and greater movement in the plants before light-activation of the herbicide (71). Under most circumstances, nitrofen does not give sufficient purple nutsedge control.

Several new herbicides such as bentazon [3-isopropyl-1H-2,1,3-benzothiadiazin(4)3H-one 2,2-dioxide], cyperquat (1-methyl-4-phenylpyridinium), and perfluidone [1,1,1-trifluoro-N-(2 methyl-4-

(phenylsulfonyl)phenyl) methanesulfonamide], are reported to give fair to good purple nutsedge control (36). However, it is too early to establish their effectiveness for general use against purple nutsedge. Other foliarly applied herbicides, such as paraquat (1,1'-dimethyl-4,4'-bipyridinium ion), dinoseb (2-sec-butyl-4,6-dinitrophenol), and phytotoxic oils have given some reduction in shoot stand, but generally unsatisfactory control (42, 58, 76).

Preemergence herbicides have generally given better control of purple nutsedge than foliar applied herbicides. The thiocarbamates, e.g., EPTC (S-ethyl dipropylthiocarbamate), butylate (S-ethyl diisobutylthiocarbamate), pebulate (S-propyl butylethylthiocarbamate), and vernolate (S-propyl dipropylthiocarbamate) have been effective for purple nutsedge control in some crops (26, 37, 42). Hauser reported almost complete control for a growing season with one application of EPTC at 11.2 kg/ha incorporated (24). Holt et al. found that EPTC at 4.5 and 9 kg/ha delayed sprouting for up to 4 weeks, and at 13.4 kg/ha it delayed sprouting for 12 weeks. Tubers exposed to EPTC at 13.4 and 17.3 kg/ha for 12 weeks were killed (32).

Dichlobenil (2,6-dichlorobenzonitrile) controls purple nutsedge well at rates of 11.2 kg/ha or greater; however, these rates are too high for most crop production (20, 47, 70). Terbacil (3-tert-butyl-5-chloro-6-methyluracil) and bromacil (5-bromo-3-sec-butyl-6-methyluracil) also give satisfactory purple nutsedge control at 11.2 kg/ha or higher (42, 49, 70).

In 1971 the Monsanto Company announced the development of a new class of herbicides with a broad spectrum of control (4). One of these, glyphosate [N-(phosphonomethyl)glycine], was developed as a commercial herbicide. It appears to interfere with the synthesis of the aromatic amino acids (35).

Foliar applications of glyphosate at 2.2 kg/ha did not affect either photosynthesis or respiration within 24 hours after application to wheat (Triticum aestivum L.) or quackgrass [Agropyron repens (L.) Beauv.]. Respiration decreased after 216 hours and photosynthesis decreased after 72 hours. Thus it appears that these processes are not directly affected (57). Glyphosate is rapidly adsorbed and/or metabolized in the soil, and has no soil activity (55, 56).

Early work with glyphosate indicated that it was very toxic to many hard-to-control perennials, such as Johnson grass [Sorghum halepense (L.) Pers.], Bermuda grass [Cynodon dactylon (L.) Pers.], quackgrass, and Paspalam spp. (2, 13, 16). Derting et al. reported that glyphosate gave 80 to 90% control of Johnson grass when application was delayed until late in the season. They attributed this to the larger receptive canopy and more active transport to the reproductive storage system (16). Parochetti et al. reported maximum kill of Johnson grass rhizomes after applications of 1.1 to 2.2 kg/ha. Control was better when the glyphosate was applied at the boot to full head stage than if applied earlier (43).

Purple nutsedge also appears to be susceptible to glyphosate. Wills reported 95% control of purple nutsedge with glyphosate at

2 kg/ha in Mississippi (74). In Tanzania, Magambo and Terry were able to control a mature stand of purple nutsedge with one application of glyphosate at 2 kg/ha for 26 weeks (40). During a prolonged dry period, they were able to extend control with 4 and 6 kg/ha for 88 weeks (66). In their study, the lack of moisture probably prevented the tubers from sprouting. Since purple nutsedge does not sprout or grow well under dry conditions, the actual length of control due to glyphosate is not clear. Hebblethwaite, in the Transvaal, conducted tests over three seasons in a mature stand of purple nutsedge. He concluded that 2 kg/ha, followed by 1 kg/ha the first season, and 1 kg/ha in following seasons, would be sufficient for satisfactory control. Higher rates, 3 to 4 kg/ha, the first season gave greater initial kill but no difference in succeeding seasons (27).

The work with glyphosate on purple nutsedge indicates that it has great potential for obtaining the level of control needed for crop production. The experiments in this study were designed to affirm these results, and to detect more efficient and effective methods of use of glyphosate for purple nutsedge control.

CHAPTER III

A COMPARISON OF GLYPHOSATE AND OTHER FOLIAR HERBICIDES ON PURPLE NUTSEDGE

Early reports about glyphosate indicated that it was active on many perennial weeds, including purple nutsedge. The following studies were carried out to compare glyphosate to other herbicides for purple nutsedge control and evaluate its effectiveness on purple nutsedge in Hawaii.

Materials and Methods

Comparison of glyphosate and paraquat for purple nutsedge control in the greenhouse. Tubers were dug from the field at the Waimanalo Research Station, Oahu, Hawaii, 1 week before the experiment began and were stored in a refrigerator at 4 C. Each treatment consisted of six tubers planted in 350 g of soil in an aluminum foil tray (8 by 4 by 5 cm). Each tuber produced at least one plant. Two-, 3-, and 4-week-old plants were treated with the isopropylamine salt of glyphosate at 4 kg/ha, or paraquat at 2.2 kg/ha. At 2 weeks, the purple nutsedge plants were 10 to 15 cm in height and some secondary basal bulbs had formed. At 3 weeks the leaves were fully expanded, and new tubers had begun to form. By the fourth week, flowering had begun and some tubers were maturing.

Fresh weight of the green leaves was taken 4 weeks after the herbicide treatment. The original and newly-formed tubers and basal bulbs were harvested and germinated in Petri dishes with 100 ppm (w/w) BA to induce all viable buds on these reproductive structures to sprout (63). Tubers were germinated in a growth

chamber at 30 C with a 12 hr photoperiod and a 21.5 Klux intensity. The total number of sprouts from tubers in each treatment was counted after 10 days. Each treatment was replicated four times.

Effect of repeat applications of glyphosate and other herbicides on purple nutsedge in the field. Purple nutsedge growing at the Waimanalo Research Station was treated with glyphosate at 2 and 4 kg/ha, paraquat at 1 kg/ha, dicamba at 1 kg/ha, or MSMA at 2 kg/ha. Untreated plots served as the control.

The experiment was designed as a randomized complete block with three replications. Each treatment plot was 1.5 by 7.5 m. The experiment was initiated in June and carried through the following April. The first stage of the experiment consisted of three successive herbicide treatments at 4 week intervals. After the third treatment the field was left undisturbed for 3 months, after which it was rotovated (worked thoroughly to a depth of 15 cm) and left for 3 weeks to allow regrowth of the purple nutsedge. The second stage of the experiment consisted of two successive herbicide treatments at 6 week intervals.

At each reapplication of herbicide, purple nutsedge plants had recovered somewhat. In the glyphosate plots, the original plants were dead, and regrowth was 5 to 10 cm high. In the MSMA plots, some of the original plants were alive but chlorotic, and new shoots were 5 to 10 cm high. After each treatment, plants in the paraquat plots regrew rapidly; the plants were 10 to 15 cm high with no chlorosis evident at reapplication. At retreatment with dicamba, many of the original shoots were alive but chlorotic; and a few new shoots that had emerged were 5 to 10 cm in height.

To assess the effectiveness of the herbicides, the number of plants from 0.4 m² within each treatment plot was counted 4 or 6 weeks after each treatment. At the termination of the experiment, the top 13 cm of soil from 0.1 m² of each treatment was excavated; and the tubers were counted. To test for tuber viability, 10 tubers from each treatment plot were germinated in Petri dishes with 100 ppm BA in the growth chamber at 30 C with 12 hour photoperiod at 21.5 Klux intensity.

Results

Comparison of glyphosate and paraquat for purple nutsedge control in the greenhouse. Glyphosate reduced fresh weight of purple nutsedge leaves below control and paraquat at all growth stages (Table 1). Plants treated with paraquat produced new leaves rapidly after being completely desiccated. Leaves of plants treated with glyphosate died slowly (several days before chlorosis was evident), but there was no regrowth of leaves.

The original tubers from plants treated with glyphosate were nearly all killed. Almost all original tubers from plants treated with paraquat and from untreated controls germinated.

Most new tubers from plants treated with glyphosate did not germinate. Tubers from 3 and 4 week old plants sprayed with paraquat had fewer sprouts than the controls, but most were alive, producing at least one sprout per tuber. Only two new tubers from 2 week old plants treated with paraquat sprouted. The rest were apparently killed, since at that growth stage the underground organs are all very closely connected to shoots.

Table 1. Effects of glyphosate and paraquat on purple nutsedge grown in the greenhouse.

Age in weeks	Treatment	Rate (kg/ha)	Fresh weight of leaves from 1 tray of 6 plants ^a (g)	Sprouts per old tuber ^b (no.)	Sprouts per new tuber ^b (no.)
2	Control		3.2 c	2.3 cd	3.2 b
	Paraquat	2.2	1.5 ab	2.5 d	0.1 a
	Glyphosate	4.0	0.3 a	0.2 a	0.0 a
3	Control		3.0 c	2.0 cd	3.3 b
	Paraquat	2.2	2.0 bc	2.3 cd	1.2 a
	Glyphosate	4.0	1.0 a	0.3 ab	0.8 a
4	Control		3.0 c	0.3 ab	6.0 c
	Paraquat	2.2	1.4 ab	1.3 bc	2.3 b
	Glyphosate	4.0	0.1 a	0.0 a	0.0 a

^aMeans in a column followed by the same letter are not significantly different at P=0.05 by Duncan's multiple range test. Figures are means of four replications.

^bTubers were germinated in petri dishes with 100 ppm BA.

These results indicate that glyphosate is superior to paraquat, a contact herbicide, in reducing fresh weight and sprouting of original and newly formed tubers from purple nutsedge plants under greenhouse conditions.

Effect of repeat applications of glyphosate and other herbicides on purple nutsedge in the field. Glyphosate reduced purple nutsedge stand to 74 and 33% of the control after single treatments with glyphosate at 2 and 4 kg/ha, respectively (Table 2); but the differences were not significant. The second and third glyphosate treatments reduced purple nutsedge stand substantially, but did not completely eliminate the population. The importance of nearly complete elimination of purple nutsedge in an infested field was clearly demonstrated in this experiment (Table 2). When the field was left undisturbed for 10 weeks after the third treatment, the purple nutsedge population increased to about 35 and 10% of the control. This clearly demonstrated the re-establishment potential of purple nutsedge if it is not controlled.

MSMA was as effective as glyphosate in reducing purple nutsedge stand. It significantly reduced the purple nutsedge stand 4 weeks after the second treatment. At other times it did not differ from the control. Paraquat and dicamba had no effects on purple nutsedge stand (Table 2).

After the field was rotovated on completion of the first stage of the experiment, the initial purple nutsedge stand did not differ between treatments (Table 3). This may be due to several factors. Rotovating the soil may have raised dormant tubers from lower levels

Table 2. Effect of three consecutive herbicide treatments on purple nutsedge stand.

Treatment	Rate (kg/ha)	Number of plants			
		Four weeks after first treatment ^a (no./0.1 m ²)	Four weeks after second treatment (no./0.1 m ²)	Four weeks after third treatment (no./0.1 m ²)	Ten weeks after third treatment (no./0.1 m ²)
Glyphosate	2	32.8 a	2.8 a	2.3 a	13.8 ab
Glyphosate	4	14.5 a	1.8 a	1.3 a	7.5 a
MSMA	2	35.0 a	13.8 a	22.8 ab	22.8 abc
Paraquat	1	31.5 a	40.3 b	37.3 ab	50.3 d
Dicamba	1	40.3 a	23.5 ab	54.3 b	32.8 bcd
Control		44.3 a	47.3 b	66.3 b	39.0 cd

^aMeans in a column followed by the same letter are not significantly different at P=0.05 by Duncan's multiple range test. Figures are means of three replications.

Table 3. Effect of two consecutive herbicide treatments on purple nutsedge stand after the field was rotovated.

Treatment	Rate (kg/ha)	Number of plants		
		Initial stand ^a (no./0.1 m ²)	Six weeks after first treatment (no./0.1 m ²)	Six weeks after second treatment (no./0.1 m ²)
Glyphosate	2	10.5 a	3.8 a	7.5 a
Glyphosate	4	10.0 a	3.3 a	6.0 a
MSMA	2	8.8 a	7.5 a	7.3 a
Paraquat	1	12.5 a	18.3 ab	27.5 b
Dicamba	1	12.8 a	17.5 ab	23.8 ab
Control		11.8 a	32.0 b	51.3 c

^aMeans in a column followed by the same letter are not significantly different at P=0.05 by Duncan's multiple range test. Figures are means of three replications.

of the soil which had not been under the influence of the herbicide treatments. Since the field was left untreated for 3 months before rotovating, the purple nutsedge may have become re-established in the treated plots. This allowed for the production of new reproductive structures, and these structures sprouted after the field was rotovated.

The reduction of purple nutsedge stand due to glyphosate and MSMA treatments, in the second stage of the experiment (Table 3) was similar to that described earlier (Table 2). Likewise, paraquat and dicamba had no effect on purple nutsedge stand.

In addition to destroying the leaves and reducing the plant population, the effect of repeated applications of glyphosate and MSMA was to reduce tuber production (Table 4). Treatments with glyphosate and MSMA reduced tuber production by 92 and 88% of the control, respectively. Paraquat and dicamba did not reduce tuber production.

The number of tubers that sprouted after treatment with glyphosate and MSMA was reduced when compared to the control (Table 4). These data indicate that foliar application of glyphosate and MSMA also affected tuber viability, as measured by BA-induced sprouting. Tubers whose viability was not affected produced as many sprouts as the control, indicating that buds on these sprouted tubers had not accumulated toxic levels of the herbicides.

Table 4. Production and viability of purple nutsedge tubers after repeated treatment with herbicides.

Treatment	Rate (kg/ha)	Tubers present ^{a, b} (no./0.1 m ²)	Tubers sprouted with BA treatment ^c (%)	Sprouts per viable tuber (no.)
Glyphosate	2	14.0 a	30 a	4.5 a
Glyphosate	4	14.5 a	33 a	5.2 a
MSMA	2	20.0 a	27 a	3.6 a
Paraquat	1	77.5 ab	85 c	3.2 a
Dicamba	1	82.0 ab	57 b	3.5 a
Control		172.0 b	82 c	4.4 a

^aTubers were obtained from the upper 13 cm of soil.

^bMeans in a column followed by the same letter are not significantly different at P=0.05 by Duncan's multiple range test. Figures are means of three replications.

^cTen tubers per petri dish sprouted with 100 ppm of BA.

CHAPTER IV

PURPLE NUTSEDGE CONTROL IN THE FIELD WITH GLYPHOSATE

Since glyphosate reduced shoot regrowth of purple nutsedge and killed many tubers, further experiments were conducted to determine the best rate of application of glyphosate, and stage of growth of purple nutsedge to obtain optimum control. Furthermore, growth of several crops after treating purple nutsedge with glyphosate was evaluated to determine whether this would be a feasible means of purple nutsedge control in annual crops.

Materials and Methods

This experiment was conducted at the Waimanalo Research Station, Oahu, Hawaii. It was initiated in July, and carried through the following summer. A field heavily infested with purple nutsedge was rotovated to a depth of 15 cm and harrowed to prepare a smooth seedbed. Three kg ai/ha each of trifluralin (α,α,α -trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) and chloramben (3-amino-2,5-dichlorobenzoic acid) granules were applied to control other weed species. These herbicides were re-applied 2 and 4 months after initial application. The field was irrigated twice weekly by overhead sprinklers. Weeds other than purple nutsedge that emerged were removed by hand.

Half of the field was rotovated again 10 weeks later, in mid-September. Two weeks later the first applications of glyphosate were made to 3 by 6 m plots. All applications were made with a one-wheel hand propelled sprayer, applying 375 L/ha spray solution at 2.1 kg/cm² pressure. Glyphosate was applied at 1, 2, or 4 kg/ha every 2, 4, 6, 12,

or 24 weeks after the last tilling, until no regrowth appeared. Thus, 2, 4, and 6 week treatments were applied at 2, 4, or 6 weeks after tilling, and repeated every 2, 4, or 6 weeks, respectively. The 12 week treatments were applied only once, since the population decreased to nearly zero, and remained there until the soil was reworked. Applications at 24 weeks were not repeated, since the experiment terminated before another 24 weeks passed.

The number of purple nutsedge shoots in 0.3 m^2 was counted every 2 weeks, for the duration of the experiment. Tubers were dug from each plot every 4 weeks, up to the second tilling. These tubers were washed and trimmed of roots and rhizomes. Twenty tubers from each plot were placed on filter paper in 15 cm diameter Petri dishes, and 15 ml of 100 ppm BA solution was added to each dish to test for viability. Then the number of sprouted tubers per dish, and the number of shoots per sprouted tuber were recorded, after incubation in the dark at 23 C for 3 weeks.

Ten months after initial tilling the glyphosate applications were discontinued and the field was rotovated again to a depth of 15 cm. Plots were rotovated lengthwise, to maintain the original plots, and to keep out tubers from other treatments. One row of each of the following crops was planted in each plot: lettuce (Lactuca sativa L. var. Anuenue), kai choy (Brassica juncea L. var. Waianae), soybean [Glycine max (L.) Merr. var. Clark 63], sweet corn (Zea mays L. var. Hawaiian Sugar), and sweet potato [Ipomoea batatas (L.) Lam. var. Waimanalo Red]. The crops were planted by seed, except the sweet potato, which was planted by stem cuttings. The crops were sidedressed with a complete

fertilizer (10-10-10) 4 and 7 weeks after planting. Crops were sprayed with diazinon every 2 weeks to control insects. Plots were hand-hoed as needed to remove all weeds except purple nutsedge. The field was irrigated twice weekly.

The crops were harvested at market-age maturity (1 meter of row per crop): lettuce and kai choy, 8 weeks after planting (fresh weight of leaves); sweet corn, 10 weeks after planting (fresh weight of ears); sweet potato, 12 weeks after planting (fresh weight of roots). Soybeans were harvested at two stages: 9 weeks after planting (fresh weight of plants from 1 meter of row at fresh vegetable stage), and 13 weeks after planting (weight of grain from four plants, dried to 12% moisture).

Results

Two and 4 kg/ha rates provided equal control of purple nutsedge in most cases (Table 5). One kg/ha was applied every 2 weeks, and after 3 months it did not differ from 2 or 4 kg/ha applied every 2 weeks. Thus, 2 kg/ha gave as good control of purple nutsedge as 4 kg/ha, and with frequent sprayings, 1 kg/ha gave as good control as 2 and 4 kg/ha.

There was some variation in the number of plants present at initial application (Table 5). Six- and 12-week-old plots had the greatest number. Maximum density is reached at about that stage, as reported by Hauser (22). At 24 weeks, the population had decreased substantially. At 3 months, the purple nutsedge populations in the 24-week plots were increasing, while all others were decreasing. The population of the 12-week treatments had dropped to nearly zero and remained there up to 7 months after treatment. This did not differ from several of the other treatments, but glyphosate was applied only once to 12-week plots. At

Table 5. Effect of glyphosate treatment on purple nutsedge plant density.

Glyphosate (kg/ha)	No. of plants in 0.3 m ²				
	At application ^a (no.)	Months after initial application			
		1 (no.)	3 (no.)	5 (no.)	7 (no.)
1 every 2 weeks	138.0 ab	147.0 ef	12.0 ab	7.0 a	6.7 abcd
2 every 2 weeks	140.3 ab	90.0 d	5.0 a	1.3 a	2.3 abc
4 every 2 weeks	143.0 ab	63.0 cd	3.0 a	1.3 a	5.3 abcd
2 every 4 weeks	140.7 ab	165.7 f	18.3 bc	9.3 a	15.7 d
4 every 4 weeks	179.0 abc	131.7 e	12.7 ab	7.7 a	11.0 bcd
2 every 6 weeks	269.0 cd	33.3 abc	11.7 ab	10.0 a	11.3 cd
4 every 6 weeks	239.3 cd	23.0 ab	10.7 ab	8.7 a	13.3 d
2 at 12 weeks	278.0 d	47.0 bc	0.3 a	0.9 a	1.3 ab
4 at 12 weeks	190.0 bcd	21.3 ab	0.5 a	0.3 a	0.7 a
2 at 24 weeks	86.7 a	11.3 a	42.7 d	67.0 b	
4 at 24 weeks	96.3 a	6.0 a	28.0 c	46.0 b	

^aMeans in a column followed by the same letter are not significantly different at P=0.05 by Duncan's multiple range test. Figures are means of three replications.

7 months after initial application, the 12-week treatments (one application) and 2-week treatments (14 applications) did not differ. More plants were present in the 4- and 6-week treatments than others, but in some cases, were not different from 2-week treatments.

Germination of tubers from these plots gave an indication of the effectiveness of the glyphosate treatments in killing the tubers. Tubers dug 1 month after initial applications showed the 12-week treatments to be the most effective (Table 6). At 3 months, the 2-, 4-, and 6-week treatments caused around 50% mortality of tubers, compared to 75% mortality for the 12-week treatments. Tubers from the 24-week treatments showed no difference in viability from controls at all dates. At 5 months, the same general pattern was present. One kg/ha every 2 weeks did not give as good control as 2 and 4 kg/ha. Four kg/ha every 6 weeks gave better tuber kill than other shorter term treatments.

The number of shoots per sprouted tuber gave an indication of the condition of the live tubers; i.e., whether tubers were weakened but not killed, or whether some buds were killed and not others on the same tuber, or whether some tubers were not affected at all by the glyphosate application. There were no differences between treatments in the number of shoots per sprouted tuber for tubers dug 1 and 3 months after initial application (Table 7). At 5 months there were slight differences with the 12-week treatments showing fewest shoots per tuber. Since the tubers dug at 1 and 3 months showed no difference between treatments in number of shoots per sprouted tuber and at 5 months only

Table 6. Germination of purple nutsedge tubers after application of glyphosate to plants in the field, and germination with BA in the lab.

Glyphosate (kg/ha)	Months after initial application ^a		
	1 (%)	3 (%)	5 (%)
1 every 2 weeks	75.0 de	48.3 bcd	78.3 de
2 every 2 weeks	31.7 abc	41.7 bcd	55.0 cd
4 every 2 weeks	48.3 cd	45.0 bcd	41.7 bc
2 every 4 weeks	85.0 e	58.3 cd	58.3 cd
4 every 4 weeks	76.7 de	66.7 de	40.0 bc
2 every 6 weeks	46.7 cd	41.7 bcd	53.3 cd
4 every 6 weeks	35.0 bc	38.3 bc	25.0 ab
2 at 12 weeks	15.0 ab	23.3 ab	10.0 a
4 at 12 weeks	3.3 a	5.0 a	5.0 a
2 at 24 weeks	83.3 e	90.0 ef	93.3 e
4 at 24 weeks	76.7 de	88.3 ef	91.7 e
0 (Control)	98.3 e	95.0 f	95.0 e

^aMeans in a column followed by the same letter are not significantly different at P=0.05 by Duncan's multiple range test. Figures are means of three replications.

Table 7. Number of shoots per sprouted tuber after application of glyphosate to plants and germination of tubers with BA.

Glyphosate (kg/ha)	Months after initial application ^a		
	1 (no.)	3 (no.)	5 (no.)
1 every 2 weeks	4.6 a	2.7 a	3.0 bcde
2 every 2 weeks	2.9 a	3.8 a	4.3 de
4 every 2 weeks	3.4 a	3.4 a	3.6 bcde
2 every 4 weeks	4.3 a	3.1 a	2.5 abc
4 every 4 weeks	4.7 a	3.7 a	2.6 abcd
2 every 6 weeks	4.7 a	3.5 a	3.5 bcde
4 every 6 weeks	4.9 a	4.5 a	4.1 cde
2 at 12 weeks	2.2 a	4.1 a	1.2 a
4 at 12 weeks	2.9 a	2.1 a	2.3 ab
2 at 24 weeks	3.9 a	5.3 a	4.7 e
4 at 24 weeks	3.8 a	4.2 a	4.0 bcde
0 (Control)	3.2 a	4.3 a	3.6 bcde

^aMeans in a column followed by the same letter are not significantly different at P=0.05 by Duncan's multiple range test. Figures are means of three applications.

slight differences, it appears that the live tubers are all in about the same condition. If some buds on a tuber were killed by glyphosate application and not others, and the tuber itself remained alive, the number of shoots per tuber would have been less with more frequent applications. Since germination rates in 2-, 4-, and 6-week treatments were about the same (about 50%) at 5 months after initial application, and tubers produced the same number of shoots per tubers, it appears that glyphosate either kills or in some way induces dormancy in the tubers. If the glyphosate dose is sufficient, the whole tuber is killed. If it is not sufficient to kill the tuber, it may weaken it or induce dormancy. If any amount of glyphosate was sufficient to kill the tubers, the germination rate of tubers from all treatments should have decreased with more frequent applications. If buds or shoots were killed and not the whole tuber, the number of shoots per tuber should have decreased. The few tubers that sprouted from the 12-week treatments may have been dormant and not connected to shoots at the time of application, thus escaping the glyphosate.

When the plots were rotovated purple nutsedge plants reinfested all plots rapidly. At 1 month after tilling, the 24-week and control plots had the largest number of shoots (Table 8). The other treatments did not differ. At 2 and 3 months after tilling, the same pattern existed. It is noteworthy that 4 kg/ha at 12 weeks consistently gave the least regrowth, and that 4 kg/ha at 24 weeks was always better than 2 kg/ha at 24 weeks. The higher rate evidently killed more tubers initially.

Table 8. Regrowth of purple nutsedge plants after tilling, subsequent to glyphosate application.

Glyphosate (kg/ha)	Number of plants in 0.3 m ²		
	One month after tilling ^a (no.)	Two months after tilling (no.)	Three months after tilling (no.)
1 every 2 weeks	32.0 a	122.0 a	143.0 ab
2 every 2 weeks	17.0 a	98.0 a	135.7 ab
4 every 2 weeks	15.0 a	72.3 a	123.0 ab
2 every 4 weeks	39.3 a	125.3 ab	145.3 ab
4 every 4 weeks	40.3 a	109.0 a	138.0 ab
2 every 6 weeks	30.3 a	128.7 ab	157.0 ab
4 every 6 weeks	17.3 a	94.7 a	133.3 ab
2 at 12 weeks	18.7 a	97.0 a	137.0 ab
4 at 12 weeks	11.3 a	57.0 a	89.7 a
2 at 24 weeks	264.3 c	314.7 c	317.3 c
4 at 24 weeks	118.3 b	214.0 b	219.7 b
0 (Control)	253.3 c	327.7 c	383.7 c

^aMeans in a column followed by the same letter are not significantly different at P=0.05 by Duncan's multiple range test. Figures are means of three replications.

The purple nutsedge plant population in untreated plots increased from tilling until 12 weeks after tilling (Table 9). It then decreased until an equilibrium was reached sometime around the 24th week after tilling. Under these field conditions in Waimanalo, flowering occurred from about 8 to 14 weeks after emergence, after which plants lost vigor and senescence set in. The condition and growth stage of the plant appear to be important factors in the level of control with glyphosate.

Results of the vegetable crop harvests were extremely variable (Table 10). In all crops, the controls and 24-week treatments had the lowest yields. In all crops, the 12-week treatments gave the highest yields, although they were not different from some of the other treatments. Since yield of crops from plots treated with glyphosate once at 12 weeks was generally as good as the other treatments, this would be the optimum treatment in terms of effectiveness and cost.

Table 9. Effect of time on population density of purple nutsedge plants. Counts were taken during the summer and fall at Waimanalo. Figures are means of three replications.

Age in weeks	Plants in 0.3 m ² (no.)
2	198
4	253
8	328
12	384
16	332
20	306
24	159
28	145

Table 10. Fresh weight of crops from 1 meter of row. Crops were harvested at market age.

Glyphosate (kg/ha)	Lettuce ^a leaves (g)	Kai choy leaves (g)	Sweet corn ears (g)	Sweet potato roots (g)	Soybean plants (g)	Soybean seeds (g)
1 every 2 weeks	398 ab	1230 ab	1854 bcd	5046 c	1286 bcd	81 abcd
2 every 2 weeks	910 cd	3187 d	2377 de	4234 bc	1437 cd	80 abcd
4 every 2 weeks	720 bc	2385 bcd	2541 de	4593 bc	1456 cd	114 d
2 every 4 weeks	416 ab	1444 abc	2531 de	4933 c	1343 cd	101 cd
4 every 4 weeks	529 bac	1495 abc	2111 cde	4328 bc	1173 abcd	65 abc
2 every 6 weeks	511 abc	2610 cd	2118 cde	3761 abc	1419 cd	72 abc
4 every 6 weeks	426 ab	2252 bcd	2180 cde	4725 c	1532 d	64 ab
2 at 12 weeks	1184 d	2427 bcd	2950 e	4328 bc	1494 d	94 bcd
4 at 12 weeks	945 cd	2326 bcd	2667 de	4800 c	1192 bcd	82 bcd
2 at 24 weeks	360 ab	561 a	735 a	2230 a	814 ab	43 a
4 at 24 weeks	240 a	662 a	1241 ab	2589 ab	927 abc	43 a
0 (Control)	95 a	198 a	1381 abc	2230 a	756 a	43 a

^aMeans in a column followed by the same letter are not significantly different at P=0.05 by Duncan's multiple range test. Figures are means of three replications.

CHAPTER V

MOVEMENT AND ACTIVITY OF GLYPHOSATE IN PURPLE NUTSEDGE

Since glyphosate is effective in controlling purple nutsedge (Chapter III), and stage of growth is an important factor in achieving this control (Chapter IV), the mechanisms within the purple nutsedge plants that affect the level of control were investigated further. Many factors need to be verified, including the relationship of plant age to its physiological age or stage of growth, the amount, direction, and speed of translocation of glyphosate in the purple nutsedge plants, and possible metabolism of glyphosate by purple nutsedge.

These studies were carried out to determine: 1. the effectiveness of glyphosate in killing the tubers and basal bulbs in chains; 2. the amount and speed of translocation, and sites of accumulation; and 3. whether glyphosate metabolism takes place in purple nutsedge.

Materials and Methods

The effect of apical dominance in rhizome-tuber chains on control of purple nutsedge with glyphosate. Purple nutsedge tubers were planted in soil in metal trays, 35 by 51 by 10 cm, 10 tubers per tray. Planting dates were such that plants were 6, 12, or 24 weeks old at application of glyphosate. Two trays of purple nutsedge were planted at each date and grown outdoors; temperatures were 25 to 28 C day, and 18 to 21 C night. Under these conditions the purple nutsedge began flowering at 6 to 8 weeks after planting.

One tray of purple nutsedge of each age was sprayed with 4 kg/ha glyphosate, and the other remained as a control. The rhizome-tuber chains (several tubers connected by rhizomes) were harvested intact

2 weeks later. Six-week-old chains contained 4 to 11 tubers and basal bulbs, 12-week-old chains had 7 to 15, and 24-week-old chains had 11 to 31 tubers and basal bulbs.

The chains were placed in plastic trays on filter paper saturated with 100 ppm BA to force all live tubers to sprout (63). The trays were covered with cellophane to prevent evaporation of the water and placed in the light at 23 C. Three weeks later the number of tubers sprouted and the number of sprouts per tuber were recorded. The chains were then broken and 20 tubers selected at random from each treatment. These were placed in two Petri dishes (10 tubers each) on filter paper with 15 ml 100 ppm BA. Three weeks later the number of sprouted tubers and the number of sprouts per tuber were recorded.

The effect of age on control of individual purple nutsedge plants with glyphosate. Purple nutsedge tubers were planted in soil in 15 by 15 cm pots and grown in the greenhouse at temperatures of 30 to 35 C day, and 20 to 22 C night. Two tubers were planted in each pot and thinned to one after they emerged. Tubers were planted every other week for 12 weeks, four pots per date. At 12 weeks, two pots from each date were sprayed with 4 kg/ha glyphosate and the others used as controls. Two weeks after spraying the tubers from all treatments were harvested and composited by treatment and date. These tubers were placed in Petri dishes and germinated with BA to test for viability. The number of sprouted tubers was recorded after 3 weeks.

Translocation of ¹⁴C-glyphosate in purple nutsedge. Purple nutsedge tubers dug from the Waimanalo Research Station, were planted in 8 by 11 cm pots in the greenhouse, 2 tubers per pot. These were thinned to

one tuber per pot after 1 week. Tubers were planted weekly for 6 weeks.

^{14}C -methyl-labeled glyphosate (specific activity of 1.51 mCi/mM) was converted from the acid to the isopropylamine salt by adding to 4.6 mg of the acid: 0.46 ml water, 1.61 mg isopropyl amine, and 2.3 mg MON 0818¹ surfactant. This gave a solution equal to 1 kg glyphosate in 182 L water, so 10 μl applied contained 0.2 μCi .

One week after emergence of the youngest plants (approximately 10 days after planting) 0.2 μCi of ^{14}C -glyphosate was applied to a recently matured leaf of each plant between two strips of lanolin. This was usually the third or fourth leaf from the apex. Plants were harvested 1, 2, 4, or 8 days after application. Three plants per age group were harvested at each date.

The spot on the leaf where the labeled herbicide was applied was wiped clean with paper to remove lanolin and remaining ^{14}C , and the leaf was wrapped with aluminum foil. Soil was then washed from the roots and rhizomes. The plants were placed in individual plastic bags and frozen, and then freeze dried.

After drying the plants were separated into treated leaf, other leaves, reproductive organs (basal bulbs, tubers, rhizomes), and roots. The separate parts of each plant were weighed, and ground to 20 mesh in a Wiley mill. The plant parts were then oxidized in a Peterson combustion apparatus and resulting $^{14}\text{CO}_2$ was captured in a liquid

¹MON 0818 is a non-ionic surfactant, manufactured by Monsanto, and included in the commercial formulation of glyphosate.

scintillation cocktail, as described by Peterson (44, 45). The residue wiped off the treated leaves was combusted with the treated leaves.

Where total weight of the plant part was greater than 200 mg, a sub-sample was taken. Samples were counted in a Packard Tri Carb 2420 liquid scintillation counter. The resulting counts were corrected for efficiency and converted to disintegrations per minute (dpm).

Autoradiography of purple nutsedge plants and tubers. Purple nutsedge tubers were planted in soil in 8 by 11 cm pots every week for 6 weeks, and grown in the greenhouse. The plants were treated with 0.1 μCi of ^{14}C -glyphosate as described above. When the ^{14}C -glyphosate had dried on the leaf, half of the plants (four from each date) were sprayed with 2 kg/ha unlabeled glyphosate. The plants were kept in the greenhouse until harvest. Two plants from each age (one sprayed with unlabeled glyphosate, one unsprayed) were harvested at 1, 2, 4, or 8 days after application. These plants were prepared for autoradiography as described by Yamaguchi and Crafts (77).

Autoradiography of individual tubers. Purple nutsedge tubers were planted in silica sand in 8 by 11 cm pots and grown in the greenhouse. They were watered daily with quarter-strength Hoagland's solution (28). Plants 4 and 8 weeks old were treated with 0.5 μCi ^{14}C -glyphosate as described above. The plants were harvested after 4 days. New tubers and basal bulbs were immediately cut into 0.5 mm thin sections on a slide microtome, and the sections quick-frozen with dry ice, freeze-dried, and mounted on paper. The mounted sections were placed on No Screen Medical X-ray film for 5 months before development.

Tubers treated with tetrazolium chloride. Four-week-old purple nutsedge plants were treated with 4 kg/ha glyphosate, and harvested after 4 days. Tubers from these plants, and unsprayed plants were cut into thin sections and placed in 0.1% tetrazolium chloride for 1 hour to test for viability. Tetrazolium chloride turns pink when reduced by the enzyme dehydrogenase, indicating that respiration is taking place and live tissue is present (50).

Glyphosate metabolism in purple nutsedge. Purple nutsedge tubers were planted in 8 by 11 cm pots and grown in the greenhouse for 4 weeks. ^{14}C -glyphosate was applied to the first two horizontal leaves between two strips of lanolin: 0.5 μCi per plant in 25 μl (half applied to each leaf). The plants were harvested 16 days after application; treated leaves were wiped clean of lanolin and ^{14}C -glyphosate remaining on the leaf, and covered with aluminum foil. The soil was washed from roots and rhizomes. The plants were frozen and freeze dried.

For extraction of glyphosate and metabolites, plants were sectioned into treated leaves, other leaves, tubers and rhizomes, and roots. These plant parts were ground in a Wiley mill to 20 mesh, and then 2 g or less was homogenized for 10 minutes in 50 ml water. The extract was filtered under vacuum. The extract was then passed through a column of Bio Rad AG1-X8 anion exchange resin to separate glyphosate from soluble plant materials, as described by Monsanto.² The column was prepared by washing 454 g of AG1-X8 (Cl^- form) with 1 L of 1 M ammonium bicarbonate (NH_4HCO_3), and then washing with

²Personal communication from Dexter Sharp, Monsanto Company.

distilled water until the wash water had a pH of 7. A column volume of 23 cc was used for 50 ml of plant extract. The column was washed with 100 ml distilled water, then eluted with 200 ml of 0.2 M NH_4HCO_3 . The eluant was collected, frozen and freeze dried, leaving glyphosate and possible metabolites in NH_4HCO_3 .

0.1 g of residue was dissolved in 0.5 ml water, and streaked on a cellulose DEAE thin layer chromatography (TLC) plate. The TLC plates were developed in a solvent system containing: 1.2 g disodium-EDTA, 100 ml ammonium hydroxide, 475 ml water, 350 ml n-propanol, 75 ml iso-propanol, 75 ml n-butanol, and 2500 ml iso-butyric acid.

After developing and drying, the TLC plates were separated into 11 strips constituting Rf positions 0.0 to 1.0, and the sections placed in liquid scintillation counting vials with 5 ml water and 15 ml Bray's solution (9). They were counted in a Packard Tri Carb liquid scintillation counter.

Results

Effect of apical dominance in rhizome-tuber chains on control of purple nutsedge with glyphosate. Chains from untreated plants 12 and 24 weeks old produced 53 and 55% sprouting of tubers, respectively, when germinated with BA (Table 11). Chains from 6-week-old plants produced 74% sprouting of tubers, indicating that these younger tubers were less dormant than the older tubers. However, when separated from the chains and exposed again to BA, tubers from 12- and 24-week-old plants increased sprouting to 80 and 90%, respectively. No increase in tuber sprouting occurred with tubers from 6-week-old plants. Thus, the chains exhibited some apical dominance in

Table 11. Effect of glyphosate when applied to purple nutsedge of different ages in trays. Tubers were germinated in chains with BA, then separated and regerminated with BA.

Age in weeks	Treatment	Tubers sprouted			Shoots per sprouted tuber	
		On chain ^a		After separation ^b	On chain	Total
		(%)	(SD)			
6	Control	74.2	4.9	77.1 d	1.5 b	2.1 c
	Glyphosate	0.0	0.0	0.0 a	0.0 a	0.0 a
12	Control	53.1	5.6	80.0 d	2.2 b	3.8 e
	Glyphosate	5.0	5.0	5.0 b	0.3 a	0.3 a
24	Control	54.5	6.3	90.0 e	1.9 b	3.3 d
	Glyphosate	31.5	7.7	31.5 c	1.7 b	1.7 b

^aThe number of tubers in the chains differed with age, so a standard deviation (SD) was calculated.

^bMeans in a column followed by the same letter are not significantly different at P=0.05 by Duncan's multiple range test. Figures are means of three replications.

maintaining dormancy of older tubers. Younger chains were less dormant and maintained less apical dominance.

Tubers on chains from 12- and 24-week-old plants treated with glyphosate sprouted at 5 and 32%, respectively. Sprouting did not increase after separation. Most of the sprouts on these tubers were very small and did not develop into full size shoots. Once the buds had germinated further development stopped. The tubers apparently were weakened but not killed by glyphosate. All the buds on a tuber reacted the same. Tubers from 6-week-old plants did not germinate on the chains after glyphosate treatment.

The controls of all ages produced more sprouts per tuber after separation of the tubers from the chains. The number of sprouts per tuber did not differ on the chain but, after separation, 12- and 24-week-old plants had more sprouts per tuber than the 6-week-old plants. This also indicated that the apical dominance of the chains was stronger in the older plants.

Tubers from plants treated with glyphosate did not produce more shoots after separation from the chains. The plants treated with glyphosate at 24 weeks produced as many sprouts per tuber as the controls, on the chains. Tubers from 12-week-old plants produced a few sprouts, but did not differ from tubers from 6-week-old plants which produced no sprouts.

Since some tubers sprouted on chains 12 and 24 weeks old, after treatment with glyphosate, but no more sprouted after separation and reapplication of BA, it appears that the rhizome-tuber chains were functional at these growth stages. Glyphosate prevented most tubers

from germinating, and appeared to kill them. The few that did sprout in chains did not increase in number of sprouts after separation. Thus, none of the tubers escaped the glyphosate; translocation may have been slower, or some tubers may have been highly dormant. Sprouting tubers were found on several chains, along with dead tubers, so there is no doubt that all chains were affected by glyphosate. Since tubers from glyphosate-treated plants did not increase in sprouting after separation from chains, as did the controls, we can conclude that they were weakened if not killed.

Effect of age on glyphosate control of individual purple nutsedge plants. No tubers from treated plants germinated except for one tuber from the 12-week-old plants (Table 12). Since no tubers from treated plants germinated, age of the plants alone is not a factor in mortality. As long as the tubers are attached to a physiologically healthy shoot, they apparently receive a toxic dose when the plants are sprayed with glyphosate at 4 kg/ha.

Translocation of ^{14}C -glyphosate in purple nutsedge. Approximately 80% of the activity applied to the plants in this experiment was recovered after combustion. Translocation of ^{14}C -glyphosate from the treated leaf increased with time up to 4 days after application (Figure 1). The amount translocated increased from 5% of the amount applied at 1 day to 19% at 4 days and decreased slightly to 15% at 8 days. The reduction in amount of ^{14}C translocated at 8 days appears to be a real effect since it was present in all growth stages. The glyphosate may have been metabolized and the $^{14}\text{CO}_2$ given off to the atmosphere. Any other metabolites containing ^{14}C should have been recovered from the plant.

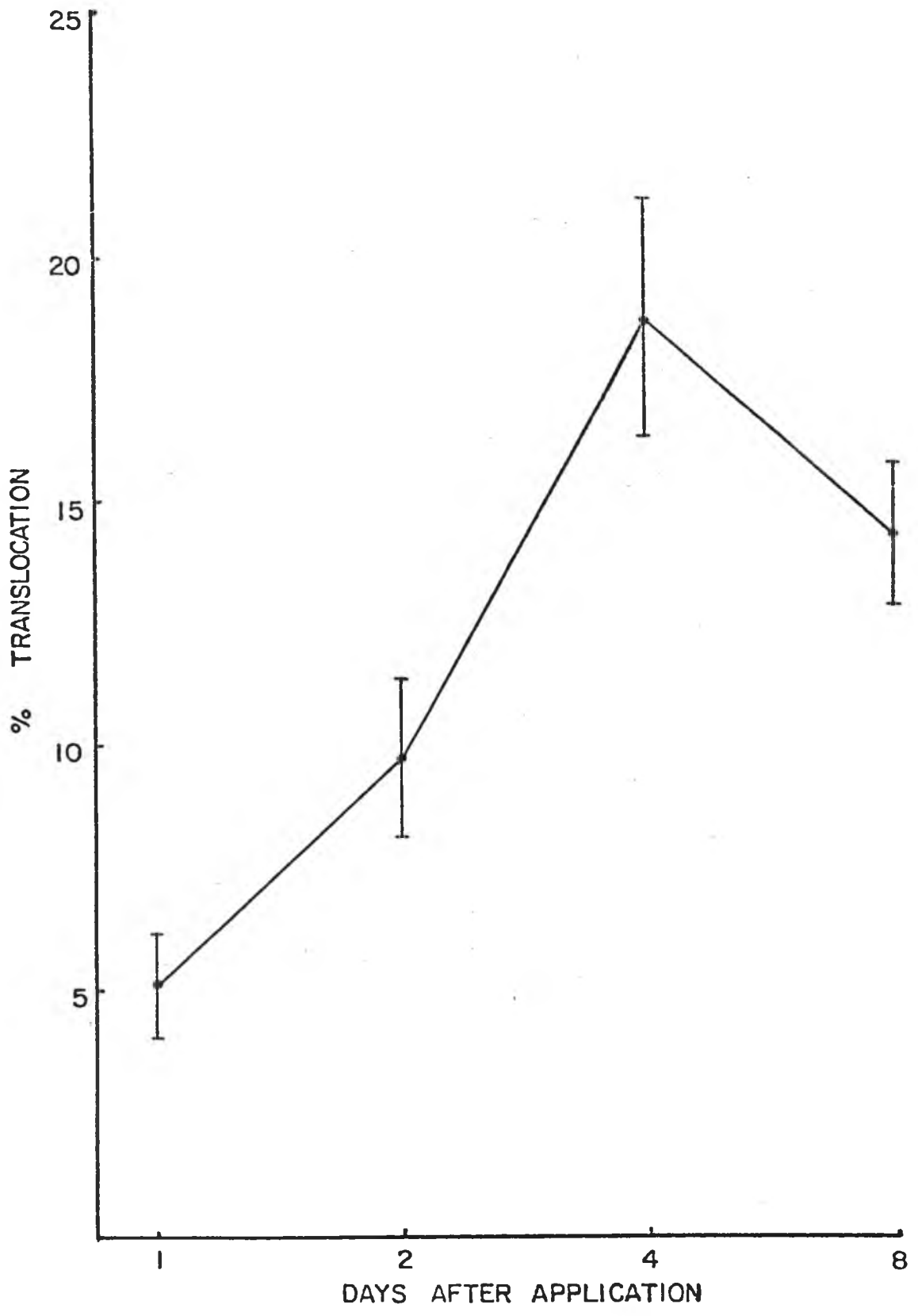
Table 12. Germination of purple nutsedge tubers in BA after foliar treatment with glyphosate.

Age in weeks	Treatment	Germination ^a (%)
2	Control ^b	50.0
	Glyphosate ^b	0.0
4	Control	80.0 c
	Glyphosate	0.0 a
6	Control	56.7 b
	Glyphosate	0.0 a
8	Control	56.7 b
	Glyphosate	0.0 a
10	Control	50.0 b
	Glyphosate	0.0 a
12	Control	43.3 b
	Glyphosate	0.3 a

^aMeans in a column followed by the same letter are not significantly different at $P=0.05$ by Duncan's multiple range test. Figures are means of three replications.

^bAt 2 weeks, only enough tubers for one rep had formed, so these treatments were not included in the analysis.

Figure 1. Percent translocation of ^{14}C -glyphosate applied, from treated leaf to the rest of the plant. Values for each time period are means for plants 2 to 6 weeks old.



Since the total amount translocated was greatest at 4 and 8 days, and since the shorter time periods greatly increased variation, the 4 and 8 day periods on the plants were composited and used for the following comparisons. The amount translocated of the total applied to the treated leaf decreased from 30% for 1-week-old plants to 19% at 2 weeks and then remained virtually the same until 6 weeks, when 16% was translocated (Figure 2).

We used two measures of the amount of labeled glyphosate translocated to various plant parts: specific activity (dpm/mg), which is a measure of the relative concentration of ^{14}C in plant parts; and total activity (dpm) in the plant parts, which tells the general direction of translocation with increasing plant age.

Since 1-week-old plants had no tubers, these factors are reported for tubers only for 2 to 6 weeks. Specific activity of ^{14}C -glyphosate in tubers decreased sharply from 306 dpm/mg at 2 weeks to 18 dpm/mg at 6 weeks (Figure 3). This dilution effect may be due to the increase in plant bulk from 2 to 6 weeks. Specific activity in leaves also decreased from 1 to 6 weeks, but was about one fourth to one third that of tubers at all growth stages. Thus, purple nutsedge plants concentrate more of the glyphosate in reproductive organs than in leaves at all ages tested.

Total activity in tubers increased with plant age while it decreased in leaves (Figure 4). Thus, while specific activity in both tubers and leaves decreased with age, total activity in tubers increased with age despite the greater production of tubers than leaves as plants

Figure 2. Percent translocation of ^{14}C -glyphosate applied, from treated leaf to the rest of the plant. Values for each age are means for plants harvested 4 and 8 days after treatment.

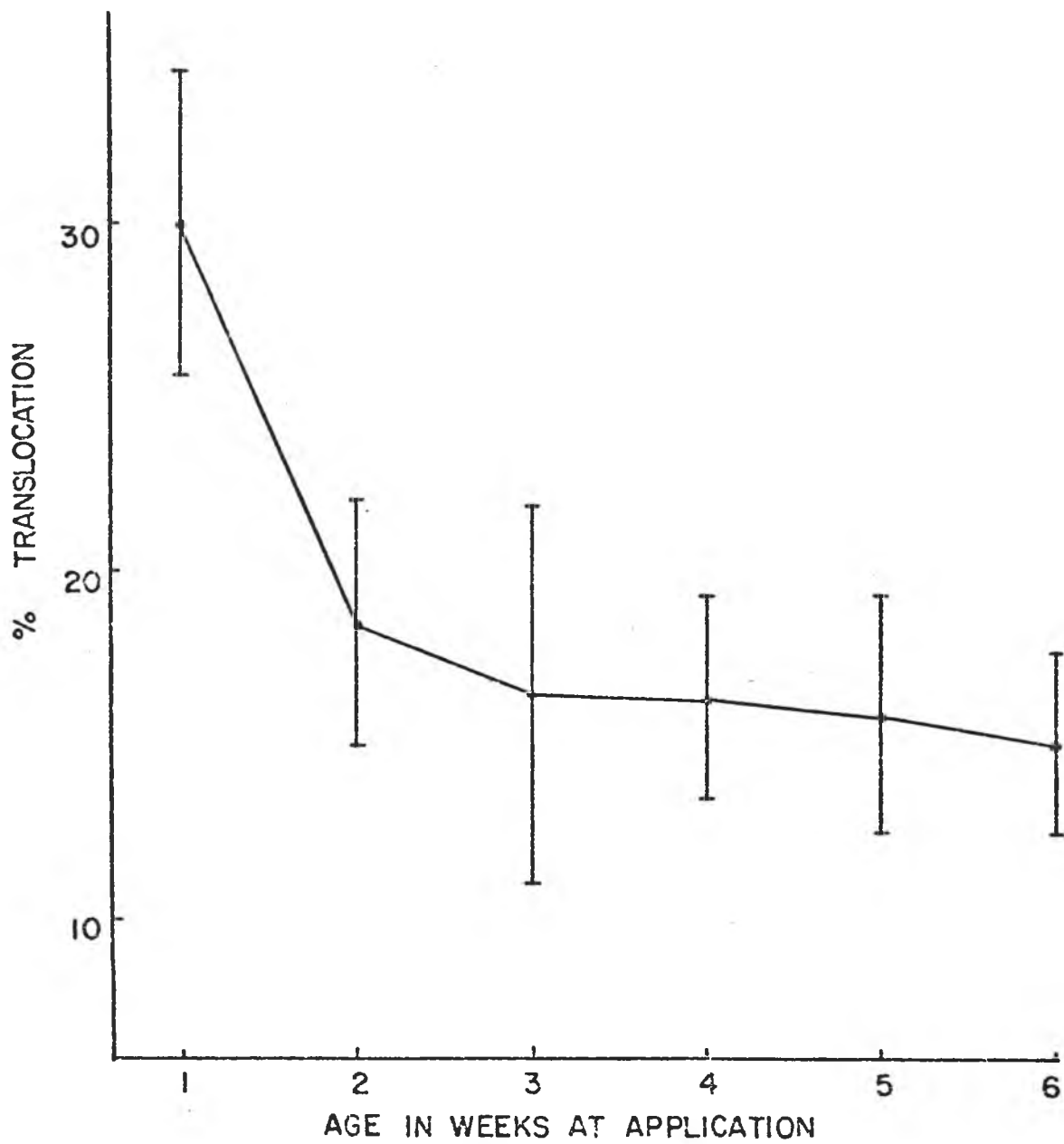


Figure 3. Specific activity of ^{14}C -glyphosate in tubers and leaves from plants treated with 0.2 μCi per plant. Values for each age are means for plants harvested 4 and 8 days after treatment.

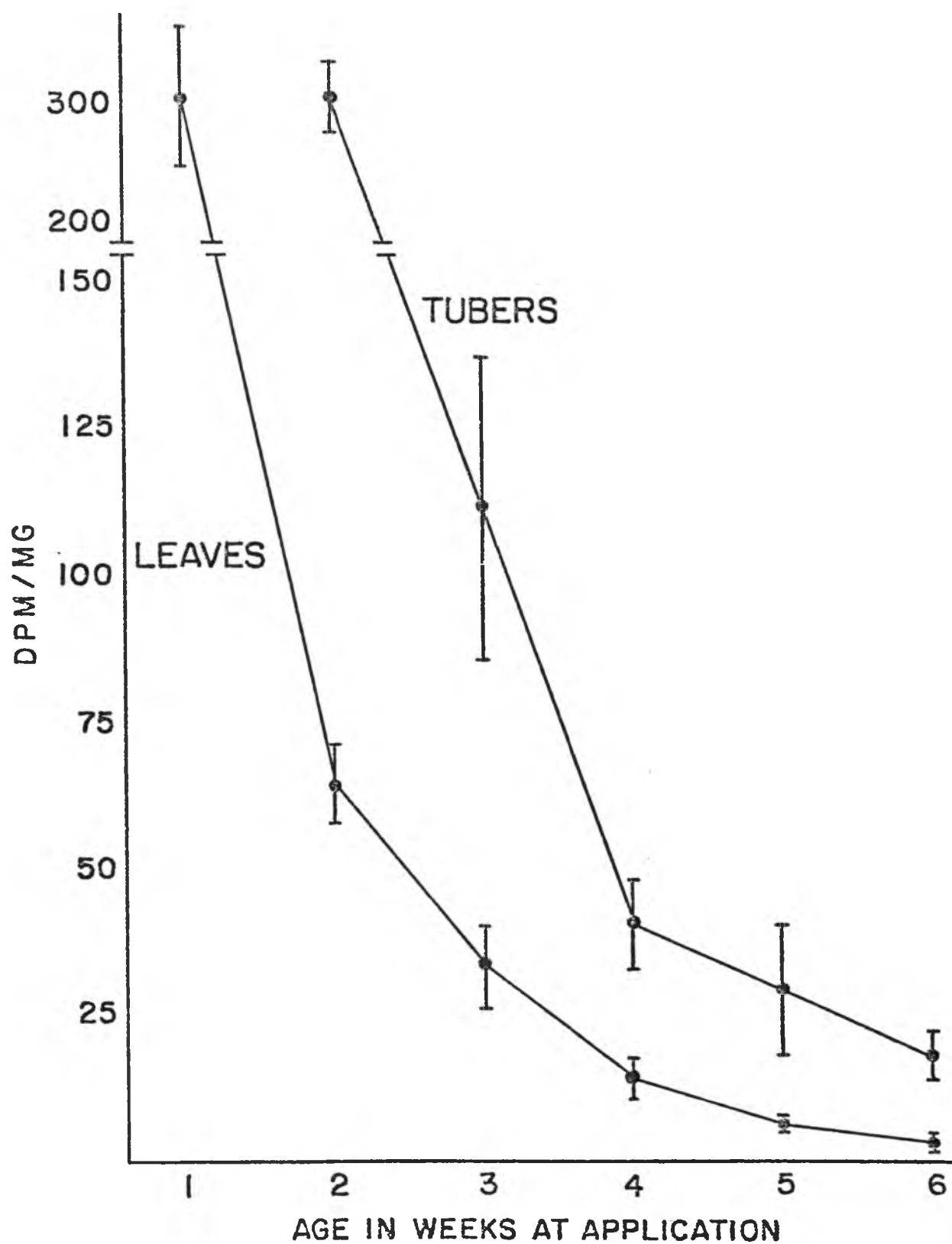
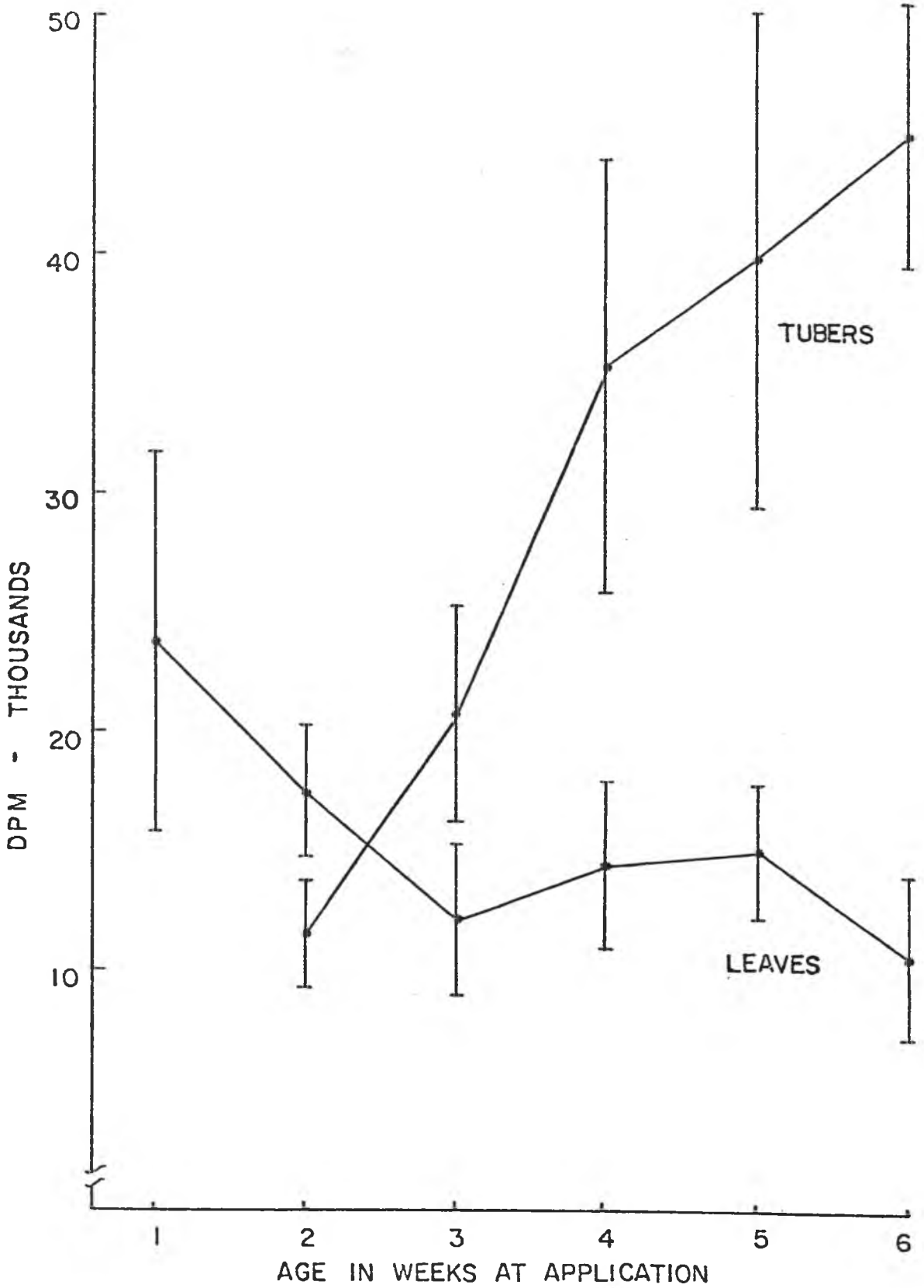


Figure 4. Total activity in leaves and tubers from plants treated with 0.2 μCi per plant. Values for each age are means for plants harvested 4 and 8 days after treatment.



mature. This indicates that the older plants transported a greater proportion of the ^{14}C to tubers than did younger plants.

Specific activity of ^{14}C -glyphosate in roots was intermediate between leaves and tubers (Table 13, Figure 3). In younger plants (1 to 3 weeks), total activity was higher in roots than in leaves or tubers but was about the same as in leaves of older plants. In young plants, roots make up a proportionately larger part of plant bulk than in older plants and therefore accumulate more of the ^{14}C . As plants mature, downward movement of the ^{14}C is more to rhizomes and tubers, and less to roots.

These results indicate: 1. Translocation from the treated leaf increases up to at least 4 days after application of glyphosate. 2. Two- to 6-week-old purple nutsedge plants translocated approximately the same amount of glyphosate. 3. As the plants developed, the tubers accumulated a larger proportion of the herbicide than tubers from younger plants. 4. Leaves accumulated less glyphosate than tubers at all growth stages when tubers were present.

Autoradiography of whole plants. The autoradiographs showed no difference in distribution of ^{14}C between plants sprayed with unlabeled glyphosate and unsprayed plants. The ^{14}C moved in the same patterns and to the same areas of accumulation for both treatments at each growth stage of purple nutsedge. Furthermore, ^{14}C distribution did not change with the time period (1, 2, 4, or 8 days) during which plants were exposed to ^{14}C -glyphosate. Young plants (1 to 2 weeks) showed fairly uniform distribution of the ^{14}C (Figure 5). As the plants got older, the ^{14}C accumulated in flowers (3 and 4 weeks)

Table 13. Movement of ^{14}C -glyphosate to roots of purple nutsedge plants.

Age in weeks	Specific activity ^a (dpm/mg)	(SD)	Total activity in roots (dpm)	(SD)
1	589	150	109,469	13,435
2	186	34	56,547	11,204
3	53	12	30,628	9,978
4	19	4	17,185	2,761
5	22	6	16,626	4,090
6	17	4	12,562	2,668

^aValues for each age are means for plants treated for 4 and 8 days.

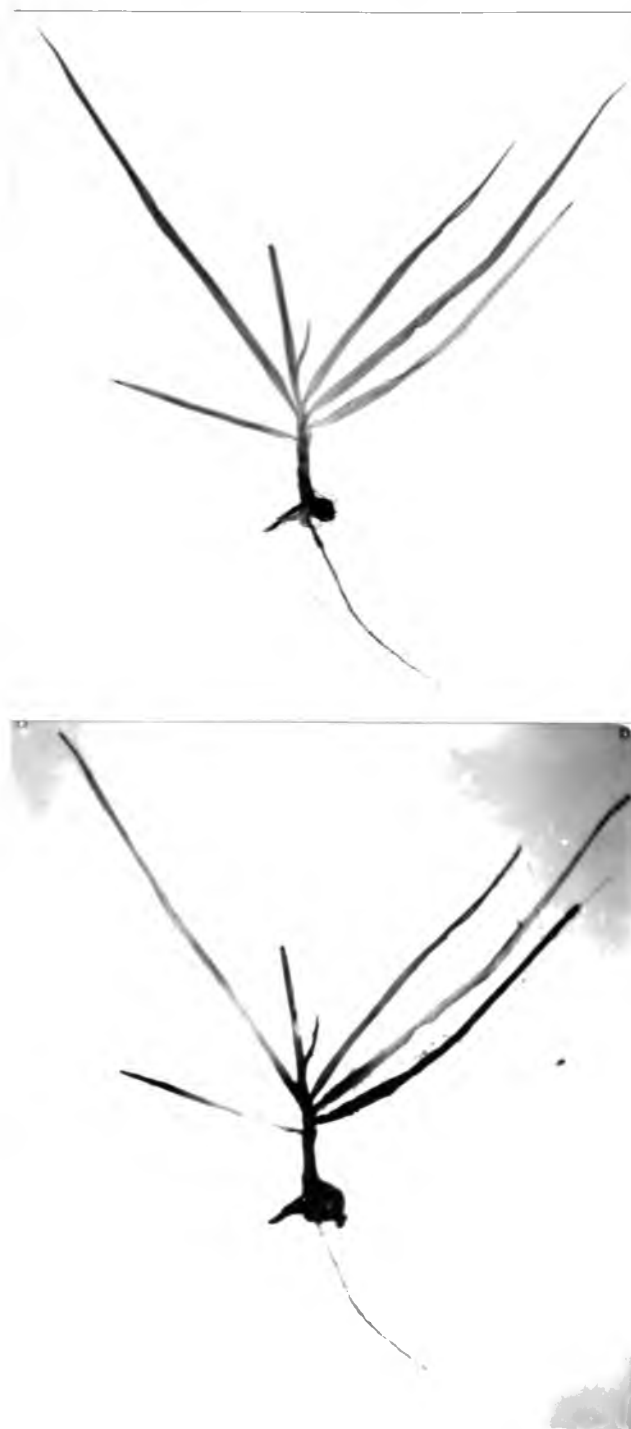


Figure 5. Translocation of ^{14}C -glyphosate in a 1-week-old purple nutsedge plant, harvested 8 days after application of $0.1 \mu\text{Ci}$.
Top: plant; bottom: autoradiograph.

(Figures 6 and 7), newly formed leaves, and tubers and basal bulbs (5 to 6 weeks) (Figure 8). The ^{14}C seems to move through mature tubers to the newly forming tubers at rhizome tips.

Autoradiography of individual tubers. The thin sections of tubers and basal bulbs treated with ^{14}C -glyphosate showed a concentration of the ^{14}C in buds and the vascular system (Figure 9). There was a greater concentration in and near the apical buds than in the rest of the tuber. Movement of ^{14}C was throughout the vascular system to areas of high metabolic activity and only secondarily to storage areas.

Tubers treated with tetrazolium chloride. After treatment with tetrazolium chloride, thin sections of tubers from plants sprayed with glyphosate showed very little metabolic activity in the vascular system, compared to control tubers (Figure 10). As glyphosate accumulated in the vascular system, it severely inhibited dehydrogenase activity. Slight metabolic activity was detected in some buds of the glyphosate-treated tubers, which means the buds were not dead. This may be a result of the weakening effect described above, which allows some buds on treated tubers to sprout but not develop. Tubers from plants harvested 4 weeks after application appeared sound externally but decay was well advanced in areas around buds and the vascular system (Figure 11).

Metabolism. Extracts from treated leaves, other leaves, and tubers all showed only one main peak at Rf 0.1 to 0.3 (Figures 12, 13, 14). This corresponded to the peak for glyphosate at Rf 0.2 (Figure 15). Most samples had considerable tailing which was probably due to a poor

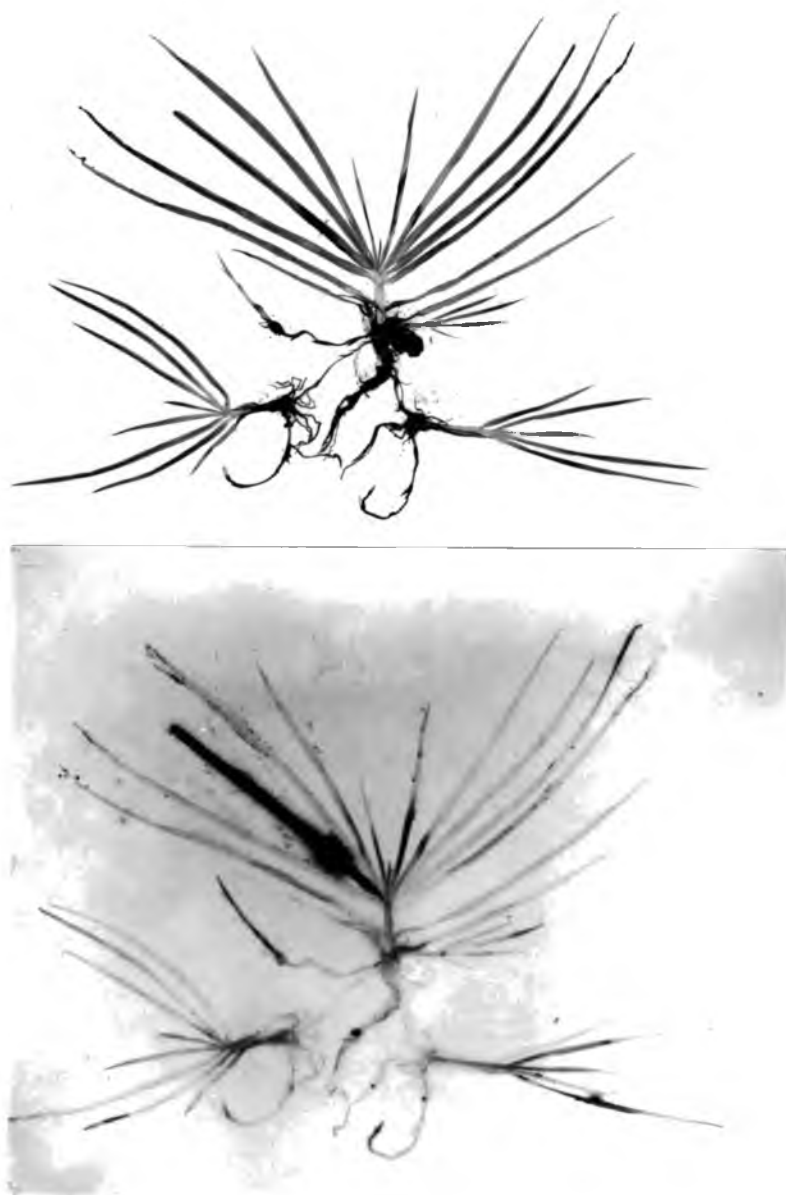


Figure 6. Translocation of ^{14}C -glyphosate in a 3-week-old purple nutsedge plant, harvested 1 day after application of $0.1 \mu\text{Ci}$. Top: plant; bottom: autoradiograph.

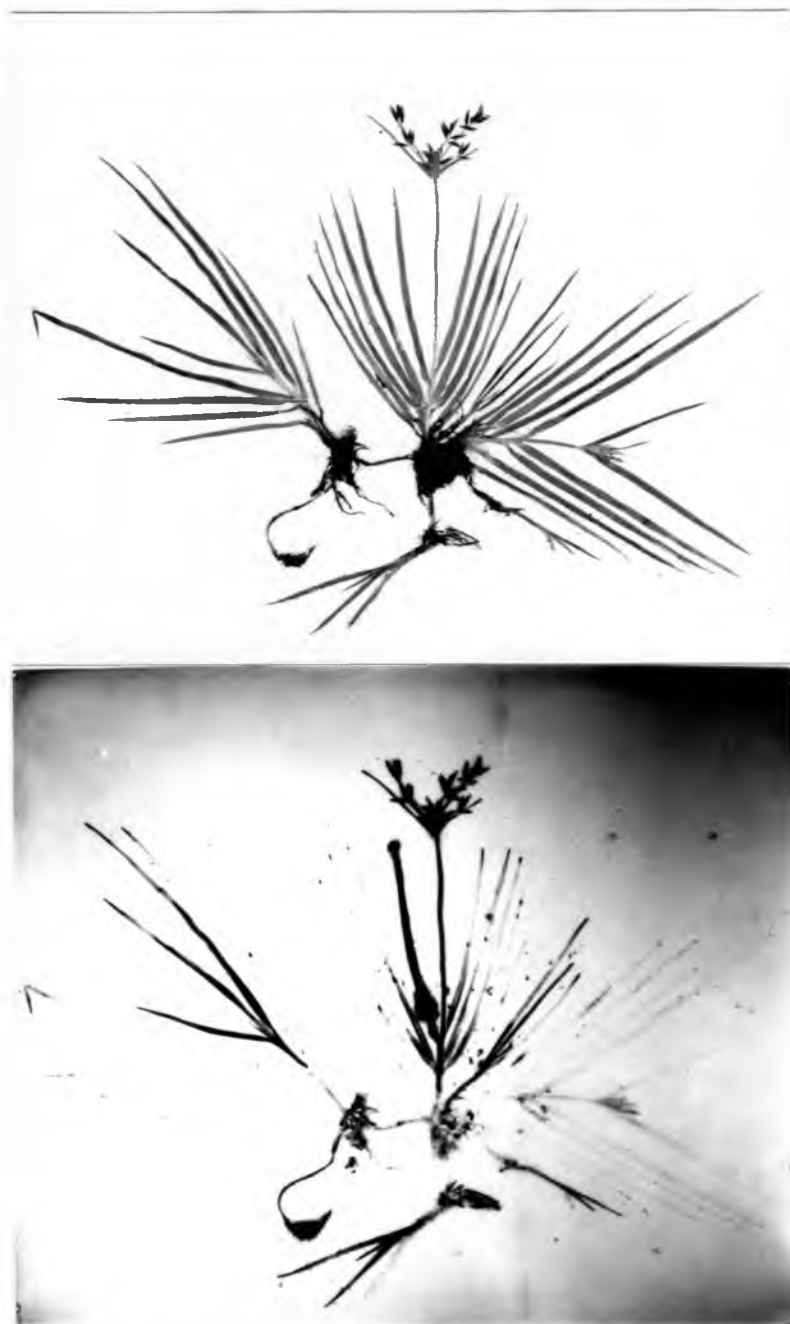


Figure 7. Translocation of ^{14}C -glyphosate in a 4-week-old purple nutsedge plant, harvested 4 days after application of $0.1 \mu\text{Ci}$. Top: plant; bottom: autoradiograph.



Figure 8. Translocation of ^{14}C -glyphosate in a 6-week-old purple nutsedge plant, harvested 2 days after application of $0.1 \mu\text{Ci}$.
Top: plant; bottom: autoradiograph.

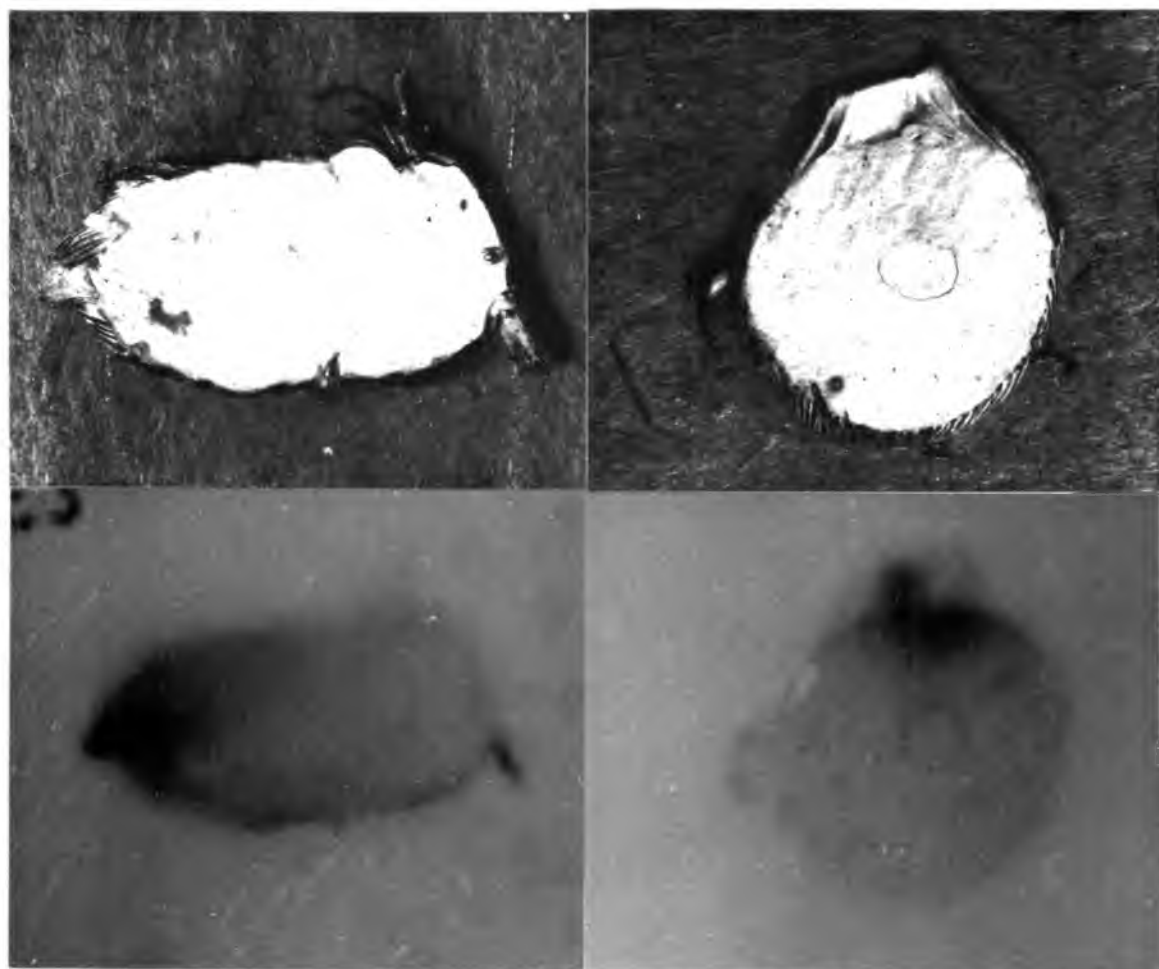


Figure 9. Distribution of ^{14}C -glyphosate in a purple nutsedge tuber from an 8-week-old plant (left), and basal bulb from a 4-week-old plant (right). Plants were treated with $0.5\ \mu\text{Ci}\ ^{14}\text{C}$, and harvested after 4 days. Top: tuber and basal bulb sections; bottom: autoradiographs.

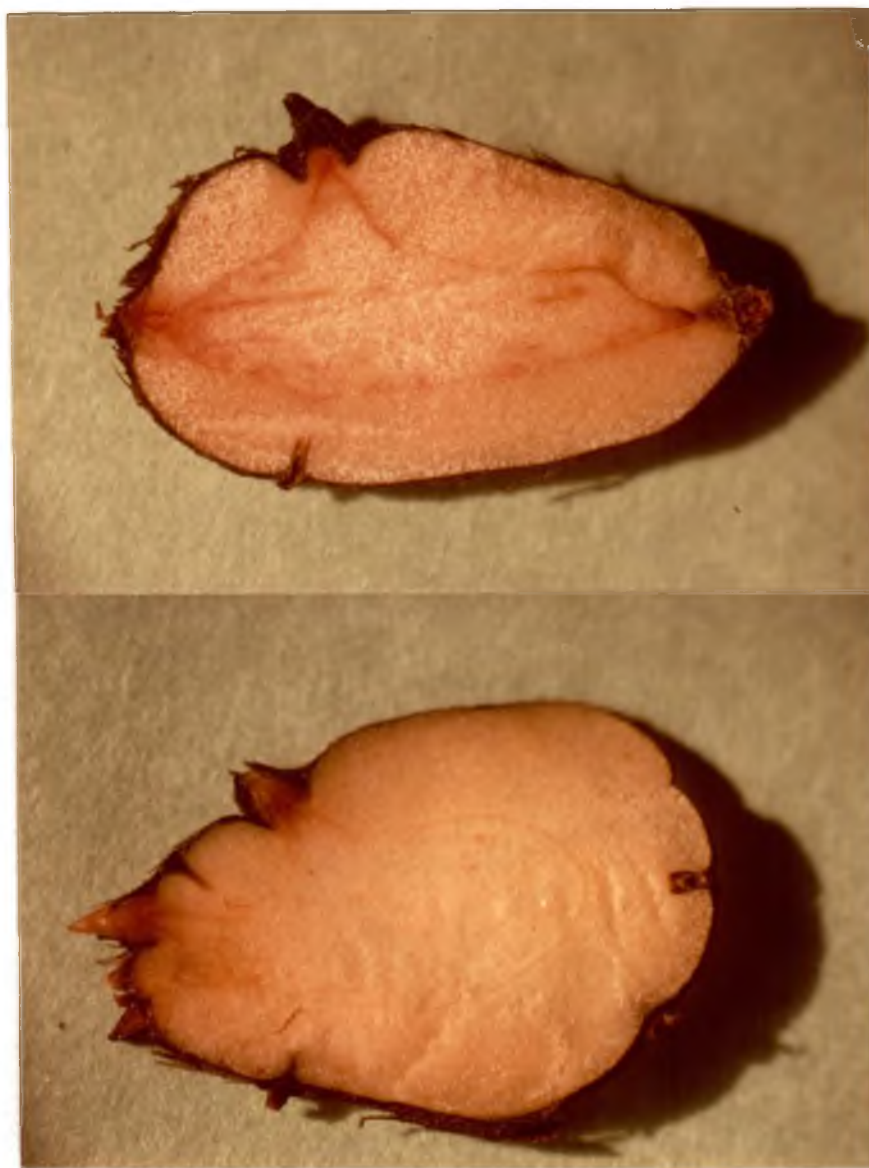


Figure 10. Purple nutsedge tubers treated with 0.1% tetrazolium chloride. Top: tuber from untreated plant; bottom: tuber taken from plant 4 days after application of 4 kg/ha glyphosate to the foliage.



Figure 11. Tuber from a 4-week-old purple nutsedge plant, harvested 4 weeks after application of 4 kg/ha glyphosate to the foliage.

Figure 12. Distribution after thin layer chromatography of ^{14}C from extracts of treated purple nutsedge leaves. Plants 2, 4, and 6 weeks old were harvested 16 days after application of ^{14}C -glyphosate.

TREATED LEAF

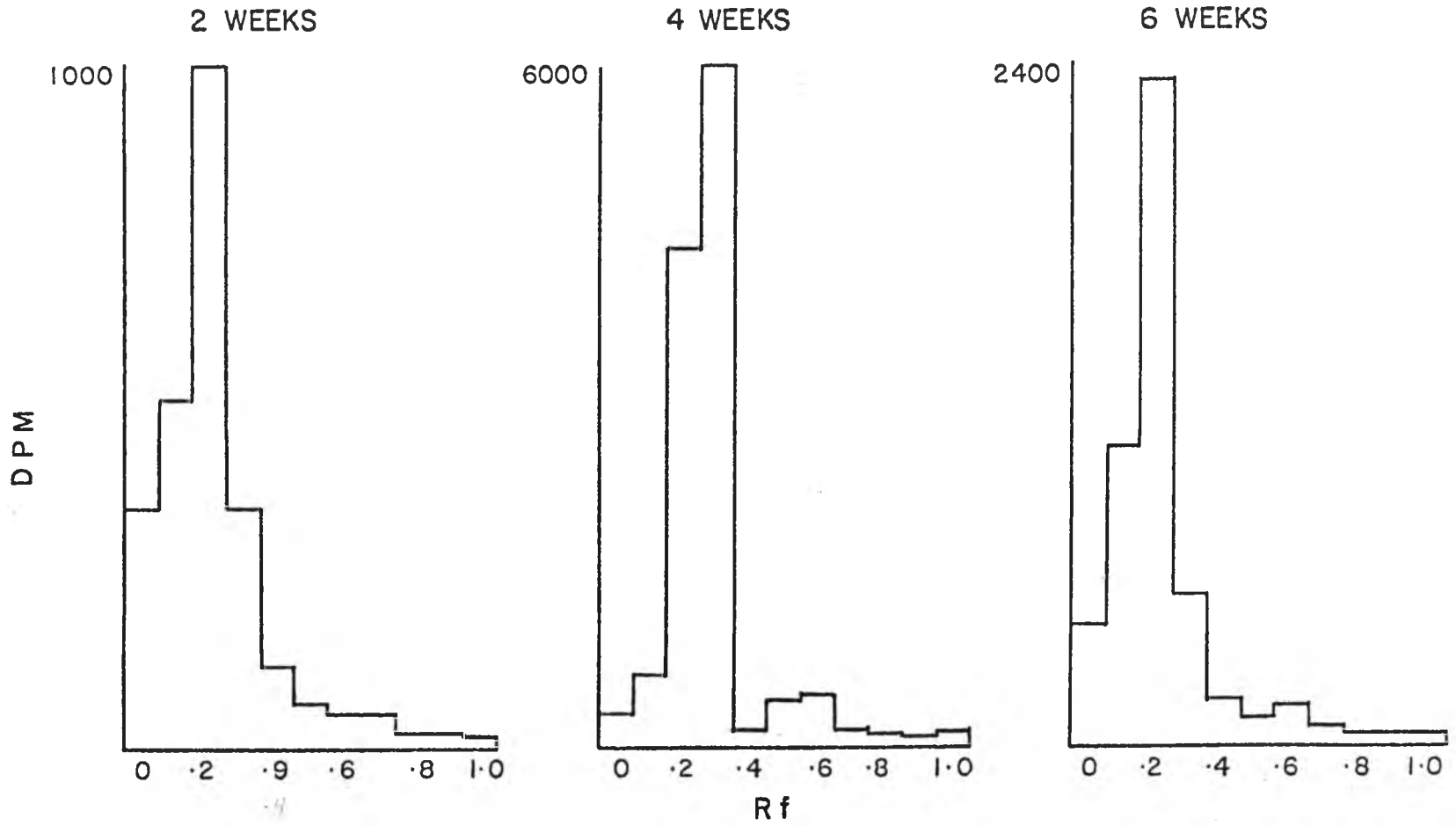


Figure 13. Distribution after thin layer chromatography of ^{14}C from extracts of other leaves of the purple nutsedge plants. Plants 2, 4, and 6 weeks old were harvested 16 days after application of ^{14}C -glyphosate.

OTHER LEAVES

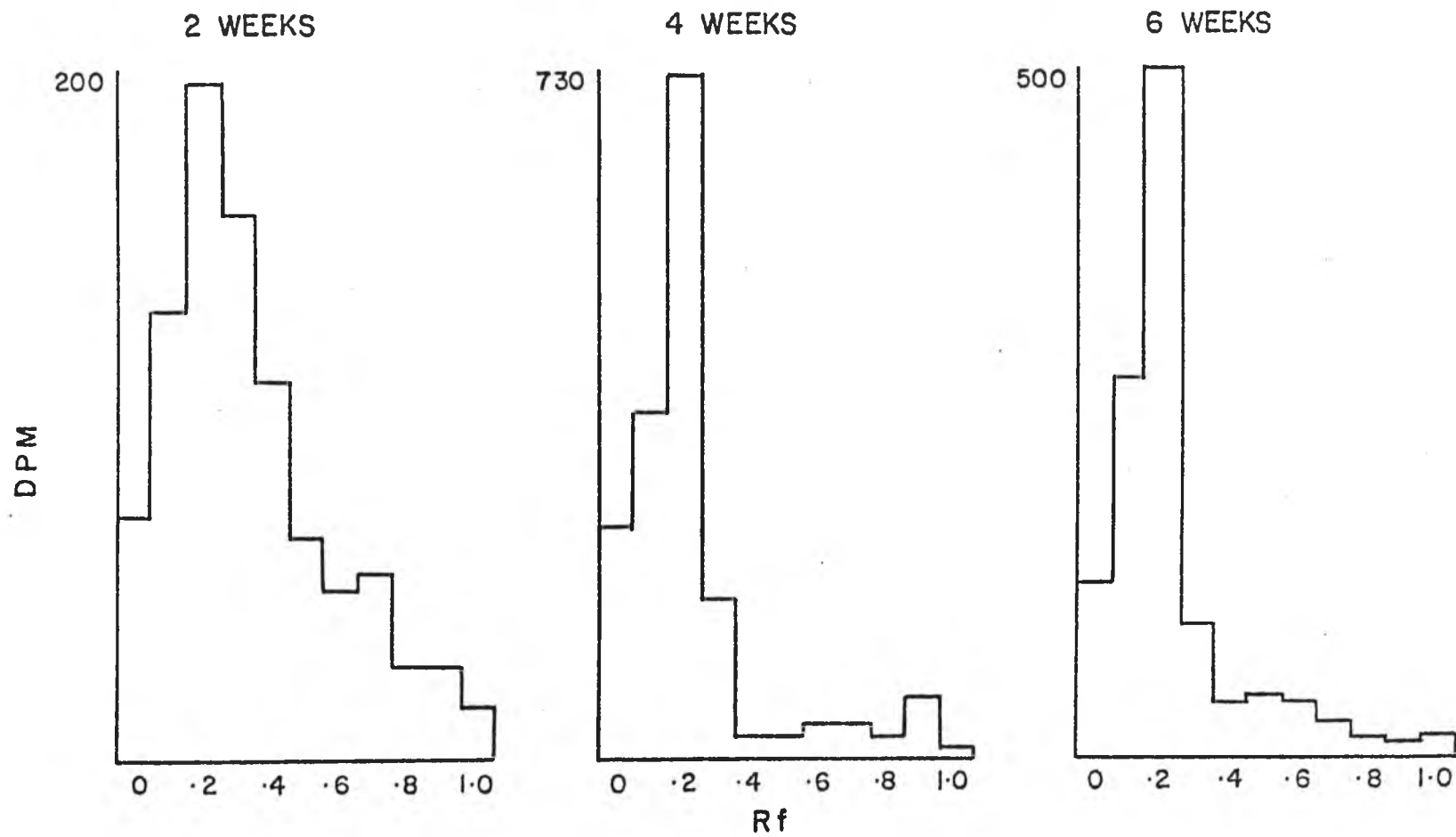


Figure 14. Distribution after thin layer chromatography of ^{14}C from extracts of tubers of purple nutsedge plants. Plants 2, 4, and 6 weeks old were harvested 16 days after application of ^{14}C -glyphosate.

TUBERS

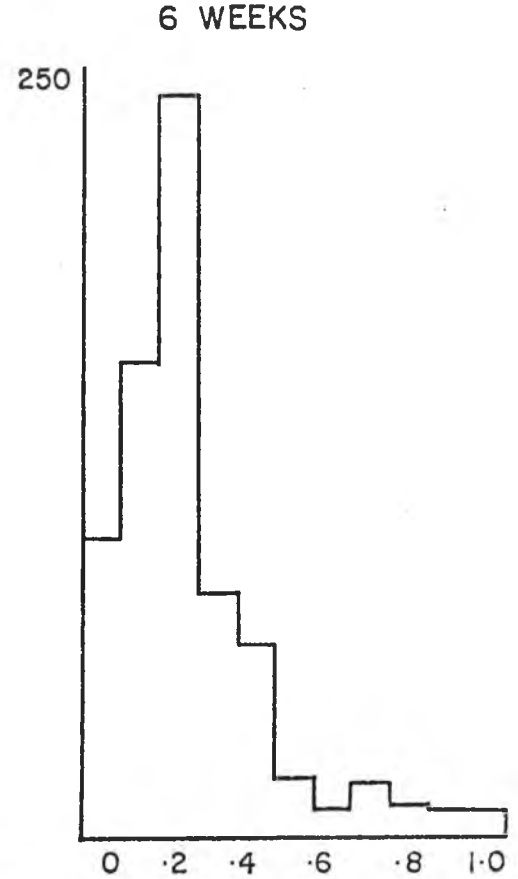
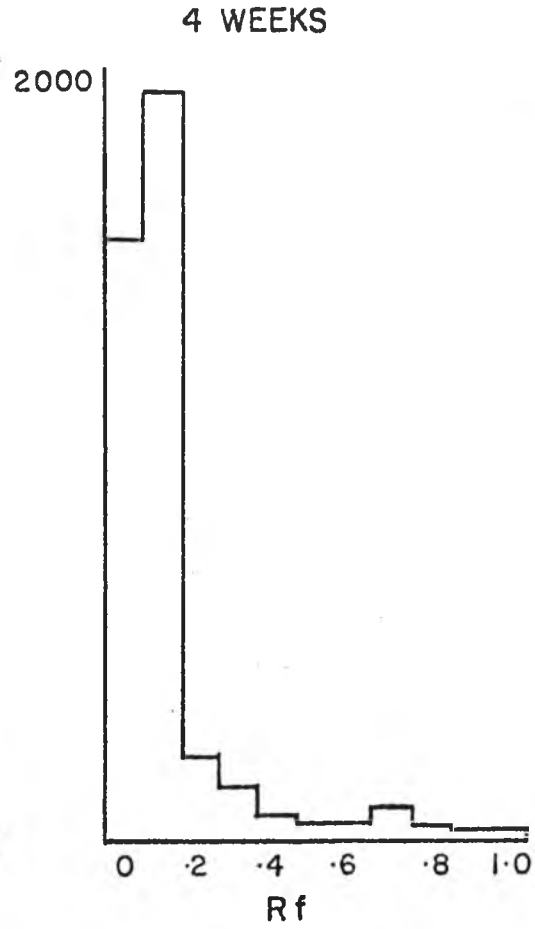
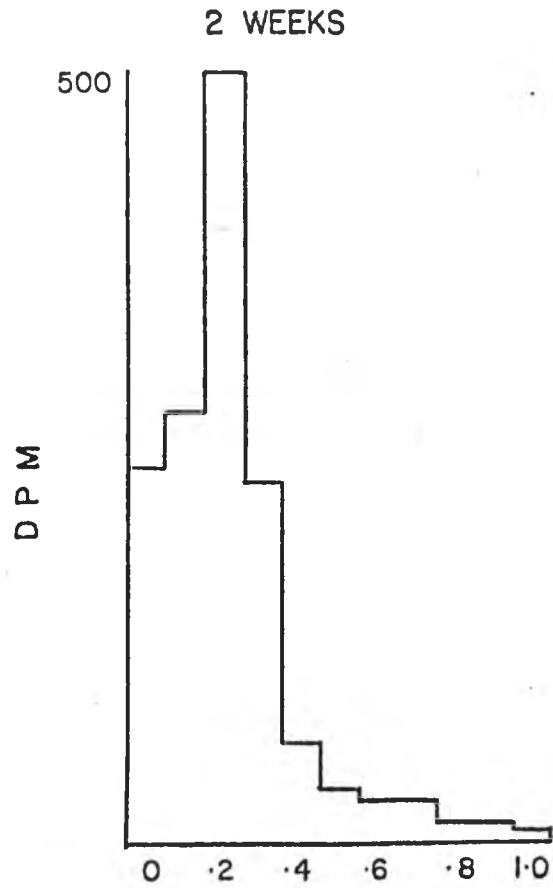
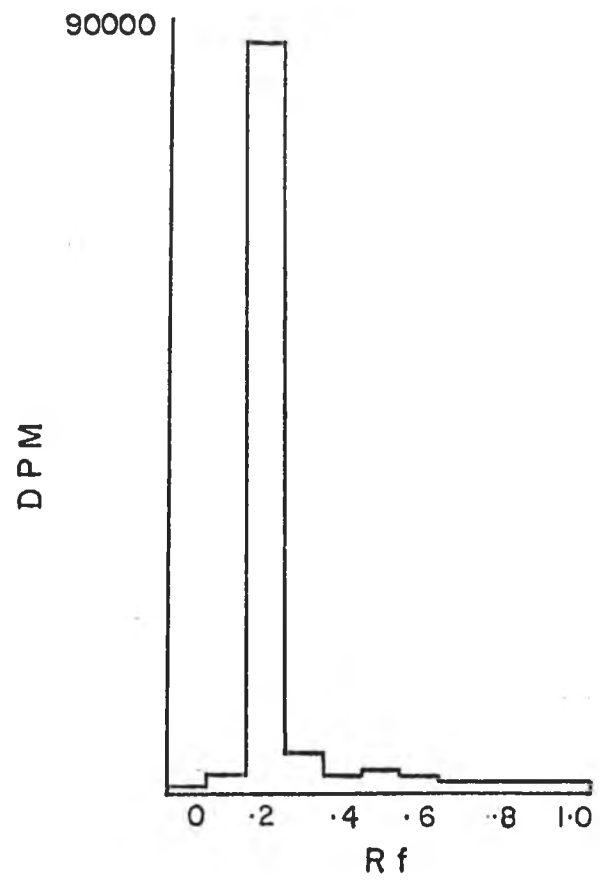


Figure 15. Distribution after thin layer chromatography of ^{14}C -glyphosate. 0.1 μCi of ^{14}C -glyphosate in water was applied at the origin.



purification process. Glyphosate was reported³ to be found at Rf 0.0 to 0.15 and the expected major metabolite (amino methyl phosphonic acid) at Rf 0.4. Even with the large tail, the peak had diminished to 1 to 2% of the total by Rf 0.4 in most cases.

It is possible that some ¹⁴C-glyphosate was completely metabolized and the ¹⁴CO₂ given off to the atmosphere. Aside from that possibility, it appears that the ¹⁴C in the purple nutsedge plants was ¹⁴C-glyphosate.

³Personal communication from Monsanto Company.

CHAPTER VI

DISCUSSION AND CONCLUSIONS

Results of our initial experiments in the greenhouse (Chapter I), are in agreement with reports of others (27, 40, 66, 74) who found that glyphosate was toxic to purple nutsedge. While foliar symptoms of purple nutsedge due to glyphosate and paraquat treatments are similar, the actual effect on the plant is much different. Paraquat is a strictly contact material, desiccating the foliage with which it comes into contact. The purple nutsedge basal bulbs and tubers appear to be unaffected by paraquat, and new leaves are produced rapidly. Treatment with glyphosate caused slower (but similar) deterioration of purple nutsedge leaves. However, foliar regrowth was slow and much less than that of paraquat-treated plants. Purple nutsedge plants treated with paraquat regrew from the same basal bulbs, which is in agreement with a recent report (58). However, basal bulbs of plants treated with glyphosate appeared to be killed, and the few new leaves came from new basal bulbs produced at the ends of rhizomes from either the mother tubers or other unaffected tubers.

Field experiments confirmed the superiority of glyphosate over paraquat in controlling purple nutsedge shoot growth (Tables 2 and 3). Repeated applications of glyphosate also reduced the number of tubers present in the soil, and the number of tubers sprouting after application of BA (Table 4).

The field experiments also compared glyphosate to dicamba, a hormone type herbicide, and MSMA, a herbicide which is translocated to some extent in purple nutsedge. Dicamba is known to move readily within

purple nutsedge plants (39, 48), and accumulates in metabolic sinks. However, control of purple nutsedge with dicamba has been only marginal (47, 51).

Repeated applications of glyphosate reduced the number of purple nutsedge shoots to a lower level than did dicamba but the difference was not always significant (Tables 2 and 3). Fewer tubers from plants treated with glyphosate germinated than did tubers from plants treated with dicamba, even though dicamba reduced germination of tubers below the level of untreated controls (Table 4). Dicamba was no better than paraquat in reducing shoot regrowth, but it reduced germination of tubers more than paraquat. It appears that the translocation of dicamba in the plants and accumulation in the metabolic sinks results in more effective control of purple nutsedge than is possible with paraquat. Since glyphosate was more effective than dicamba in reducing germination of tubers, it may have been due to better translocation to rhizomes and tubers.

MSMA gave results similar to glyphosate in reducing number of shoots and germination of tubers. MSMA is translocated to all parts of the purple nutsedge plant, including tubers, basal bulbs, and adjoining plants (38). Holt suggested that the reduction in regrowth of purple nutsedge is probably due to depletion of food supplies in the reproductive organs, after repeated sprouting, rather than to direct toxicity of the arsenic to the plant tissue (31). If this is true, it could explain our results which show a slower reduction in plant numbers with MSMA than with glyphosate. Our work indicates that MSMA probably has some direct toxic effects on the purple nutsedge

plants. If the reduction in plant numbers was only due to a reduction in food supplies, paraquat should also have reduced plant numbers after repeated applications, but this was not the case.

Translocation of a herbicide into rhizomes and tubers of purple nutsedge is necessary to obtain control. But translocation alone is not sufficient, as seen from the results with dicamba, which is translocated somewhat to tubers but is not very effective in killing them. Sufficient amounts of a herbicide toxic to the tubers have to accumulate to kill the tubers and prevent buds from sprouting. Our work indicated that glyphosate was rapidly translocated to reproductive organs, resulting in a high degree of toxicity and little regrowth.

Having established that glyphosate was toxic to purple nutsedge tubers, the most effective method for its use in the field was investigated. This included determining optimum rate of application, most susceptible age of purple nutsedge plants at application, most advantageous timing of repeated applications, and the relationship of age to growing conditions. ^{14}C -glyphosate was used to examine translocation and accumulation of glyphosate in purple nutsedge plants of different ages.

Two kg/ha of glyphosate was as effective as 4 kg/ha in reducing the number of live purple nutsedge plants (Table 5). At 3 months after initial application, only the 24-week treatments showed differences between the rates. After 5 and 7 months there was no difference within any of the growth stages. In addition to the 2 and 4 kg/ha rates, 1 kg/ha was applied every 2 weeks. After 3 months 1 kg/ha applied every 2 weeks was as good as 2 or 4 kg/ha applied every 2 weeks, in reducing the number of live shoots.

Two kg/ha reduced germination of purple nutsedge tubers as well as 4 kg/ha (Table 6). In most cases there was less germination of tubers from plants treated with 4 kg/ha than with 2 kg/ha, but the differences were not significant. Generally, more tubers from plants treated with 1 kg/ha germinated than from plants treated with 2 or 4 kg/ha, even though the effect on purple nutsedge stand was similar after 3 months.

After the plots were rotovated and the purple nutsedge regrew, there were no differences in the number of plants between rates within ages except for plants treated at 24 weeks (Table 8). In the 24-week applications, 4 kg/ha reduced plant population more than did 2 kg/ha at 1, 2, and 3 months after tilling. Two kg/ha did not differ from untreated controls at 1, 2, and 3 months after tilling.

Under the conditions of this experiment 2 kg/ha gave as good control as 4 kg/ha. One kg/ha did not give sufficient control until it was repeated several times, but it may be effective as a follow-up treatment after 2 kg/ha. The slight increase in control with 4 kg/ha does not justify the additional cost. The only exception to this might be for plants which are past maturity and no longer actively growing, as in the case of the plants treated at 24 weeks. Then the added glyphosate gave an increase in control. This compares to the work of Wills who reported satisfactory control of purple nutsedge with 2 kg/ha (74). Magambo and Terry obtained control of a mature stand of purple nutsedge under coffee for 26 weeks with 2 kg/ha (40). Their application was followed by the dry season, so regrowth was inhibited by lack of water also. They later reported extending control with

4 kg/ha for 88 weeks during a long dry period (66). Evidently the lack of moisture was as important as the rate of glyphosate in reducing regrowth of purple nutsedge plants. Hebblethwaite was not able to maintain long-term control with a single application of glyphosate at 2 kg/ha (27). However, with a second application of 1 kg/ha he was able to maintain control for the rest of the growing season. Higher rates did not increase his long-term control.

The condition of the purple nutsedge plant at the time of glyphosate application is an important factor in its control. Under conditions in which good control is possible, 2 kg/ha glyphosate will give satisfactory control. If external factors (moisture, light, nutrients) or internal factors (age, stage of growth) are not optimum, increasing the rate of glyphosate above 2 kg/ha will increase initial kill of foliage and will reduce later regrowth (Table 8).

Maximum control of purple nutsedge with a single application of glyphosate was achieved at 12 weeks after emergence (Tables 5 and 6). In the field, with adequate moisture and nutrients, purple nutsedge began to flower at 8 weeks after emergence. By 12 weeks most plants were in flower, and maximum plant density was reached (Table 9). By 16 weeks plant density was declining.

It seems that after most of the shoots have flowered and the purple nutsedge plants have occupied all vacant area, the plants begin a slow senescing process in which few flowers are formed and shoot density declines to a level of less than half of the maximum density. A few new shoots continue to form, and old shoots die. If left in this state for a long period, grass and broadleaf weeds will

eventually crowded out the purple nutsedge. However, when the soil is tilled again the purple nutsedge comes back in large numbers. William and Warren reported the same phenomena of rapid growth and senescence (72). Under the conditions of their experiment, maximum density was reached at 7 weeks after field preparation. They also noted that once the plants had flowered, senescence set in rapidly.

This "physiological maturity" (i.e., when the plants had flowered and leaf area was at its maximum) was reached at 12 weeks. It appears to be the critical factor in achieving successful control of purple nutsedge, rather than age itself. Purple nutsedge grown in pots and trays confirmed the necessity of applying glyphosate at physiological maturity to obtain kill of tubers. Purple nutsedge grown in trays under crowded conditions flowered at about 6 weeks after planting. By 12 weeks the plants had declined in vigor, and some tubers were not killed by the application of glyphosate (Table 11). Individual plants grown in pots were not as crowded as the plants grown in the trays, and no tubers from plants treated with glyphosate sprouted, except a few from 12-week-old plants (Table 12). Under these conditions physiological maturity was reduced somewhere between 6 and 10 weeks after planting.

Other workers have also commented on the effects of age on weed control with glyphosate. Derting et al. obtained the best control of Johnson grass with glyphosate when application was delayed until late in the season (16). They attributed the higher kill with older plants to the greater amount of translocation downward. Parochetti et al. found that maximum kill of Johnson grass was obtained when glyphosate

was applied at the boot to full head stage (43). If applied earlier the rhizomes were not killed, and new shoots were formed. Neither Derting nor Parochetti reported a decrease in control beyond maturity of Johnson grass, as with purple nutsedge in this study.

There are two possible reasons that control was not achieved in the field when glyphosate was applied at intervals less than 12 weeks:

1. Many purple nutsedge tubers in the soil are dormant, and germinate very slowly. Since plant density increases up to 12 weeks, it appears that most dormant tubers that will sprout have in fact sprouted by 12 weeks. There are still some that are highly dormant remaining in the soil.
2. Foliage of very young plants (less than 2 weeks old) may be killed, allowing the mother tuber to produce additional shoots. Or the vascular system in very young plants may not be sufficiently developed to transport toxic quantities of glyphosate to the mother tubers. A sub-lethal dose of glyphosate may induce some type of dormancy in these tubers, allowing them to maintain viability even after shoot numbers have declined (Tables 5 and 6).

The radiotracer work supports the contention that glyphosate is translocated downward to basal bulbs, rhizomes, and tubers to a greater extent as plants mature. The specific activity of ^{14}C -glyphosate was always three to four times higher in tubers than in leaves (Figure 3). Since 1-week-old plants had not produced any tubers, the amount of ^{14}C in the leaves was high at that stage. However, at 2 weeks there was greater movement downward to the rhizomes and tubers that were just beginning to develop, resulting in a higher concentration of glyphosate, than to leaves, which were also developing

at that stage. This is extremely important since the weight of underground organs increases more rapidly than weight of leaves as plants mature, and the higher specific activity of ^{14}C -glyphosate in tubers was still maintained.

Total activity in tubers was lower than in leaves of 2-week-old plants. However, in older plants (3 to 6 weeks old) total activity increased in tubers and decreased slightly in leaves. The ^{14}C -glyphosate evidently was moved to areas of high metabolic activity and to storage areas. Thus, while plants are growing vigorously, and photosynthesis is at a high level, more of the glyphosate is moved downward. If enough glyphosate accumulates in tubers, they are killed. If lesser amounts are accumulated, the tubers may be weakened, or dormancy induced.

Some tubers obviously escaped the glyphosate treatment in the field. These were probably dormant at the time of application. When tubers were dug from plots and germinated with BA, there were only slight differences between treatments in the number of shoots produced by the live tubers (Table 7). There were fewer live tubers in plots treated with glyphosate at 12 weeks, but these tubers maintained their viability. When the field was rotovated 7 months after the initial applications of glyphosate, purple nutsedge reinfested these plots rapidly (Table 8). While control was maintained by one application of glyphosate at 12 weeks for 7 months, control was lost as soon as the soil was disturbed.

This means that control of purple nutsedge with glyphosate in short-term crops will be difficult, but not impossible. Treatments

every 2, 4, or 6 weeks, or at 12 weeks reduced plant density to a low level. But purple nutsedge in all plots regrew rapidly and caused some competition with vegetable crops. The untreated controls and 24 week treatments showed the greatest reduction in yield due to competition (Table 10). The single applications at 12 weeks gave as high yields as any other treatments, and were usually among the highest in yields.

Sweet corn, sweet potato, and soybean were able to compete fairly well with the purple nutsedge; consequently, yields in the best plots were only about twice as large as in control plots. Lettuce and kai choy were poor competitors, and suffered a greater reduction in yield. The best plots of these crops produced about 10 times as much as the untreated controls. There is no doubt about the deleterious effects of purple nutsedge competition on vegetable crop yields, as reported by William and Warren (72). Since a single application of 2 kg/ha at 12 weeks after field preparation gave as good results as other treatments, it would be the most economical in terms of cost of glyphosate.

Regrowth in plots treated at 12 weeks began after tilling: there was virtually no regrowth for 7 months. Therefore, to maintain the land free of purple nutsedge plants, the crops would have to be planted without tilling the soil, or with shallow tilling. Land under long-term crops, such as tree crops, is better suited for purple nutsedge control with glyphosate since the land is generally not tilled for several years. The long-term control of purple nutsedge with glyphosate reported by other workers was in a tree culture (40, 66).

Eradication of purple nutsedge with glyphosate is probably not possible, due to the dormant tubers. However, the number of tubers in the ground could probably be reduced considerably by allowing the purple nutsedge to grow until mature, spraying with glyphosate and allowing the plants to die for 4 days, then tilling the soil and repeating the process. This requires good growing conditions with adequate water, nutrients, and light. This is, of course, expensive, because of non-productivity of the land during the fallow period.

Several possible methods for using glyphosate to obtain maximum yields with minimum cost in vegetable crops can be suggested. One method would be to prepare a field, allow the purple nutsedge to emerge, and apply glyphosate at 12 weeks after emergence. Then the soil could be tilled, and a good competitor, such as corn, soybean, or sweet potato, planted. All of these can produce good yields with some competition, and all have a 3- to 4-month harvest cycle. Thus, at harvest time of the crops the purple nutsedge in the field would be at about physiological maturity and ready for another application of glyphosate. Four days after applying the glyphosate the field could be tilled, and another crop planted. After two successive applications at 12 weeks much of the purple nutsedge would be dead, and less competitive crops such as lettuce and kai choy could produce a crop.

Another possible method for growing vegetable crops would be to treat the purple nutsedge at physiological maturity with glyphosate, allow the plants to die 4 days, then plant directly into the soil with as little disturbance as possible. The dead purple nutsedge plants on the surface will serve as a mulch to slow down germination of other

weeds. If other weeds are a serious problem, preemergence herbicides should be used at initial field preparation, and again when crops are planted. A transplanter could penetrate the weed mass if the soil is soft. For small seeded crops, a fluted colter could precede the seed wheel to make a narrow seed bed through the dead plants on the soil surface. This would result in minimum soil disturbance and minimum regrowth of purple nutsedge. The vegetable crops could then mature with very little competition from the purple nutsedge.

Glyphosate has been demonstrated to be a very active herbicide on purple nutsedge. If applied at the optimum stage of growth of purple nutsedge, it will give a level of control unattainable with any other foliar applied herbicide. It has great potential for controlling purple nutsedge in undisturbed soil cultures. And if used properly, it can be used to reduce purple nutsedge competition and allow for good returns in vegetable crops as well.

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